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Development and Evaluation of Solid Self-Microemulsifying Drug Delivery System for Solubility Enhancement of Valsartan.

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ABSTRACT:

Valsartan is orally active, and specific angiotensin II antagonist acting on the AT1 receptor subtype (angiotensin receptor blocker). It is a lipophilic in nature and is feebly aqueous soluble drug with absolute bioavailability of 25%. The goal of the present study was to develop Solid self-micro emulsifying Drug Delivery System (S-SMEDDS) of valsartan to improve its oral bioavailability. The formulations were explored on the basis of solubility, FTIR study and excipient compatibility, emulsification efficiency, particle size and zeta potential. Solubility of valsartan was determined in various vehicles and maximum solubility was found in Castor oil as (oil), Tween 60 as (surfactant) and Kollisolv PG as (co-surfactant). These elements were used to design pseudo-ternary phase diagrams to identify the micro emulsion zone. Solid-SMEDDS were prepared by adsorption technique using Aerosil 200 (1% w/w) and were evaluated for micromeritic properties, scanning electron microscopy, X-ray diffraction, FTIR study and Drug content. The designed Solid-SMEDDS were further evaluated for stability study. Solid-SMEDDS may be considered as a better solid dosage form as solidified formulations are more ideal than liquid ones in terms of its stability. These results suggest the potential use of SMEDDS and solid-SMEDDS to improve the dissolution and hence oral bioavailability of poorly water-soluble drugs like valsartan through oral route.

KEYWORDS: Valsartan, Bioavailability, Lipophilicity, Microemulsion, L-SMEDDS, S-SMEDDS.

1. INTRODUCTION:

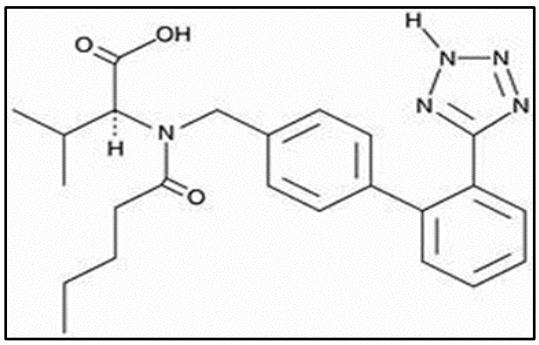
Self-microemulsifying drug delivery system (SMEDDS) are the newly emerging techniques for the enhancement of lipophilic drug delivery. Primary challenge of any oral formulation design program is to maintain drug solubility within G.I tract and particularly maximizing drug solubility within primary absorption site of the gut.[1] For lipophilic drug compounds which exhibit dissolution rate limited absorption self microemulsifying drug delivery systems (SMEDDS) can offer great step-up in rate and extent of absorption, leading to reproducible blood time profiles of BCS class II drugs in particular. [2]

According to Lipinski's rule of five for oral absorption trends, it predicts that poor permeation or poor absorption is more likely when there are more than 5-H bond donors, more than 10 H-bond acceptors, with molecular weight >500 and log P>5. [3]

SMEDDS are isotropic mixtures of oils, surfactants and co-surfactants, which form oil-in-water micro emulsion in aqueous media under gentle agitation. The finely divided oily droplets, with a droplet size less than 50 nm, provide a large surface area for drug release and absorption. The oily phase allows the drug to be present in its solubilised state, thereby avoiding the slow and rate-limiting dissolution process of a hydrophobic drug. [4] It can be prepared either in liquid form or encapsulated in hard or soft gelatine capsule. Nevertheless, it has some drawbacks also such as instability, leakage, precipitation of drug, and ageing of shells of the capsules.[5] to solve these above obstructions, the researchers have successfully developed solid SMEDDS using solid carriers and demonstrated their usefulness in dissolution and bioavailability. Recently the spray drying method for solidification of SMEF has been reported using different adsorbent materials for enhancement of solubility and bioavailability. [6]

Valsartan is a potent, orally active nonpeptide tetrazole derivative and selectively inhibits Angiotensin II Receptor type 1 which causes reduction in blood pressure and is used in treatment of hypertension. [7] It is a lipophilic drug and possesses moderate onset of action than other drugs of the same category. It is soluble in the neutral pH range. Valsartan is 3-methyll-2-[pentanoyl-[[4-[2-(2H-tetrazoyl-5-yl)phenyl]phenyl]methyl]amino]-butanoicacid (Structure 1) with empirical formula $C_{24}H_{29}N_5O_3$. Its molecular weight is 435.519g/mol.[8]Valsartan is a white coloured powder that is freely soluble in ethanol, methanol, and sparingly soluble in water. The partition coefficient of Valsartan is 0.033 (log P=1.499), suggesting that the compound is hydrophilic at physiological pH. The compound is stable under storage in dry conditions. Valsartan has bioavailability of about 25% due to its acidic nature. Being acidic in nature it is poorly soluble in the acidic environment of GIT and is absorbed from the upper part of GIT that is acidic in nature and where its solubility is low. [9]

Fig.1: Molecular Structure of Valsartan.



The main aim of the study was to develop valsartan SMEDDS to improve upon the solubility of the valsartan which will have some bearing on the bioavailability. The SMEDDS consists of an isotropic mixture of drug, lipid, surfactant, and typically a co-surfactant or co-solvent. When exposed to the fluids of the gastrointestinal (GI) tract, these precursor solutions spontaneously emulsify to form highly dispersed microemulsions. These dispersions commonly have been shown to enhance the oral bioavailability of lipophilic drugs. The ease of dispersion and the very small particle size of the resultant colloidal microemulsion have

historically been viewed as the principal reasons for their utility in the delivery of lipophilic drugs. [10]

2. MATERIALS AND METHODS:

2.1 MATERIALS:

Valsartan was purchased from Dhamtec Pharma ltd. Navi Mumbai. Tween 60, Etocas, Isopropyl myristate was a kind gift from Croda India Company pvt. Ltd, Navi Mumbai, Maharashtra. Kollisolv PG was kind gift from BASF India, Ltd. Navi Mumbai. Acrysol EL-135 and Acrysol K-150 was a kind gift received from Corel Pharma Chem Pvt. Ltd. Ahmedabad, Gujarat. All other chemicals used were of analytical reagent grade.

2.2 METHODS:

2.2.1 Solubility of the oil phase, surfactant and co-surfactant:

The solubility study was performed to select the suitable oil (O), surfactant (S), and co-surfactant (Co-S) that possesses high solubilizing capacity for valsartan Selection of the oil phase was based upon the maximum solubility of the drug. Different oils like Oleic acid, Etocas, long-chain triglycerides (Soyabean, Sunflower, Castor oil, and Coconut Oil) and Isopropyl Myristate. Surfactants like Tween 20, Tween 40, Tween60, Tween80, Span 20, Acrysol EL-135, and Acrysol K-150. Co-surfactants like Kollisolv PG, Polyethylene Glycol 200 and Polyethylene Glycol 400 were selected and their solubility was determined by shaking flask method.[11] The excess amount of drug was placed in 5.0 mL screw cap glass bottle having 2.0 mL of each oil, surfactant, and co-surfactant. The mixture vials were then kept at 25 ± 1.0 °C in an isothermal shaker for 72 hr. to reach equilibrium. The equilibrated samples were removed from shaker and centrifuged at 3000 rpm for 20 min. The supernatant was taken and filtered through a 0.45 µ m membrane filter. The concentration of Valsartan was determined in oils and water using UV Spectrophotometer at wavelength of 248 nm. [12] The data is shown in the Table 3.1, 3.2, 3.3 and Figure 3.1, 3.2 and 3.3.

2.3 Drug-Excipients Compatibility:

The Drug – Excipients Compatibility Studies were performed in order to confirm the drug-excipients compatibility. The study mainly include FT-IR study. Mixture of Drug+ Oil, Drug + Surfactant, and Drug + Co-Surfactant. [13]

2.4 Construction of Pseudo-ternary phase diagram:

On the basis of the solubility study of drug, oil, surfactants, co-surfactants and aqueous phase were used for construction of phase diagram. Oil, surfactant, and co-surfactant are grouped in four different combinations for phase studies. Surfactant and co-surfactant (Smix) in each group were mixed in different weight ratio (1:1, 1:2, 2:1, 3:1). [15] These Smix ratios are chosen in increasing concentration of surfactant with respect to co-surfactant and in increasing concentration of co surfactant with respect to surfactant for detail study of the phase diagram for formulation of micro emulsion. For each phase diagram, oil, and specific Smix ratio are mixed thoroughly in different weight ratio from 1:9 to 9:1 (1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2, 9:1) in different glass vials.[16] Different combination of oils and Smix were made so those maximum ratios were covered for the study to delineate the boundaries of phase precisely formed in the phase diagrams. Pseudo-ternary phase diagram was developed using aqueous

titration method. Slow titration with aqueous phase is done to each weight ratio of oil and Smix and visual observation is carried out for transparent and easily flow able o/w micro emulsion. The physical state of the micro emulsion was marked on a pseudo-three-component phase diagram with one axis Data of aqueous phase, the other Data of oil and the third Data of a mixture of surfactant and co-surfactant at fixed weight ratios (Smix ratio). [17] Each of these ratios was mixed with increasing percentage of oil, i.e., 10%, 20%, 30%, 40% up to 90% of oil to get phase diagram. To determine the effect of drug addition in SMEDDS, phase diagrams were also constructed in presence of drug. In order to prepare SMEDDS, selection of microemulsion region from phase diagram was based on the fact that solution remains clear even on infinite dilution. [18]

2.5 Selection of Formulation from Pseudo ternary Phase Diagram:

From each phase diagram, constructed, different formulations were selected from micro-emulsion. Selected formulations were subjected to different thermodynamic stability and Dispersibility tests. [19]

2.5.1 Thermodynamic stability studies: [20]

It was determined by carrying heating cooling cycle, centrifugation test and freeze thaw cycle.

a. Heating cooling cycle:

Six cycles between refrigerator temperatures 4°C and 45°C with storage at each temperature for not <48 hr. was studied. If SMEDDS stable at these temperatures was subjected to centrifugation test.

b. Centrifugation test:

Passed SMEDDS were centrifuged at 3500 rpm for 30 min using digital centrifuge (Remi motors Ltd). If SMEDDS did not show any phase separation was taken for freeze-thaw stress test.

c. Freeze-thaw cycle :

Three freeze-thaw cycles between −21°C and +25°C with storage at each temperature for not < 48 h was done for SMEDDS.

2.5.2 Dispersibility Studies: [21]

The dispersibility test of SMEDDS was carried out to assess to compatibility to disperse into emulsion and the size of resulting globules to categorize them as SMEDDS. It was carried by using a standard USP Paddle type dissolution test apparatus, formulation was added to 500 ml of water at 37±0.5°C and the paddle was rotated at 50 rpm. On titration with water the SMEDDS formulation forms a mixture which was of different type. Depending upon which the in vitro performance of formulation can be assessed.

Grade	Dispersibility and Appearance
A	Rapidly forming (Within 1 min) microemulsion having a clear or bluish appearance
B	Rapidly forming, slightly clear emulsion having a bluish white appearance
C	Fine milky emulsion that formed within 2 min

D	Dull, greyish white emulsion having slightly oily appearance that is slow to emulsify (longer than 2min)

Those formulations that passed the thermodynamic stability and also Dispersibility test in Grade A, and Grade B was selected for further studies.[22]

2.6 Preparation of SMEDDS:

A series of microemulsions of SMEDDS were prepared with varying ratios of oil, surfactant, and co-surfactant. Formulations 1, 2, and 3 were prepared using Castor oil as oil, Tween 60 as surfactant, and Kollisolv PG as co-surfactant. In all the formulations, the level of Valsartan was kept constant (i.e. 20mg). The amount of SMEDDS should be such that it should solubilize the drug (single dose) completely.[23] The Valsartan (20 mg) was added in the mixture. Then the components were mixed by gentle stirring and mixing, and heated at 37˚C.The mixture was stored at room temperature until used. So, prepared SMEDDS was the concentrate of oil, surfactant, co-surfactant and drug. The composition of formulations is given in Table 1, 2 and 3.[24]

Table No. 2.1: Composition of Formulation 1

Formulation 1 (1:1)	Valsartan (mg)	Castor Oil (w/w)	Tween 60 (w/w)	Kollisolv PG (w/w)
M1	20	40	30	30
M2	20	30	35	35
M3	20	20	40	40
M4	20	10	45	45

Table No. 2.2: Composition of Formulation 2

Formulation 2 (2:1)	Valsartan (mg)	Castor Oil (w/w)	Tween 60 (w/w)	Kollisolv PG (w/w)
M1	20	40	40	20
M2	20	30	46.10	23.30
M3	20	20	53.3	26.7
M4	20	10	60	30

2.7 Physicochemical characterization of self-microemulsifying drug delivery system:

a) Appearance:

The prepared microemulsion was inspected visually for clarity, colour and presence of any particulate matter.

b) FT-IR Study

In this study FTIR instrument was used. FTIR spectra for the drug and the excipients of the optimized formulations were obtained.[25]

c) Drug content:

Self-microemulsifying drug delivery system containing valsartan 10 mg was added in 10 mL methanol and mixed well with shaking and was sonicated for 10-15 min. Further was centrifuged and supernatant was further was further diluted with suitable quantity of fresh methanol and drug content was determined using UV-spectrophotometer at λmax 248 nm. [26]

d) Robustness to dilution:

Robustness to dilution was studied by diluting SMEDDS to 50, 100 and 1000 times with water, 0. 1 N HCl and phosphate buffer pH 6.8. The diluted SMEDDS were stored for 12 h and observed for any signs of phase separation or drug precipitation. [27]

e) Self-Emulsification Time:

The emulsification time of SMEDDS was determined according to USP 22, dissolution apparatus each formulation added drop wise to 500ml purified water at 37°C. Gentle agitation was provided by a standard stainless steel dissolution paddle rotating at 50 rpm. Emulsification time was assessed visually. [28]

f) Viscosity:

The viscosities were measured to determine rheological properties of formulations. Brookfield viscometer at 30˚C used to serve this purpose. [29]

g) Refractive index :

Refractive indices of the prepared micro emulsions were determined at 25°C by Abbe's refractometer by placing one drop of micro emulsion on the slide. [30]

h) % Transmittance:

The percent transmittance of various formulations was measured at 248 nm using UV spectrophotometer keeping water as a blank.[31]

i) Cloud Point Measurement:

The formulated SMEDDS was diluted with 50ml water in a beaker which was placed on a water bath with gradually increasing temperature until the diluted formulation turned cloudy. It mainly insists about the stability of microemulsion at the temperature of body. [32]

j) Particle Size Determination:

Particle size of the prepared microemulsion was determined using Dynamic Light Scattering (DLS) method. For DLS particle sizing, the sample needs to be crystal clear to very slightly hazy. If the solution is white or too hazy, it should be diluted further before attempting a DLS size measurement. When the solution is ready for analysis and transfer it in the cuvette, care should be taken to avoid bubbles which are formed on the walls of the cuvette. Slowly tilting or tapping the cuvette on a hard surface may help also. Once the solution was homogenous and ready for DLS measurement, the cuvette containing the solution was placed in the instrument. The instrument was run and solution was analysed for particle size. [33]

k) Zeta Potential:

Zeta Potential of the prepared microemulsion was determined using Light Scattering method. For Zeta Potential determination, the sample needs to be crystal clear. When the solution is ready for analysis and transfer it in the cuvette, care should be taken to avoid bubbles which are formed on the walls of the cuvette. Slowly tilting or tapping the cuvette on a hard surface may also help to remove the bubble formed. Then the electrode was dipped inside the cuvette containing sample solution. Care should be taken to avoid bubbles in between the electrodes.

The cuvette containing the solution was be placed in the instrument. The instrument was run and solution was analysed for Zeta Potential.[33]

l) Polydispersity Index (PI) :

Polydispersity Index (PI) of the prepared microemulsion was determined using Dynamic Light Scattering (DLS) method. For DLS method, the sample needs to be crystal clear to very slightly hazy. If the solution is white or too hazy, it should be diluted further before attempting a DLS size measurement. When the solution was ready for analysis and transfer it in the cuvette, care should be taken to avoid bubbles which are formed on the walls of the cuvette. Slowly tilting or tapping the cuvette on a hard surface may also help to remove the bubbles formed. Once the solution was homogenous and ready for DLS measurement, the cuvette containing the solution was be placed in the instrument. The instrument was run and solution was analysed for Polydispersity Index .[33]

m) *in vitro* Dissolution Studies:

In vitro dissolution study of was performed by using USP Dissolution Apparatus II. The dissolution vessel was fitted with 900 mL dissolution media 0.1 N HCl and 6.8 buffer and kept at 37 ± 0.5∘ C with a rotating speed of 50 rpm. The aliquot of 5.0 mL was withdrawn at 5, 15, 30, and 60 min and filtered through 0.45 μm Whatman membrane filter. The volume withdrawn was replaced each time by fresh dissolution media. [34]

2.8 Preparation of Solid SMEDD:

The optimized liquid self microemulsifying formulation was transformed into free flowing granules using Aerosil 200 colloidal porous carriers as adsorbent. The L-SMEDDS and Aerosil 200 were taken in ratio 1:1 w/w to optimize the drug loading on colloidal silica. The mixture was further dried to obtain the free flowing powder.

2.9 Evaluation of Solid SMEDDS Formulations:

1. **Micromeritics Properties:**

Prepared solid-SMEDDS was evaluated for micromeritics properties such as angle of repose, bulk and tapped density, compressibility index and Hausner ratio (HR).[35]

2. **Scanning electron microscopy:**

Scanning electron microscopy (SEM) for Valsartan and prepared solid-SMEDDS was taken using scanning electron microscope (Philips, XL-30) at accelerating voltage at 3-5 kV to study surface topography. [36]

3. ***in vitro releas*e studies :**

Dissolution study was carried out using USP Type II apparatus (Paddle method) at 50 rpm, and at 37°C ± 0.5°C. The dissolution medium was 0.1 N HCl and 6.8 pH Phosphate Buffer and. Prepared solid-SMEDDS with equivalent amount of drug 20 mg were placed in 900 ml of dissolution medium respectively. A sample of 5 ml were withdrawn at regular time interval of 5, 15, 30, and 60, and filtered using 0.45 µm filter. An equal volume of respective dissolution medium was added to maintain sink conditions. Drug content from sample was analysed using UV-spectrophotometer at 248 nm.[37]

4. **X-ray diffraction study:**

The X-ray diffraction (X-RD) of Valsartan were obtained using X-RD instrument Bruker AXS, D8 Advance with Ni-filtered Cu radiation, at a voltage of 45 kV and current of 40 mA. The scanning speed was 2°/min between 5θ and 50θ. [38]

5. **Drug content:**

S-SMEDDS equivalent to 20mg was diluted in suitable quantity of methanol. The sample was mixed thoroughly to dissolve drug in methanol by stirring. Drug content in the solvent extract is filtered through 0.45 um membrane filter. Drug content analysed by suitable analytical method against the standard solvent solution of drug. [39]

6. **Fourier transform-infrared spectroscopy :**

In this study FTIR instrument was used. FTIR Spectra was determined of Solid SMEDD.

2.10 Stability Study:

Stability studies for solid-SMEDDS were studied at different temperature conditions according to ICH guidelines at room temperature i.e. 25°C ± 2°C/60% ± 5% relative humidity (RH). The samples were withdrawn at different time intervals as 0, 30, 60, 90 days. Formulation was evaluated for Appearance, equivalent to 20 mg of the drug was dissolved in methanol, diluted approximately and estimated for the drug content spectrophotometrically at 248 nm using methanol as blank. Effect of storage conditions on drug release was also studied.[41]

3. RESULT AND DISCUSSIONS:

3.1. Screening of Excipients:

3.1.1 Solubility Study:

Table 3.1: Data for solubility of Valsartan in various oil phase

Sr No	Oil Phase	Solubility (mg/ml)
1	Oleic Acid	11.38
2	Castor oil	12.35
3	Isopropyl myristate	11.33
4	Soyabean Oil	5.15
5	Coconut Oil	3.79
6	Etocas	11.71
7	Sunflower Oil	3.98

SMEDDS of Valsartan, it should possess good solubility in the oil, surfactants and co-surfactants of system. The solubility of Valsartan in various oils, surfactants and co-surfactants was investigated. Valsartan had significantly higher solubility in castor oil (12.35 mg/ml) than, Sunflower oil, Coconut oil, soyabean oil, etocas, isopropyl myristate, oleic acid. Among surfactants and co-surfactants, tween 60 (12.38 mg/ml) and Kollisolv PG (12.378mg/ml)

respectively showed highest solubilities .Therefore, castor oil was screened as oil phase , Tween 60 as surfactant and Kollisolv PG as co-surfactant based on solubility studies. Table 3.1, 3.2, 3.2 respectively and it is represented graphically in Figure 3.1, 3.2, 3.3.

Table 3.2: Data for solubility of Valsartan in various surfactants

Sr No	Surfactants	Solubility (mg/ml)
1	Cremophor RH 40	10.13
2	Tween 20	10.92
3	Tween 60	12.38
4	Tween 80	11.22
5	Span 20	11.88
6	Acrysol K-150	9.934
7	Acrysol EL-135	9.625

Table 3.3: Data for solubility of Valsartan in various co-surfactants

Sr. No.	Co-Surfactants	Solubility (mg/ml)
1.	Kollisolv PG	12.37
2	Polyethylene Glycol 200	9.52
3	Polyethylene Glycol 400	10.08

Figure 3.1: Solubility of Valsartan in Various Oil Phases

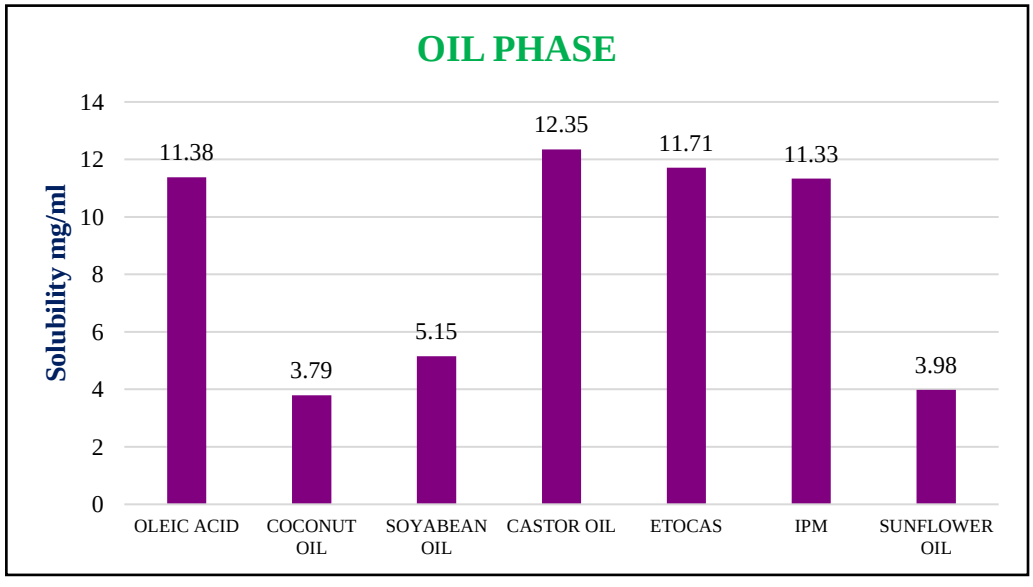


Figure 3.2: Data for solubility of Valsartan in various surfactant

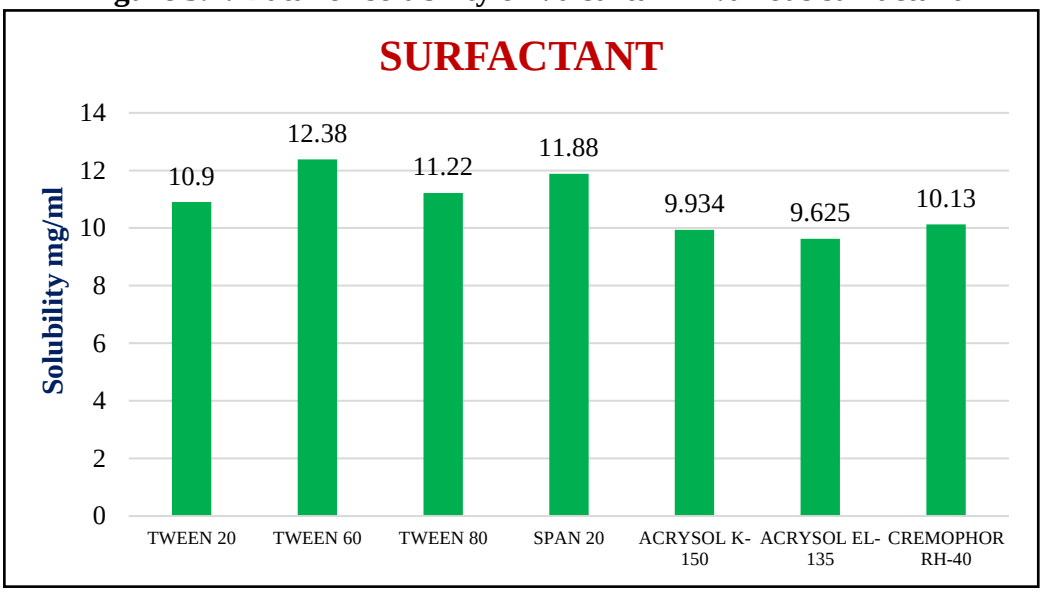
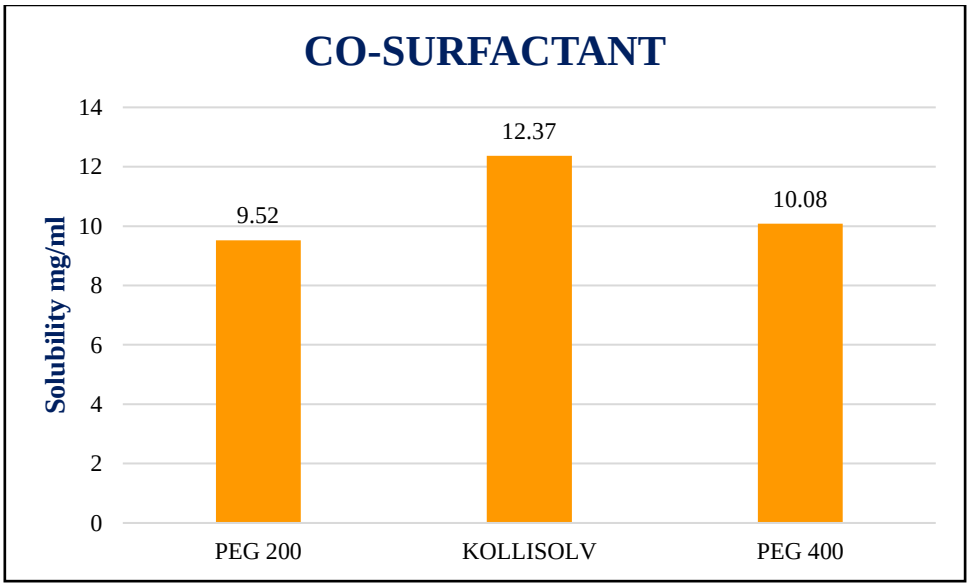


Figure 3.3: Data for solubility of Valsartan in various co-surfactants



Based on the results of Solubility screening, one distinct system was selected which was: Castor Oil as oily phase, Tween 60 as surfactant, Kollisolv PG as co-surfactant for further studies.

3.2 Drug – Excipients Compatibility Study:

The scanning range was 400 to 4000 cm-1 and resolution was $1cm^{-1}$. The major peaks in recorded spectra were compared with standard spectra given in figure below. So it can be concluded that the spectra of pure drug valsartan and the combination of drug with additives,

that all the characteristic peaks of valsartan were present in the combination spectrum, thus indicating compatibility of the drug and additives.

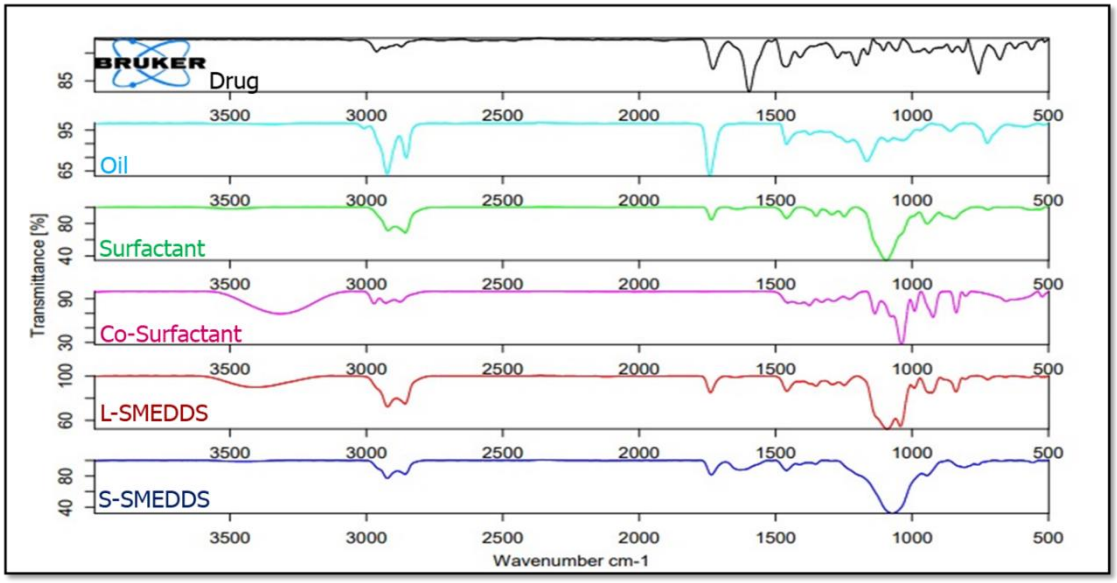
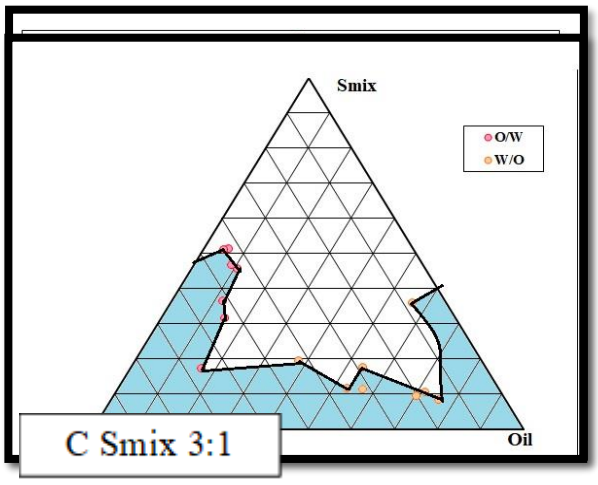
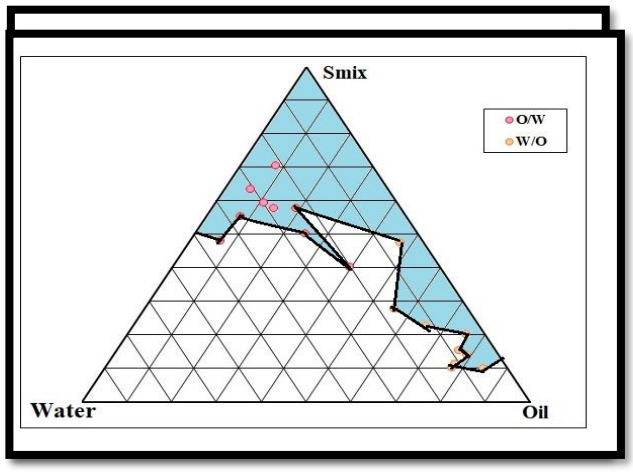


Figure 3.4: FT-IR Spectra of Overlay for compatibility study.

3.3 Construction of Pseudo ternary phase diagram:

The consideration for screening formulation of SMEDDS usually involves: the formulation composition should be simple, safe, and compatible; it should possess good solubility; a large efficient self-microemulsification region which should be found in the pseudo-ternary phase diagram, and have efficient droplet size after forming microemulsion. Thus, pseudo-ternary phase diagrams were constructed to identify the self-microemulsifying regions with maximum drug loading and to optimize the concentration of oil, surfactant and co-surfactant in the SMEDDS formulations and to obtain transparent and stable O/W micro-emulsions .The shaded areas in the pseudo-ternary phase-diagrams shown in fig 3.9 represented the existence field of stable, clear and transparent O/W microemulsions containing Castor oil as oil and with the Tween 60: Kollisolv PG fixed mixing ratio, respectively. For any selected composition of





D Smix 1:2

surfactant and co-surfactant ratio from self-microemulsifying region of ternary phase diagram (shaded) the addition of great volumes of continuous phase allowed the clear system.

Figure 3.5: Phase diagram of Castor oil (oil), Smix (Tween 60 and Kollisolv PG) were water system having different Smix ratio.

3.4 Selection of Formulation from Pseudo ternary Phase Diagram:

After the construction of Pseudo ternary phase diagram 1.1, 2:1 and 3.1 Smix ratios, maximum area was selected and also which indicate that the area covers the maximum number of formulation. The phase diagram of selected formulation is shown in Fig 3.9. The Smix ratios 1:2 and 3:1 was discarded due to smaller microemulsion region and excess of surfactant concentration which cause GIT irritation. Hence it was discarded. Ratio 1:1 and 2:1 were taken for further studies.

3.4.1 Thermodynamic stability studies:

Microemulsions are thermodynamically stable systems and are formed at a particular concentration of oil, surfactant and water, with no phase separation, creaming or cracking. It is the thermostability which differentiates microemulsion from emulsions that have kinetic stability and will eventually phase separate .Thus, the selected formulations were subjected to different thermodynamic stability testing by using heating cooling cycle, centrifugation and freeze thaw cycle stress tests. Those formulations, which passed thermodynamic stability tests, were taken for dispersibility test. (Table 3.8, and 3.9). Thus it was concluded that the efficiency of surfactant and co-surfactant mixture was unaffected after exposing to extreme conditions.

3.4.2 Dispersibility test:

When infinite dilution is done to microemulsion formulation, there is every possibility of phase separation, leading to precipitation of a poorly soluble drug as microemulsions are formed at a particular concentration of oil, surfactant and water. For oral microemulsions the process of dilution by the GI fluids will result in the gradual desorption of surfactant located at the globule interface. The process is thermodynamically driven by the requirement of the surfactant to maintain an aqueous phase concentration equivalent to its CMC. In the present study, we used distilled water as a dispersion medium because it is well reported that there is no significant difference in the microemulsions prepared using non-ionic surfactants, dispersed in either water or simulated gastric or intestinal fluid. Formulations in Group I (Table 3.8) and Group II (Table 3.9) that passed dispersibility test in Grade A, B and C were taken for further study, as Grade A and B formulations will remain as microemulsions when dispersed in GIT. Formulation falling in Grade C could be recommended for self microemulsifying drug delivery formulation.

So from the study, total four formulations were selected for further study two from each group i.e. M3 and M4 from Group I and M3 and M4 from Group II.

Table 3.4 - Data for Thermodynamic stability test and Dispersibility test of different formulations selected from Group I

Group I Smix 1:1	Observations based on the preparation, thermodynamic stability studies and dispersibility tests				Inference
Formulation	**Heating Cooling**	**Centrifugation Test**	**Freeze Thaw**	**Dispersibility**	
M1	X	X	X	D	Rejected
M2	✓	X	X	C	Rejected
M3	✓	✓	✓	A	Selected
M4	✓	✓	✓	A	Selected

Table 3.5 - Data for Thermodynamic stability test and Dispersibility test of different formulations selected from Group II

Group II Smix 2:1	Observations based on the preparation, thermodynamic stability studies and dispersibility tests				Inference
Formulation	**Heating Cooling**	**Centrifugation Test**	**Freeze Thaw**	**Dispersibility**	
M1	✓	X	X	B	Rejected
M2	✓	✓	X	C	Rejected
M3	✓	✓	✓	A	Selected
M4	✓	✓	✓	B	Selected

3.5. Preparation of Liquid SMEDDS Formulations:

Formulations selected in section 3.8 and 3.9 were prepared as per the composition reported in Table 2.1 and 2.2 and found to be thermodynamically stable.

3.6. Evaluation of Liquid SMEDDS Formulations:

a. Appearance:

Appearance of the prepared microemulsion was inspected visually and all the batches of Valsartan were Clear, Colourless, and free from any particulate matters.

b. FTIR Spectra:

The scanning range was 400 to 4000 cm-1 and resolution was $1cm^{-1}$. So it can be concluded that the spectra of pure drug valsartan and the Liquid SMEDDS spectrum, that all the characteristic peaks of valsartan were present in the Liquid SMEDDS spectrum.

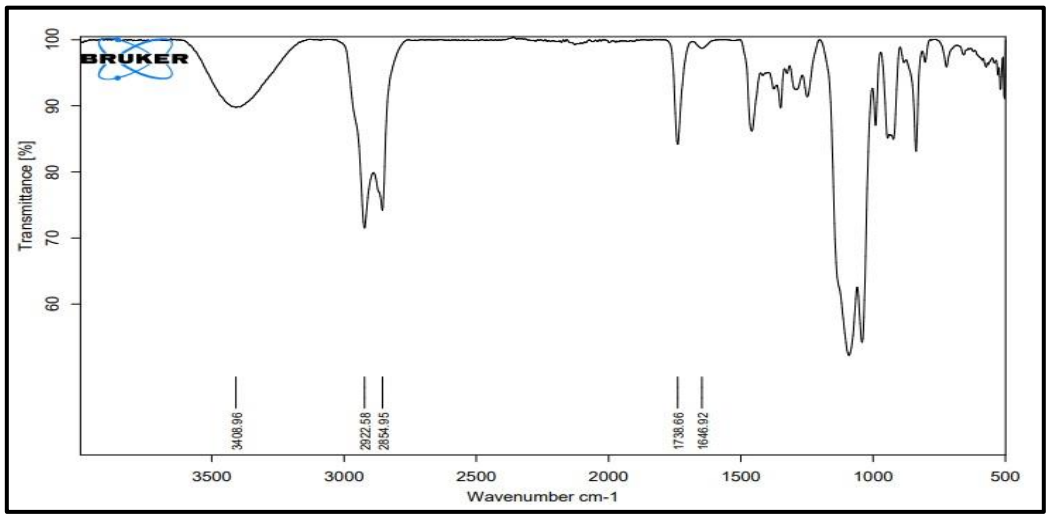


Fig 3.6: FTIR Spectra of Optimized Liquid SMEEDS

c. Drug content:

The drug content at 250 nm was found to be in the range of in the selected batch of Group I formulation M3 and M4 as well as Group II formulations M3 and M4. The data is shown in the table no. 3.6

Formulation Code	Group I	Group II
M3	91.01±0.01	97.77±0.16
M4	94.08±0.22	97.48±0.08

Table 3.6: Drug Content of Selected formulation.

d. Robustness to dilution:

After diluting SMEDDS to 50, 100 and 1000 times with water, 0.1 N HCl and buffer pH 6.8 and storing for 12 h, it was observed that there was no sign of phase separation or drug precipitation in formulations.

Formulation	Drug Precipitation or Phase Separation		
Group I	**Water**	**0.1 N HCl**	**6.8 pH buffer**
M3	-	-	-
M4	-	-	-
Group II			
M3	-	-	-
M4	-	-	-

Table 3.7: Data of the Robustness to dilution.

e. Self-Emulsification Time:

The emulsification time of liquid SMEDDS are presented in Table 3. Among the tested formulations, formulations of Group I`s M4 and Group II `s both formulation M3 and M4 showed shortest emulsification time than Group I`s M3.

Formulation Code	Group I	Group II
M3	Within 2 min	Within 1 min
M4	Within 1 min	Within 1 min

Table 3.8: Data of self-emulsification time.

f. Viscosity:

Formulation Code	Group I	Group II
M3	13.20±1.3 cps	18.00±1.2 cps
M4	14.40±1.2 cps	19.20±1.6 cps

All formulations of Group I and Group II were found to have rather low viscosities, ranging from to cps. The viscosity of the micro emulsion increased with increasing concentration of the surfactant.

Table 3.9: Data of Viscosity of Group I and Group II.

g. Refractive Index (RI):

The refractive index was carried out by Abbe refractometer was found to be in the range of 1.45 to 1.49 of Group I to Group II formulations along with the plain formulation which is closely related to the RI of water.

Formulation Code	Group I	Group II
M3	1.4971	1.4591
M4	1.4886	1. 4692

Table 3.10: Data of Refractive Index of Group I and Group II.

h. % Transmittance:

Formulation Code	Group I	Group II
M3	93.28±0.04	98.40±0.01

M4	94.77±0.01	97.76±0.01

The percent transmission was found to be in the range of 98.23 % to 99.37 % for formulations of Group I and Group II along with the plain formulation which confirms good transparent nature of formulations.

Table 3.11: Data of % Transmittance of Group I and Group II.

i) **Cloud Point Measurement:**

Cloud point of prepared SMEDDS formulations Group I and Group II was found to be higher than 70°C, which indicates that micro emulsion will be stable at physiological temperature without risk of phase separation.

Formulation	Particle Size (nm)	Zeta Potential
Group I		
M3	219	-40.78
M4	217	-41.95
Group II		
M3	191	-45.60
M4	215	-43.60

Table3.12: Data of Cloud Point of Group I and Group II.

j) Particle Size Determination:

Particle Size Determination Particle size of the prepared Valsartan microemulsion was determined using Dynamic Light Scattering (DLS) method. Particle size determination results for all the prepared batches of Valsartan microemulsion are presented in the Table 3.13 and all the Graph obtained are reported in the Figure 3.7

Table 3.13: Data of Particle Size and Zeta Potential values of Group I and Group II

k) Zeta Potential:

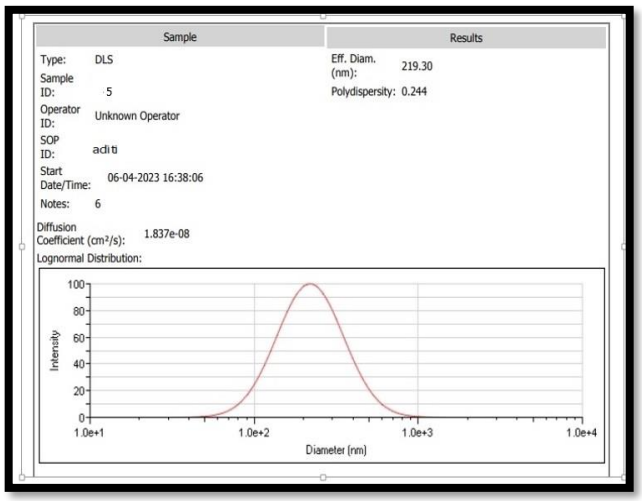
Zeta Potential of the prepared Valsartan microemulsion was determined using Light Scattering method. Zeta Potential results for all the prepared batches of Valsartan microemulsion are presented in the Table 3.13 and all the Graph obtained are reported in the Figure 3.8

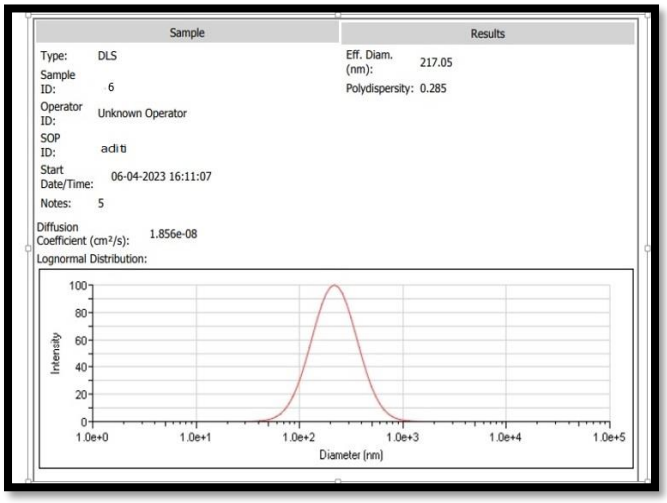
l) Polydispersity Index (PI):

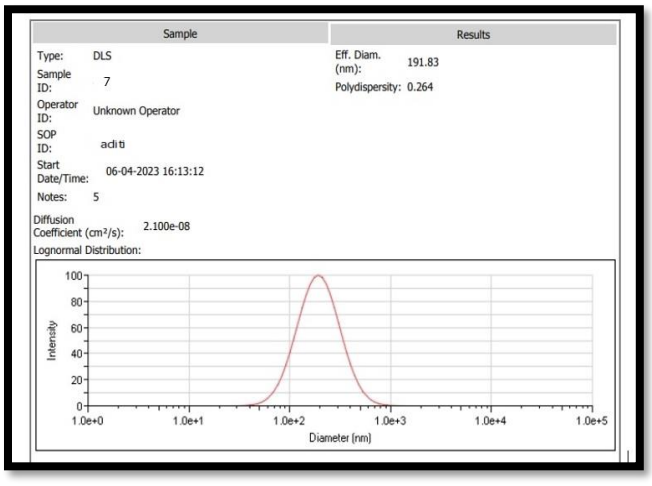
Polydispersity Index (PI) of the prepared microemulsion was determined using Dynamic Light Scattering (DLS) method. Result of Polydispersity Index (PI) is reported in the Table 3.14 and Figure 3.7

Formulation Code	Polydispersity Index
Group I	
M3	0.244
M4	0.285
Group II	
M3	0.264
M4	0.276

Table 3.14: Polydispersity Index of Group I & Group II







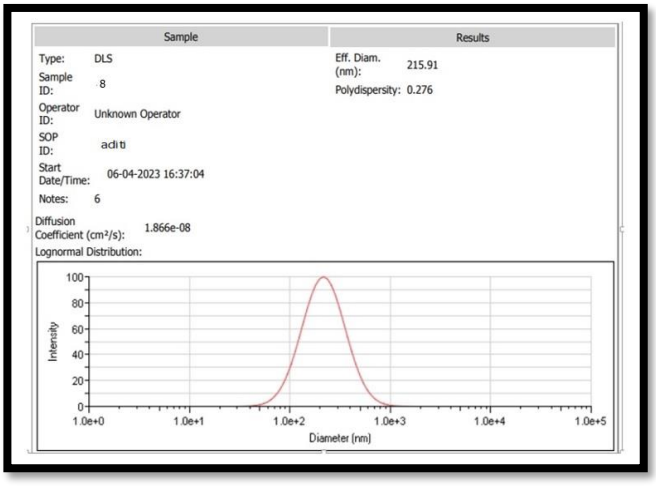


Figure 3.7: Particle Size Analysis of all the Formulation.

Where, Sample 5 and Sample 6 indicate Group I M3 and M4 respectively whereas Sample 7 and Sample 8 indicate Group II M3 and M4 respectively.

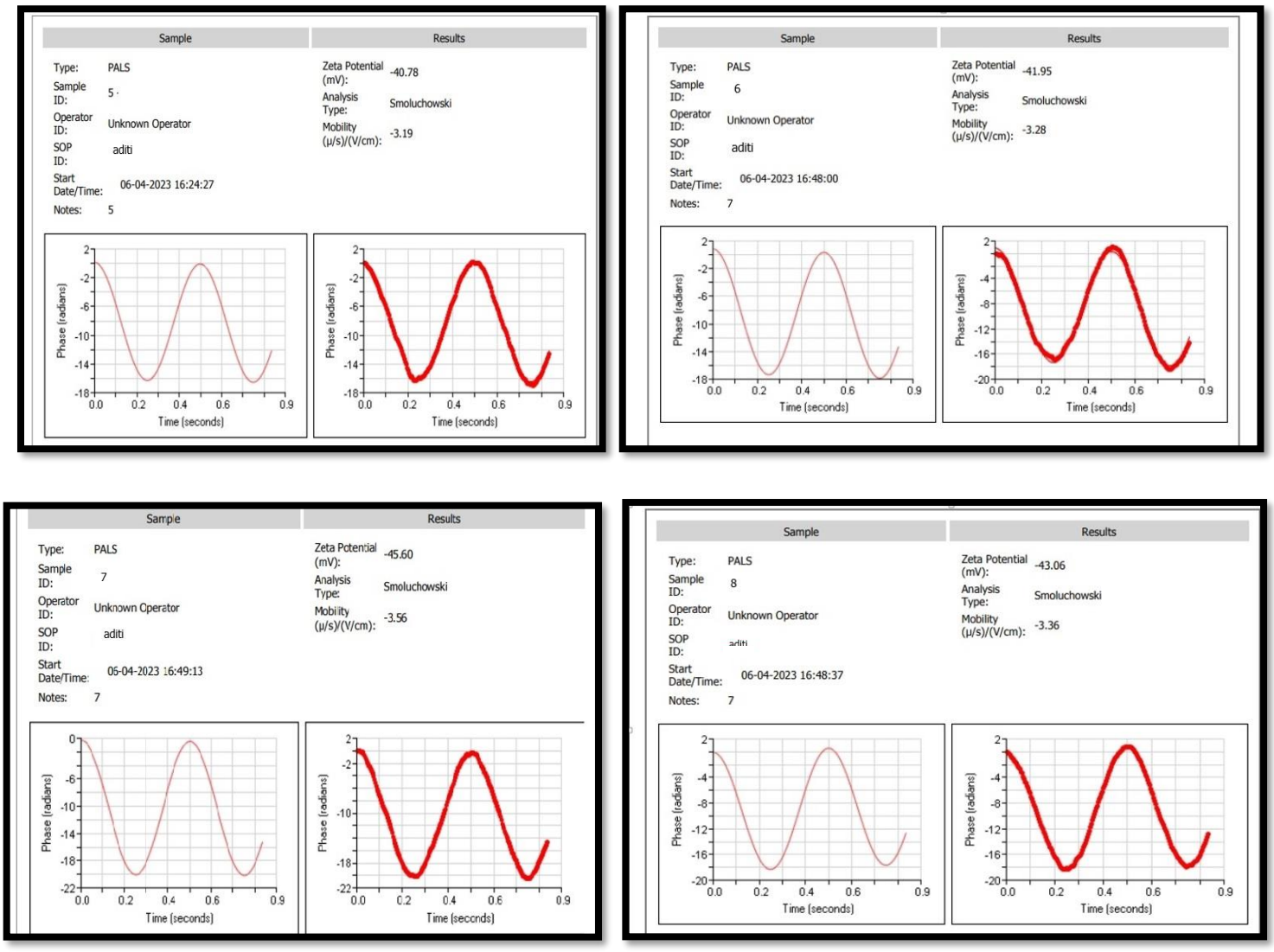


Figure3.8: Data of Zeta Potential of Group I & Group II

Where, Sample 5 and Sample 6 indicate Group I M3 and M4 respectively whereas Sample 7 and Sample 8 indicate Group II M3 and M4 respectively.

m) *in-vitro* drug release:

The in-vitro drug release study for the batches for Valsartan drug and microemulsion was carried out using paddle method (USP apparatus II). Data for in-vitro drug release study is presented in the following Table and the graphical representation of Percentage Drug Release vs. Time graph is shown in the Figure and. The release study was carried out in both 0.1N HCl and 6.8 pH phosphate buffer. The data showed that release of valsartan was faster in phosphate

buffer of pH 6.8 than other media. The pH-dependent solubility of drug can be responsible for higher release.

- ***in-vitro* drug release in 0.1 N HCl :**

Time	% Drug Release		
(min)	Valsartan	SMEDDS(1:1)	SMEDDS(2:1)
0	0	0	0
5	15.1±1.2	17.53±0.89	22.71±0.94
15	20.25±1.04	22.19±2.5	24.69±0.32
30	23.7±1.05	24.12±0.75	26.56±0.26
45	25.96±1.1	28.99±0.23	29.65±0.51
60	29.93±1.02	30.91±1.2	31.89±0.47
75	31.85±1.02	32.90±0.68	33.89±1.3
90	32.79±1.05	34.93±1.01	36.14±2.2
105	34.16±1.01	36.64±1.06	39.61±1.2
120	35.6±1.02	37.57±1.22	41.37±0.79

Table 3.15: Dissolution data for Liquid SMEDDS in 0.1N HCl

- ***in-vitro* drug release in 6.8 pH Phosphate Buffer:**

Formulation	Percent Drug Release				
	0 min	5 min	15 min	30 min	60 min
Valsartan	**0**	26.26±1.02	28.37±1.28	32.06±1.17	42.39±2.1
M4(Group I)	**0**	76.34±1.4	78.75±1.02	81.56±1.08	83.79±2.0
M3 (Group II)	**0**	84.48±1.2	86.26±1.09	90.23±1.10	92.33±2.1

Table 3.16: Dissolution data for Liquid SMEDDS in 6.8 pH buffer

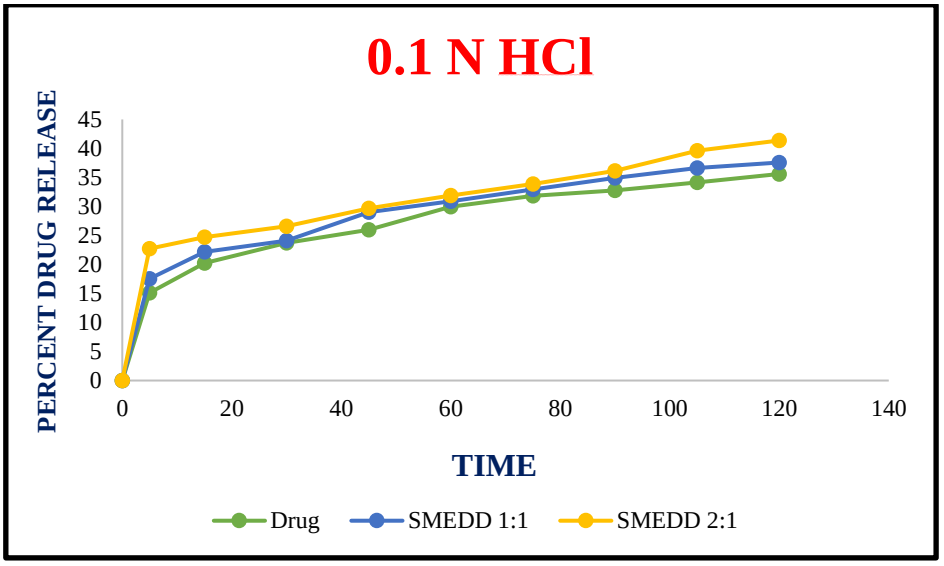


Figure 3.9: In- vitro drug release profile of Liquid SMEDDS and Valsartan (API), M4 (Group I), M3 (Group II) in 0.1N HCl.

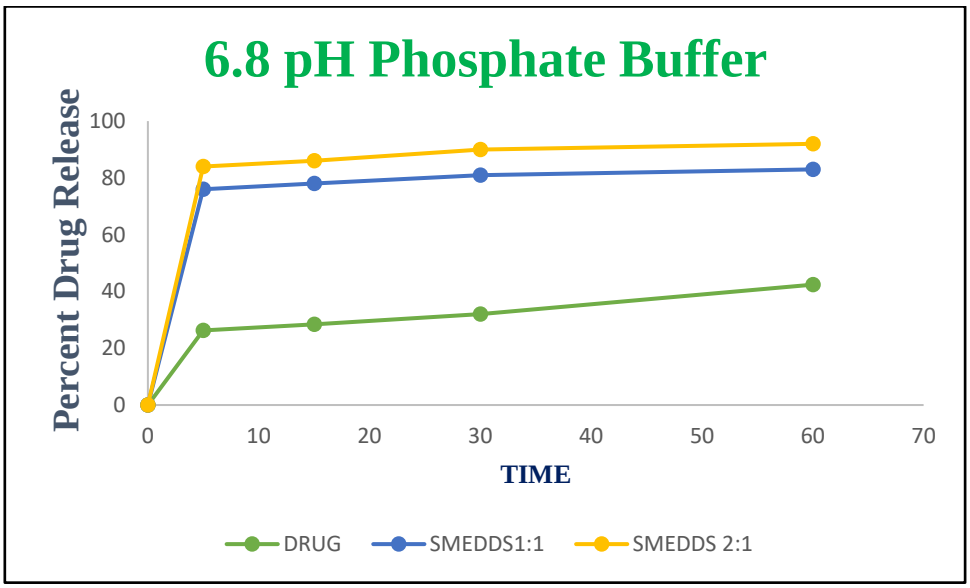


Figure3.10: - In- vitro drug release profile of Liquid SMEDDS and Valsartan (API), M4 (Group I), M3 (Group II) in 6.8 pH phosphate buffer.

3.7. Preparation of Solid SMEDDS:

Solid SMEDDS were prepared as per the composition reported

3.8. Evaluation of Solid SMEDDS Formulations:

1. Micromeritics properties:

The formulation indicated angle of repose < 30 which showed that they had excellent flow properties. Bulk density and tapped density was evaluated to study Carr's index and Hausner`s Ratio. Results indicated in the table 3.17.

Formulation Code	Angle of repose	Bulk density	Tapped density	Carr`s index	Hausner`s ratio
S1	25.64°	0.47m/ml	0.54gm/ml	12.96%	1.12

Table 3.17 : Micromeritic properties of Solid SMEDD.

2. Scanning electron microscopy:

Solid-SMEDDS appeared as smooth surfaced particles, indicating that the liquid SMEDDS is adsorbed onto the Aerosil 200 with a lesser amount of aggregation which showed effective particle size reduction of SOLID SMEDDS as compared to the drug. It is indicated in figure 3.11.

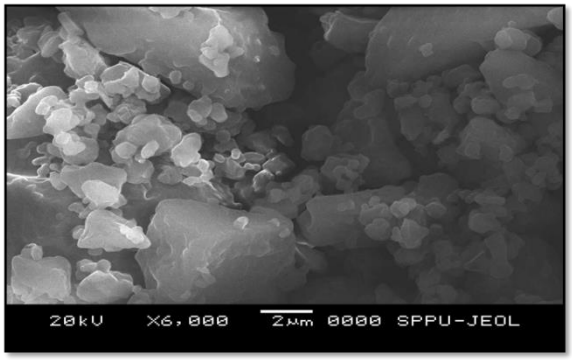


Fig 1: SEM of Valsartan

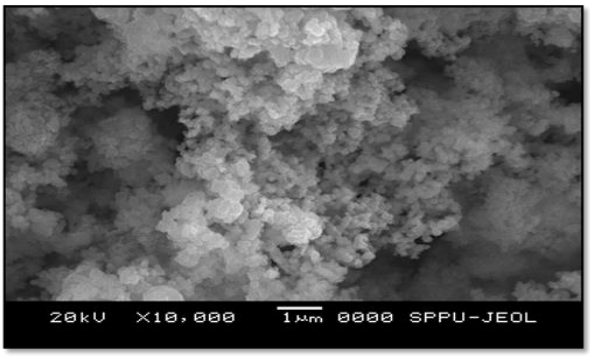


Fig 2: SEM of Solid SMEDDS

Figure 3.11: Scanning Electron Microscopy of Valsartan and Solid SMEDDS.

3. *in-vitro releas*e studies:

The release study of SOLID SMEDDS was carried out in both 0.1N HCl and 6.8 pH phosphate buffer. The data showed that release of valsartan was faster in phosphate buffer of pH 6.8 than other media. Data for in-vitro drug release study is presented in the following Table 3.18 and 3.19 and the graphical representation of Percentage Drug Release vs. Time graph is shown in the Figure 3.12 and 3.13.

- **in-vitro drug release 0.1 N HCl:**

Time (min)	% Drug Release of Solid SMEDD
0	0
5	20.505±0.76
15	23.225±1.02
30	25.948±1.05
45	29.409±1.1
60	30.668±1.02
75	32.664±1.03
90	34.661±1.02
105	36.661±0.49
120	39.644±1.03

Table 3.18: Dissolution data for Solid SMEDDS in 0.1N HCl.

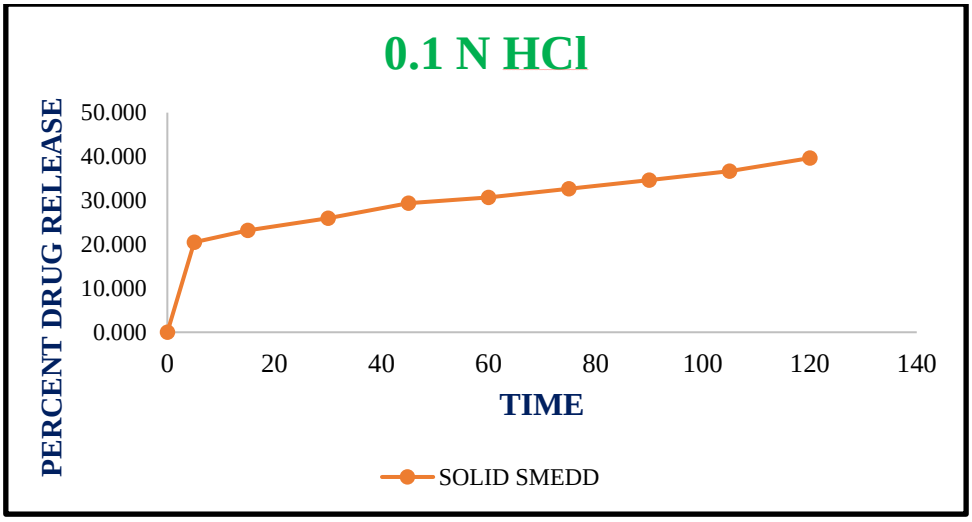


Figure 3.12: - In- vitro drug release profile of Solid SMEDDS in 0.1N HCl.

- **in-vitro drug release 6.8 pH phosphate buffer:**

Formulation	Percent Drug Release				
S1	0 min	5 min	15 min	30 min	60 min
	0	83.82 ±1.02	85.99 ±1.05	88.99 ±2.1	91.58 ±1.02

Table 3.19: Dissolution data for Solid SMEDDS in 6.8 pH phosphate buffer

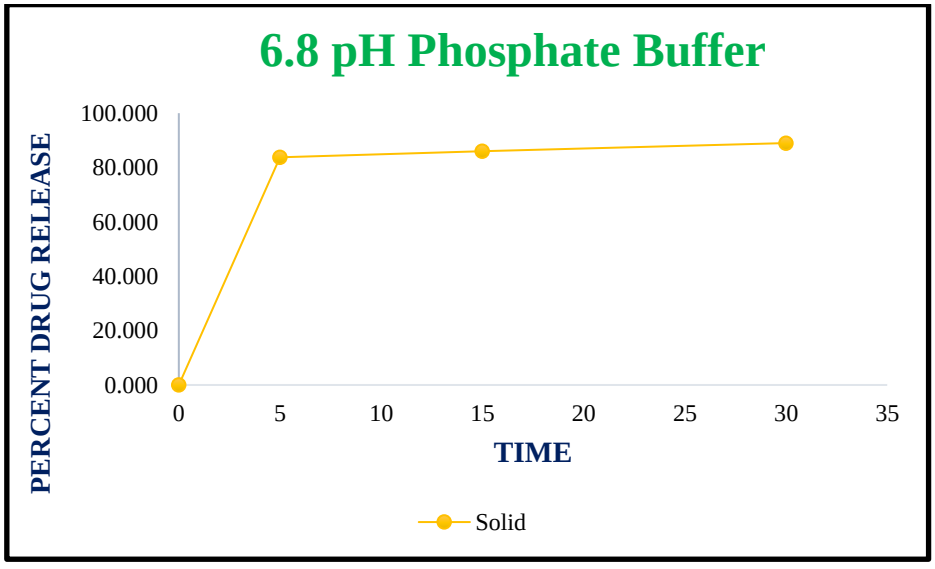
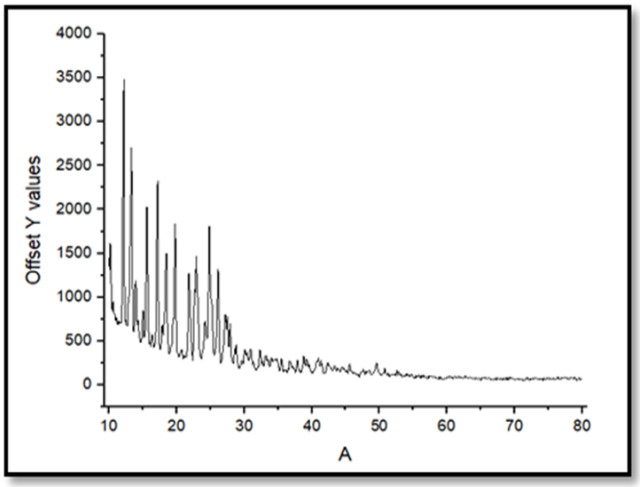


Figure 3.13: In- vitro drug release profile of Solid SMEDDS in 6.8 pH phosphate buffer.

4. X-ray diffraction study:

The diffraction pattern of valsartan revealed several sharp high-intensity peaks at diffraction angles 2θ suggesting that the drug existed as crystalline material. There were few

characteristic peaks of valsartan with a considerable reduction in the peak intensity. This diminished peak suggests conversion of the drug into an amorphous form. This marked reduction in peak intensities provides may increase dissolution rates of Solid-SMEDDS preparation. It is indicated in figure 3.14.



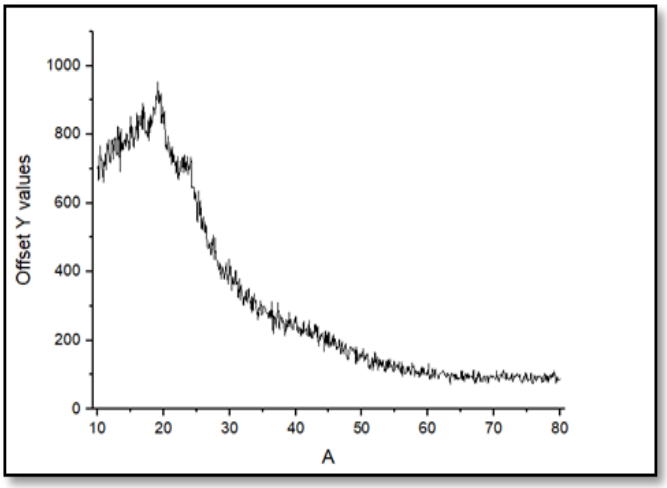


Fig: X-ray Diffraction of Valsartan

Fig: X-ray Diffraction of Solid SMEDDS

Figure 3.14: X ray diffraction of Valsartan and Solid SMEDDS

5. Drug Content:

The drug content of Solid SMEDDS formulation was found at 248 nm. The data is shown in the Table no 3.20

Formulation Code	Drug Content
S1	97.73±1.05

Table 3.20: Drug Content of Solid SMEDDS formulation.

6. FTIR Spectra:

The scanning range was 400 to 4000 cm-1 and resolution was 1cm^{-1}. So it can be concluded that the spectra of pure drug valsartan and the Solid SMEDDS spectrum, that all the characteristic peaks of valsartan were present in the Solid SMEDDS spectrum.

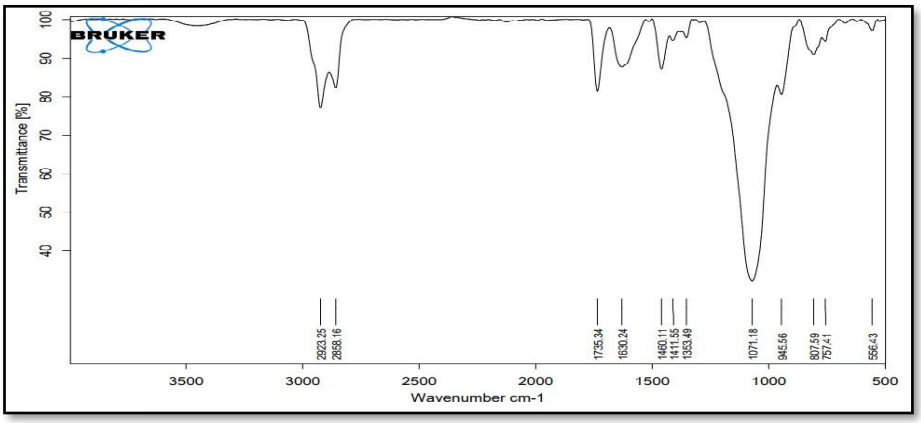


Figure3.15: FT-IR Spectra of Solid SMEDDS.

7. Stability study:

The results of stability studies depicted that the solid-SMEDDS formulation remained clear even after a period of 3 months at temperature 25°C ± 2°C & 60% ± 5%. All the formulations were found to be consistent with respect to their drug content and appeared clear on reconstitution.

Temperature& Humidity	Month	Appearance	Drug Content
25±2°& 60% ± 5%	0	Clear	97.73±1.5
25±2°& 60% ± 5%	1	Clear	97.56±1.2
25±2°& 60% ± 5%	2	Clear	97.49±2.5
25±2°& 60% ± 5%	3	Clear	97.37±2.1

Table 3.21: Stability Study of S-SMEDDS

CONCLUSION:

In the present study, Valsartan an antihypertensive drug who has low aqueous solubility was formulated in the form of Self-Microemulsifying Drug Delivery System (SMEDDS) to increase its solubility which will result in enhancement in Dissolution Rate and Bioavailability of the drug. Firstly the solubility was checked in various oil, surfactant and co-surfactant. Liquid SMEDDS were formulated from which the optimized micro emulsion formulation M3 containing Castor oil as oil, Tween 60 as surfactant, and Kollisolv PG as co-surfactant and distilled water was a transparent, clear and low viscosity system, with particle size 191 nm. The optimized formulation was converted into solid by adsorption on a Solid Carrier (Aerosil 200). The *in-vitro* release of drug was checked in both the medium 0.1N HCl and 6.8 pH Phosphate buffer. It was found out that valsartan has more solubility in the 6.8 pH phosphate buffer. Optimized SMEDDS showed good in vitro release which is increased more than 90%. Solid-SMEDDS were preferred over SMEDDS in terms of stable dosage form. It can be concluded that valsartan solid-SMEDDS offer more predictable and more extensive drug release/absorption than the corresponding conventional formulations. The results from the study showed the utility of solid-SMEDDS to enhance solubility and bioavailability of sparingly soluble compounds like valsartan, which can be helpful to reduce dose and related side effects of the drug. The present research work successfully illustrates the prospective advantage of Solid-SMEDDS for the delivery of poor aqueous soluble compounds.

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Formulation and Evaluation of Solid Self Micro-Emulsifying Drug Delivery System of Olmesartan medoxomil

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ABSTRACT - Olmesartan medoxomil (OLM) is a prodrug of Olmesartan, a selective AT1 subtype angiotensin-II receptor antagonist used widely for the treatment of hypertension. Although OLM has excellent performance against the treatment of hypertension, but its low bioavailability (BA), approximately 26% in humans, due to its low water solubility and efflux by drug resistance pumps in the gastrointestinal tract limits its use in pharmaceutical industry. OLM being class II drug has low solubility and thus this leads to poor absorption and low bioavailability. To increase the therapeutic efficacy of OLM, the solubility of OLM should be increased in aqueous systems. Solid Self micro-emulsifying drug delivery system (S-SMEDDS), which is easily emulsified in aqueous media under gentle agitation and digestive motility, was formulated to increase the solubility and in turn increase the oral BA of OLM. Among the surfactants, co-surfactants and oils studied, Tween 80, PEG 400 and Oleic acid were chosen for preparing SMEDDS. Liquid SMEDDS was prepared by dissolving OLM in various S_{mix} concentrations. The prepared formulations were characterized for Compatibility, Self emulsification time, Viscosity, Drug content, Dissolution studies, Droplet size, Zeta potential and Stability studies. FT-IR study revealed no interaction between drug and excipients. After evaluation, F6 formulation was found to be optimized. Thus, F6 was solidified using adsorption onto carrier technique using Aerosil 200 as adsorbent. The dissolution of the drug was enhanced significantly from the S-SMEDDS formulation as compared to pure drug. Optimized Batch SF6 showed 96.70±0.3% drug Release in 60 min while Pure Drug Showed 42.63±0.71% drug Release in 60 min. The physical state of the drug in S-SMEDDS powder was revealed by X– ray powder diffraction studies which indicated the presence of the drug in the dissolved form in the lipid excipients. These findings were supported by scanning electron microscopy studies which did not show the evidence of precipitation of the drug on the surface of the carrier.

KEYWORDS – Olmesartan medoxomil, Solid self micro-emulsifying drug delivery system, Adsorption, Dissolution.

1. INTRODUCTION –

Olmesartan medoxomil (OLM), is a selective and competitive angiotensin-II receptor blocker that has been approved to treat hypertension. Chemically OLM is (5-methyl-2-oxo-1,3-dioxol-4-yl)methyl-5-(2-hydroxypropan-2-yl)-2-propyl-3-[[4-[2-(2*H*-tetrazol-5-yl)phenyl]phenyl] methyl]imidazole-4-carboxylate (figure. 1.1) [1]. It is a prodrug that is rapidly hydrolyzed to form Olmesartan by esterase's found in plasma, gastrointestinal tract, and liver during absorption. Olmesartan, the active metabolite causes dose-dependent reduction of blood pressure, vasodilation and sodium retention. However, OLM is hampered by its poor water solubility with an oral bioavailability of merely 26% in healthy humans [2]. This is due to its high lipophilicity with a Log P value of 5.55. Its poor bioavailability is also caused by the unfavourable breakage of OLM in GI fluids to Olmesartan. Olmesartan, the parent molecule, has poor permeability with a Log P of 1.2 at pH 7. Efflux pumps (P-glycoprotein) that are found in the GI tract also hamper the absorption of OLM [3].

Lipid based formulations represents a unique solution to delivery of poorly soluble compounds. A lipid dosage form typically consists of one or more drugs dissolved in a blend of lipophilic excipients such as triglycerides, partial glycerides, surfactants or co-surfactants. [4]. Among the lipid-based system, Olmesartan medoxomil, solid self-micro emulsifying drug delivery system, is a promising technology to improve the rate and extent of **OLM** absorption of poorly water-soluble drugs.

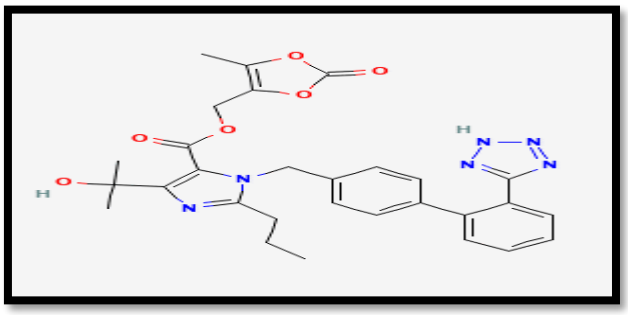


Figure 1.1 Structure of Olmesartan medoxomil

Self-micro emulsifying drug delivery system (SMEDDS) are mixtures of oils and surfactants, ideally isotropic, sometimes including cosolvents, which emulsify under conditions of gentle agitation, similar to those which would be encountered in the gastro-intestinal tract. Hydrophobic drugs can often be dissolved in SMEDDS allowing them to be encapsulated as unit dosage forms[5]. When such a system is released in the lumen of the gastrointestinal tract, it disperses to form a fine emulsion (micro/nano) with the aid of GI fluid. This leads to in situ solubilization of drug that can subsequently be absorbed by lymphatic pathways, bypassing the hepatic first pass effect [6].

OLM being under BCS Class II, which has low solubility and high permeability require solubility enhancement as an integral part of the formulation strategies. Thus, SMEDDS are

beneficial since it is a simple process and the drugs are in a pre-dissolved state and the energy input associated with a solid–liquid phase transition is avoided, thus overcoming the slow dissolution process after oral intake[7]. Thus, OLM becomes an ideal candidate to formulate into SMEDDS to enhance the solubility and dissolution rate of the formulation, which may further increase the overall bioavailability of drug. Thus, an attempt was made to formulate a SMEDDS formulation for oral drug delivery of OLM and the liquid formulation was converted into solid for filling into capsule by adsorption onto a solid carrier technique.

2. MATERIAL AND METHOD

2.1. Material

Olmesartan medoxomil, was obtained as gift sample from CTX Life Sciences, Gujarat. Acrysol 150, & Acrysol EL-135 was Gifted sample from Corel Pharma Chem, Ahmedabad. Tween 20/60/80, Span 80, Etocas was obtained as gift sample from Croda India. PEG 400 was obtained as gift sample from BASF, India. Other excipients such as Oleic acid, Olive oil, Cottonseed oil, Sunflower oil, Soyabean oil, Arachis oil, Sweet almond oil, Coconut oil, Lemon oil, Dill oil, Coriander oil, Anise oil, Span 20, PEG 200, Propylene glycol, were purchased from Research lab, Mumbai.

2.2. Screening of Excipients

2.2.1. Solubility study [8,9,10]

The solubility of OLM in various oils, surfactants, and co-surfactants was measured, respectively. An excess amount of OLM was added into 3ml of each of the selected oils, surfactants, co-surfactants and distilled water in 5-ml stoppered vials separately, and mixed by vortexing. The mixture vials were then kept at 25 ± 1.0°C in an isothermal shaker for 72 h to reach equilibrium. The equilibrated samples were removed from shaker and centrifuged at 3000 rpm for 15 min. The supernatant was taken and filtered through a 0.45 µm membrane filter. The concentration of OLM was determined in oils, surfactants, co-surfactants and water using UV- spectrophotometer at 256nm

2.2.2. Preliminary screening of surfactants [11]

500 µL of each surfactant was added to 500 µL of the selected oil. The mixtures were gently heated at 50 ″C for 2 min to attain homogenization. From each mixture, 100 µL were then diluted with distilled water up to 50 mL in glass stoppered flask. The stoppered flasks were inverted several times and the number of flask inversions required to form a homogenous microemulsion (with no turbidity or phase separation) was counted. Furthermore, the formed emulsions were allowed to stand for 2hr and their percentage transmittance was assessed by means of UV–Vis Spectrophotometer using distilled water as blank.

2.2.3. Preliminary screening of co-surfactants [11]

The selected oily phase and surfactant were used for further screening of the different cosurfactants (PEG 200, PEG 400) for their emulsification efficiency. Mixtures of 200 µL of cosurfactant, 400 µL of selected surfactant and 600 µL of selected oil were prepared and evaluated in the same manner as described in preliminary screening of surfactants.

2.3. Drug – Excipients Compatibility Study [12,13]

The Drug – Excipients Compatibility Studies were performed in order to confirm the drug-excipients compatibility. This study mainly include FT-IR Study. The samples of Drug and physical mixture of Olmesartan medoxomil with each excipient obtained after physical compatibility studies of one month was analysed using FTIR spectrophotometer in the range of 400–4000 cm^{-1} .The spectra so obtained were compared with spectra of pure drug for the chemical compatibility.

2.4. Construction of Pseudo-ternary phase diagram [14,15]

On the basis of the solubility studies of drug, select the oil phase, surfactants and cosurfactants. Water was used as an aqueous phase for the construction of phase diagrams. Surfactant and cosurfactant (Smix) are mixed in different weight ratios 1:1, 2:1, 3:1, 1:2. These Smix ratios were chosen in increasing concentration of surfactant with respect to cosurfactant and increasing concentration of cosurfactant with respect to surfactant for detailed study of the phase diagrams for formulation of microemulsion. For each phase diagram, oil and specific Smix ratio was mixed thoroughly in different weight ratios from 1:9 to 9:1 in different glass vials. Seventeen different combinations of oil and Smix, 1:9, 1:8, 1:7, 1:6, 1:5, 1:4, 1:3, 1:2, 1:1, 2:1, 3:1, 4:1, 5:1, 6:1, 7:1, 8:1, 9:1 were made so as to cover possible combinations for the study to delineate the boundaries of phases precisely formed in the phase diagrams. Pseudo ternary phase diagrams were developed using aqueous titration method. Slow titration with aqueous phase was done to each weight ratio of oil and Smix and visually observed for transparent and easily flowable o/w microemulsions. The physical state of the micro- emulsion was marked on a pseudo-three-component phase diagram with one axis representing aqueous phase, the other representing oil and the third representing a mixture of surfactant and cosurfactant at fixed weight ratios (Smix ratio). Based on the results, appropriate percentages of oil, surfactant and co-surfactant were selected and correlated in the phase diagram and then were used for preparation of SMEDDS. Pseudo-ternary phase diagram was constructed by using Microsoft Excel and were reported in section 3.3.

2.5. Selection of Formulation from Pseudo ternary Phase Diagram [16]

From each phase diagram constructed different formulations were selected from micro-emulsion region, so that drug could be incorporated into the oil phase on the following bases.

- The oil concentration should be such that it solubilizes the drug (single dose) completely depending on the solubility of the drug in the oil. 10 mg of OLM will dissolve easily in 1 mL of oil.
- To check if there was any effect of drug on the phase behaviour and microemulsion area of the phase diagram.
- The minimum concentration of the S_{mix} used for that amount of oil was taken.

Selected formulations were subjected to different thermodynamic stability and Dispersibility tests.

2.5.1. Thermodynamic stability studies [17,18]

1. Heating cooling cycle

Six cycles between refrigerator temperature 4^0C and 45^0C with storage at each temperature of not less than 48h was studied. Those formulations, which were stable at these temperatures, were subjected to centrifugation test.

2. Centrifugation

Passed formulations were centrifuged at 3000 rpm for 30 min. Those formulations that did not show any phase separation were taken for the freeze thaw stress test.

3. Freeze thaw cycle

Three freeze thaw cycles between -21˚C and 25 ˚C with storage at each temperature for not less than 48 h was done for the formulations.

Those formulations, which passed these thermodynamic stress tests, were further taken for the Dispersibility test for assessing the efficiency of self-emulsification.

2.5.2. Dispersibility test [19]

The efficiency of self-emulsification was assessed using a standard USP XXII dissolution apparatus 2 (Disso TDT 08L, Electrolab). One millilitre of each formulation was added to 500 mL of water at 37±0.5^{0}C. A standard stainless steel dissolution paddle rotating at 50 rpm provided gentle agitation. The in-vitro performance of the formulations was visually assessed using the following grading system:

Grade A: Rapidly forming (within1min) Nano emulsion, having a clear or bluish appearance.

Grade B: Rapidly forming, slightly less clear emulsion, having a bluish white appearance.

Grade C: Fine milky emulsion that formed within 2 min.

Grade D: Dull, greyish white emulsion having slightly oily appearance that is slow to emulsify (longer than 2min).

Grade E: Formulation, exhibiting either poor or minimal emulsification with large oil globules present on the surface.

Those formulations that passed the thermodynamic stability and also Dispersibility test in Grade A, Grade B was selected for further studies.

2.6. Preparation of Liquid SMEDDS Formulations [20]

The formulations were prepared by dissolving the formulation amount of OLM (10 mg) in the mixture of surfactant, oil and co-surfactant (Table 2.1). Oleic acid, Tween 80, Polyethylene glycol 400 (PEG 400), and OLM were accurately weighed and transferred into a borosilicate glass vial. Using magnetic stirrer, the ingredients were mixed for 10 min at 60–65^{0}C until a yellowish transparent formulation was attained. OLM SMEDDS formulations were then allowed to cool to room temperature before they were used in subsequent studies

Table 2.1- Data for Preparation of Liquid SMEDDS Formulations

Ingredients	Group I (S_{mix} 2:1)		Group II (S_{mix} 3:1)	
	F5	F6	F9	F10
OLM (mg.)	10	10	10	10
Oleic acid (% w/w)	10	20	10	20
S_{mix} (% w/w)	90	80	90	80

Where S_{mix} is Tween 80 and PEG 400

2.7 Characterization of Liquid SMEDDS

1. Appearance [17]

The prepared liquid SMEDDS were inspected visually for clarity, colour and presence of any particulate matter.

2. Determination of self-emulsification time [21]

The emulsification time of SMEDDS was determined according to United State Pharmacopeia (USP) XXIII, dissolution apparatus II. In brief, 0.5mL of each formulation was added drop wise to 500mL of purified water at 37^0C. Gentle agitation was provided by a standard stainless steel dissolution paddle rotating at 50 rpm. The emulsification time was assessed visually.

3. FT-IR of Liquid SMEDDS

The SMEDDS sample was analysed using FTIR spectrophotometer in the range of 400–4000 cm^{-1}. The spectra so obtained was compared with spectra of pure drug for the chemical compatibility.

4. Cloud Point [17,22]

Cloud point is the temperature above which an aqueous solution of a water-soluble surfactant becomes turbid. The Cloud point of non-ionic surfactant is the temperature at which the mixture starts to phase-separate, and two phases appear, thus becoming cloudy. Dilute the formulation 1 ml with 100 ml of water in beaker and placed on a water bath with gradually increasing the temperature until the diluted formulation turned to cloudy or turbid. It gives the information about the stability of the microemulsion at body temperature.

5. Viscosity Determination [11]

The viscosities were measured to determine rheological properties of formulations. Brookfield viscometer with a CPE 18 spindle at 10 rpm was used to serve this purpose.

6. Robustness to dilution [23]

In order to simulate in vivo dilution behavior, effect of dilution on emulsion characteristics was studied. This test was performed by diluting 1 mL of each formula 10, 100 and 1000 times with distilled water, 0.1 N HCl and phosphate buffer pH 6.8. The diluted systems were mixed

using a magnetic stirrer at 100 rpm and 37 °C to simulate body temperature to complete homogeneity. These systems were stored at an ambient temperature for 24 h then visually observed for any signs of phase separation.

7. Determination of Refractive Index and Percent Transmittance [17,24]

The refractive index was measured using Abbes refractometer. The percent transmittance of the system is measured by diluting 1 ml of formulation with 100 fold water and % transmittance was determined using UV spectrophotometer at particular wavelength keeping distilled water as blank. Due to higher particle size, oil globules may reduce the transparency of microemulsion and thereby values of %T.

8. Determination of Drug Content [14,25]

Liquid SMEDDS containing OLM, each equivalent to 10 mg was dispersed in suitable quantity of methanol. The samples were mixed thoroughly to dissolve the drug in methanol, centrifuged at 3000 rpm for 15 min to separate the undissolved excipients. The supernatant was suitably diluted and analyzed spectrophotometrically at 256 nm using UV-visible spectrophotometer.

9. Determination of Droplet size, PDI & Zeta-potential [26,27,28]

For the determination of droplet size and zeta potential the prepared formulations were suitably diluted with distilled water. To ensure complete dispersion of the formulation, the samples were inverted twice. Following complete dispersion, the mean droplet size, zeta potential (charge of surface) were directly measured using Laser Light Scattering Particle Size Analysis Technique with Zeta-Sizer. The principle involved is due to Brownian motion of droplets as a function of time which is determined due to fluctuation in light scattering, and it determined by photon correlation spectroscopy. PDI determination is done after 100 folds dilution with distilled water. The globule size distribution was expressed in terms of polydispersity index, which is a measure of the width of the globule size distribution. Zeta potential is used to identify the charge of the droplets. In conventional SMEDDS, the charge on an oil droplet is negative due to presence of free fatty acids.

10. In-vitro drug release study [21]

Drug release studies from Liquid SMEDDS were performed using USP XXIII, dissolution apparatus II with 900 mL of 0.1N HCl as medium at 37 ± 0.5^0C. The speed of the paddle was adjusted to 50 rpm. Hard gelatin capsules, size 0 filled with pure drug (10 mg) and preconcentrate (equivalent to 10 mg OLM) were put into dissolution media. Samples were withdrawn at regular time intervals (5, 15, 30, 45 and 60 min) and filtered using a 0.45 μm filter. An equal volume of the dissolution medium was added to maintain the volume constant. The samples were analysed using UV spectrophotometer at 256nm.

2.8 Conversion of liquid SMEDDS into Solid SMEDDS [17]

Various options are available for transformation of liquid SMEDDS into solid like adsorption on to solid carriers, spray drying, freeze drying and other techniques. The adsorption process is simple and just involves addition of the liquid formulation onto carriers by mixing

in a blender. The resulting powder may then be filled directly into capsules or alternatively, mixed with suitable excipients before compression into tablets. The adsorption process was adopted in the present study for preparing solid-SMEDDS for which the carrier chosen was Aerosil 200. Thus, the liquid SMEDDS containing OLM were adsorbed onto Aerosil 200 by mixing in a mortar and pestle till uniform distribution of blend and after sieving, it was dried and stored till evaluation tests.

2.9 Characterization of S-SMEDDS

1. Determination of micromeritic properties [17,29]

The bulk density, tapped density, Carr's Compressibility Index and Hausner's ratio were determined for the optimized solid-SMEDDS. The angle of repose of self- micro emulsifying powder was determined by funnel method. Briefly the sample was poured through a funnel with its tip positioned at a fixed height (h) on a horizontal surface until apex of pile touches the tip of the funnel. The angle of repose was calculated using the formula $\tan \theta = h/r$ where r is radius of the pile of powder.

2. Determination of Self-emulsification time [14,17]

The emulsification time of S-SMEDDS was determined according to United State Pharmacopeia (USP) XXIII, dissolution apparatus II. In brief, S-SMEDDS formulation was added to 500mL of purified water at 37^0C. Gentle agitation was provided by a standard stainless steel dissolution paddle rotating at 50 rpm. The emulsification time was assessed visually

3. FT-IR of S-SMEDDS

The S-SMEDDS sample was analysed using FTIR spectrophotometer in the range of 400–4000 cm^{-1}. The spectra so obtained was compared with spectra of pure drug for the chemical compatibility.

4. Determination of Droplet size, PDI & Zeta-potential [23]

The S-SMEDDS formulations were subjected to sonication prior to globule size, zeta potential and PDI determination after 100 times dilution with distilled water. Globule size, PDI and Zeta potential was determined by photon correlation spectroscopy using Zetasizer.

5. Determination of Drug content [17]

Solid-SMEDDS containing OLM, each equivalent to 10 mg was dispersed in suitable quantity of methanol. The samples were mixed thoroughly to dissolve the drug in methanol, centrifuged at 3000 rpm for 15 min to separate the undissolved excipients. The supernatant was suitably diluted and analyzed spectrophotometrically at 256 nm.

6. In-vitro drug release study of S-SMEDDS [21]

Drug release studies from S-SMEDDS were performed using USP XXIII, dissolution apparatus II with 900 mL of 0.1N HCl as medium at 37 ± 0.5^0C. The speed of the paddle was adjusted to 50 rpm. Hard gelatin capsules, size 0 filled with S-SMEDDS (10 mg) were put into dissolution media. Samples were withdrawn at regular time intervals (5, 15, 30, 45 and 60 min)

and filtered using a 0.45 μm filter. An equal volume of the dissolution medium was added to maintain the volume constant.

7. Powder X-Ray Diffraction Study [30]

X-ray powder scattering measurements of the OLM and that of solid self- micro-emulsifying powder were carried out with X-ray diffractometer .The Powder X-ray diffraction patterns were recorded at room temperature using monochromatic CuKα-radiation (k=1.5406 Å) at 40 mA and at 45 kV over a range of 2 θ angles from 3° to 50° with an angular increment of 02° per second.

8. Scanning electron microscopy (SEM) Study [31]

Scanning electron microscopy (SEM) was used to determine the particle morphology of pure drug and optimized SMEDDS. The outer macroscopic structure of the drug and solid SMEDDS was investigated by Scanning Electron Microscope (SEM) operating at 10 kV .

2.10 Stability Study [20,32]

Stability study was conducted as per ICH guidelines for final selected solid SMEDDS formulation. Hard Gelatin Capsule filled with final selected solid SMEDDS of Olmesartan medoxomil were stored in air-tight screw capped containers protected from light and maintained under real time (25 ± 2 ºC / 60± 5% RH) for 3 months. Samples were taken on 30th day, 60th day , 90 th day and evaluated for appearance, self-emulsifying properties and drug content. The results are reported in section 10.11.

3. RESULT AND DISCUSSIONS

3.1. Screening of Excipients

3.1.1. Solubility study

The self-emulsifying formulations consisted of oil, surfactants, co-surfactants and drug should be clear and monophasic liquids at ambient temperature when introduced to aqueous phase and should have good solvent properties to allow presentation of the drug in solution. Solubility studies were aimed at identifying suitable oily phase and surfactant/s for the development of OLM SMEDDS. Identifying the suitable oil, surfactant/cosurfactant having maximal solubilizing potential for drug under investigation is very important to achieve optimum drug loading . The solubility of OLM in various oily phases, surfactants and cosurfactant is reported in Table 3.1, 3.2, 3.2 respectively and it is represented graphically in Figure 3.1

The Solubility study demonstrated that solubility of the lipophilic drug-Olmesartan medoxomil was found to be highest in Oleic Acid followed by Anise oil. All the surfactants showed good solubility of the drug. Among the surfactants tested in this study, Tween 80, with HLB 15 was selected as appropriate surfactant because non-ionic surfactants are less toxic than ionic surfactants, has good biological acceptance, is powerful permeation enhancer, is less affected by pH and ionic strength, and highest solubility was also obtained. Furthermore,

Polyethylene glycol 400 (PEG 400) was selected as a co-surfactant because of their potential to solubilize the drug.

Table 3.1 - Data for Solubility study of OLM in Various Oils

Sr No	Oil	Solubility of OLM (mg/ml)
1	Olive oil	4.07
2	Cottonseed oil	4.41
3	Sunflower oil	3.23
4	Oleic acid	11.70
5	Arachis oil	5.68
6	Sweet almond oil	4.36
7	Coconut oil	2.92
8	Lemon oil	6.69
9	Dill oil	10.08
10	Coriander oil	9.82
11	Anise oil	10.35
12	Soyabean oil	3.20

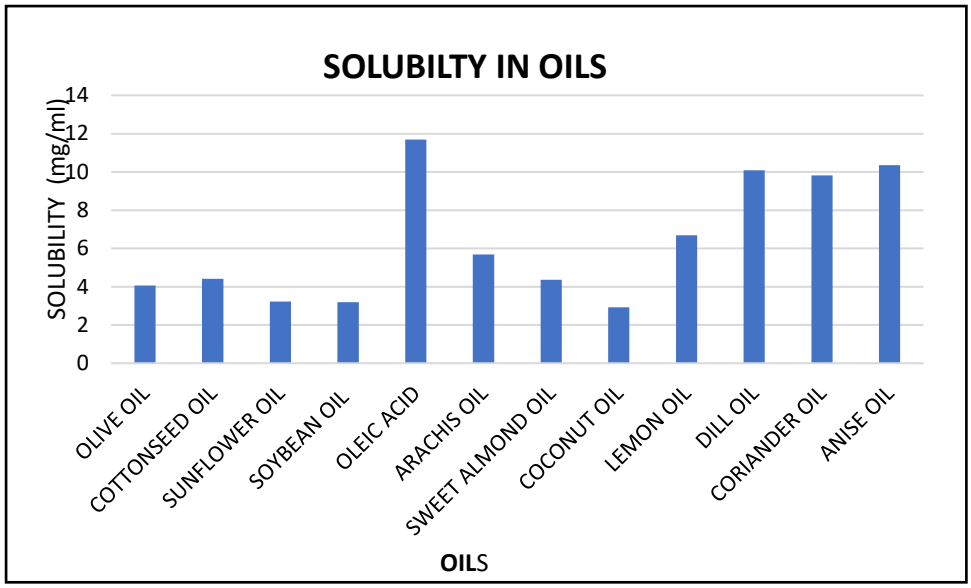
Table 3.2 - Data for Solubility study of OLM in Various Surfactants

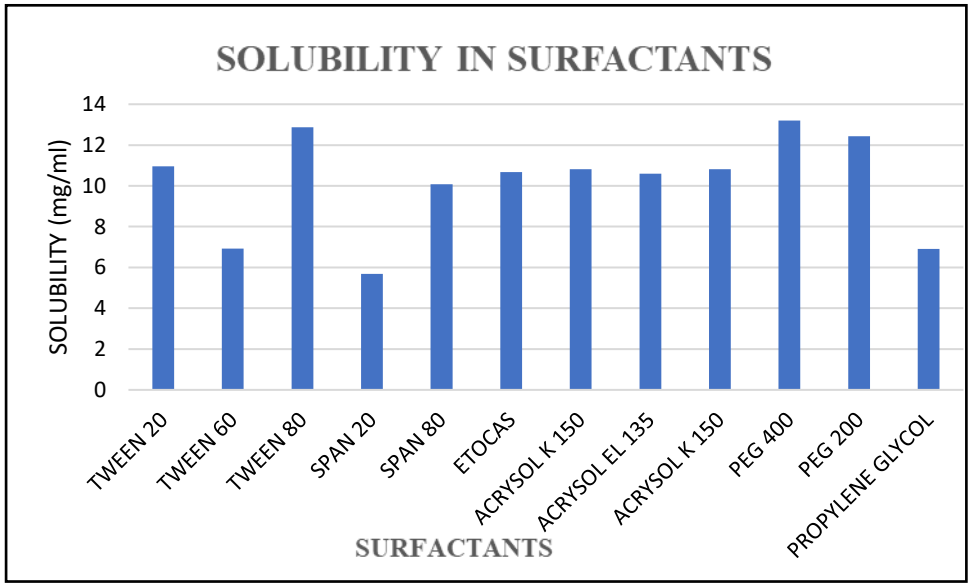
Sr No	Surfactant	Solubility of OLM (mg/ml)
1	Tween 20	10.95
2	Tween 60	6.92
3	Tween 80	12.87
4	Span 20	5.68
5	Span 80	10.08
6	Etocas 35	10.68
7	Acrysol EL 135	10.59
8	Acrysol K 150	10.82

Table 3.3 - Data for Solubility study of OLM in Various Co-Surfactants

Sr No	Co-Surfactant	Solubility of OLM (mg/ml)

1	PEG 200	12.44
2	PEG 400	13.20
3	Propylene Glycol	6.91





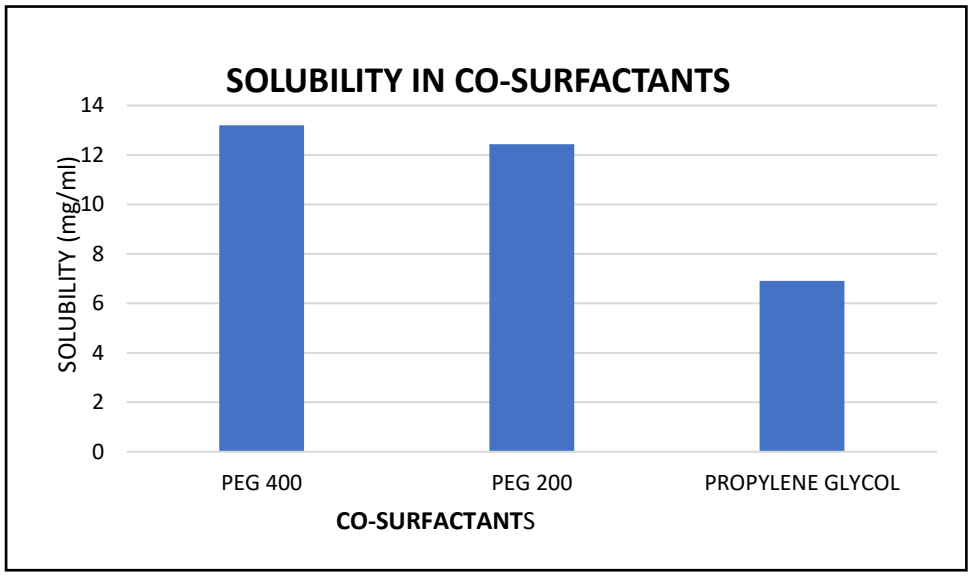


Figure 3.1 – Solubility of OLM in various Oils, Surfactants and Co-surfactants

3.1.2. Preliminary screening of surfactants

Non-ionic surfactants are generally considered less toxic than ionic surfactants. They are usually accepted for oral ingestion. The surfactants were compared for their emulsification efficiencies using oily phase. It has been reported that well formulated SMEDDS is dispersed within seconds under gentle stirring. Transmittance values of different mixtures are demonstrated in Table 3.4. From results it was inferred that the oily phase Oleic acid exhibited the highest emulsification efficiency with Tween 80, requiring only 5 flask inversions for homogenous emulsion formation. Therefore, mentioned results suggested the use of Oleic acid as an oily phase with Tween 80 as a surfactant for further study.

Table 3.4 - Data for Emulsification efficiency of surfactant

Sr. No.	Oils	% Transmittance
		Oleic acid
1.	Tween 80	96.52±0.27
2.	Tween 20	90.15±0.33

3.1.3. Preliminary screening of co-surfactants

Addition of a co-surfactant to the surfactant-containing formulation was reported to improve dispersibility and drug absorption from the formulation. In view of current investigation, two co-surfactants, Polyethylene Glycol 400, Polyethylene Glycol 200 were compared for ease of emulsification. As reported in Table 3.5, Oleic acid exhibited good emulsification with both co-surfactants, with PEG 400 showing maximum transmittance followed by PEG 200.

Table 3.5 - Data for Emulsification efficiency of Co-surfactant

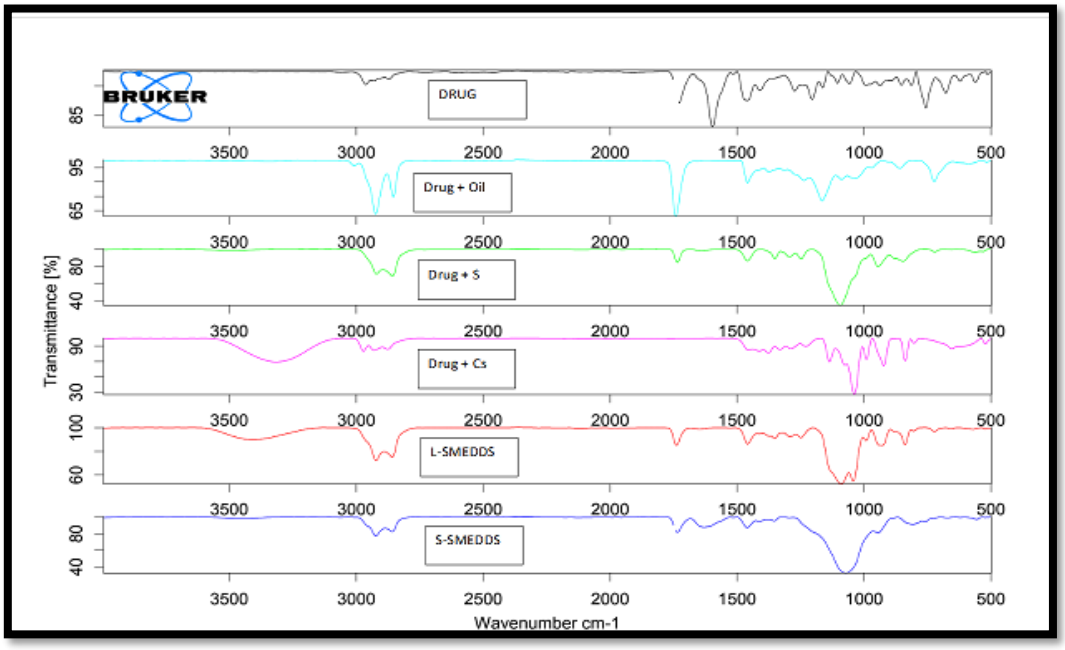
Sr. No.	Co-surfactants	% Transmittance
		Oleic Acid + Tween 80
1.	PEG 400	97.79±0.28
2.	PEG 200	93.32±0.17

Based on the results of preliminary screening, one distinct system was selected which was: **Oleic acid** as oily phase, **Tween 80** as surfactant, **Polyethylene Glycol 400** as co-surfactant.

3.2. Drug – Excipients Compatibility Study

Compatibility of drug and excipients was determined by FT-IR Spectroscopical analysis and drug and excipients were found to be compatible and is shown in figure 3.2

Figure 3.2 – FT-IR Spectra of OLM and Excipients and SMEDDS



3.3. Construction of Pseudo ternary phase diagram

The consideration for screening formulation of SMEDDS usually involves: the formulation composition should be simple, safe, and compatible; it should possess good solubility; a large efficient self-emulsification region which should be found in the pseudo-ternary phase diagram, and have efficient droplet size after forming microemulsion. Thus, pseudo-ternary phase diagrams were constructed to identify the Self-micro-emulsifying regions with maximum drug loading and to optimize the concentration of oil, surfactant and co-surfactant in the SMEDDS formulations and to obtain transparent and stable O/W micro-emulsions.

The shaded areas in the pseudo-ternary phase-diagrams shown in below figures represented the existence field of stable, clear and transparent O/W micro-emulsions containing Oleic acid as oil and with the Tween 80: PEG 400 fixed mixing ratio, respectively. For any selected composition of surfactant and co-surfactant ratio from self micro-emulsifying region of ternary phase diagram (shaded) the addition of great volumes of continuous phase allowed the clear system.

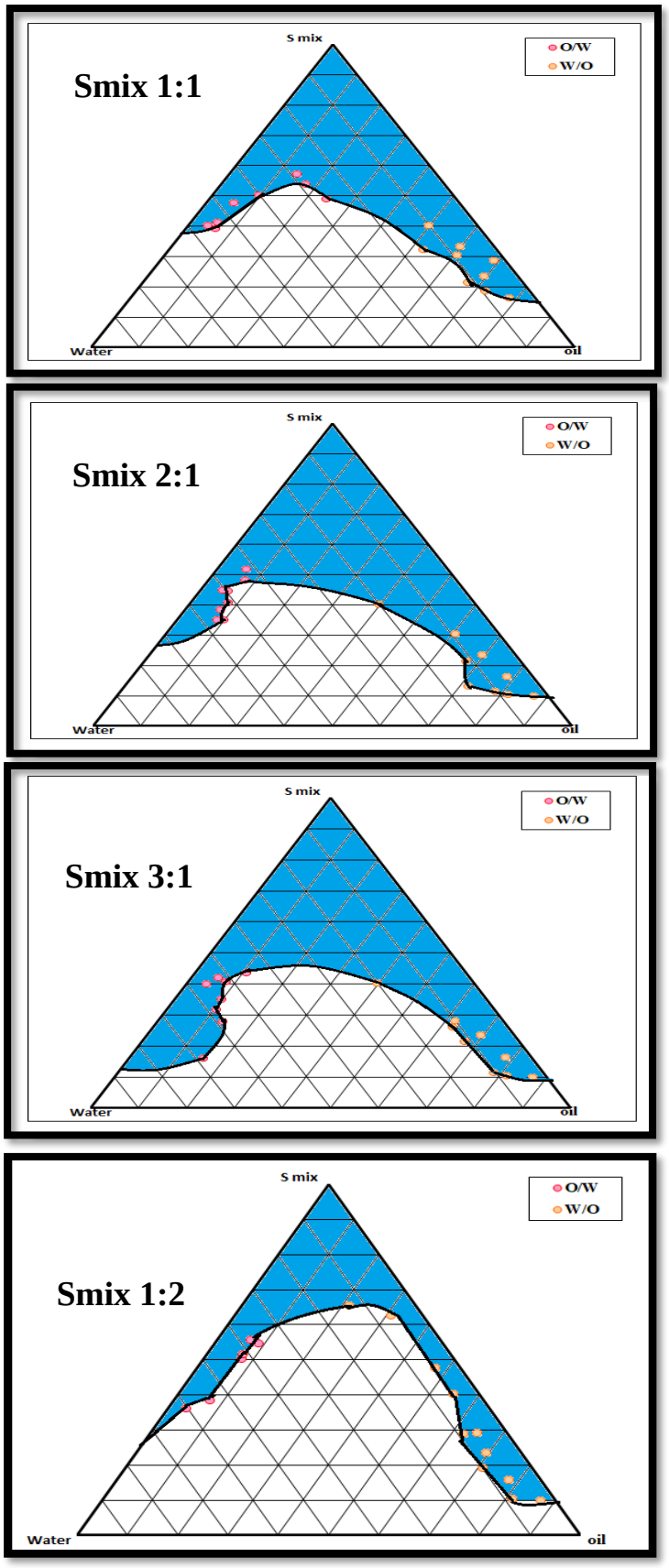


Figure 3.3 -Pseudo ternary Phase diagram of Oleic acid(oil)-S_{mix} (Tween 80 and Polyethylene glycol 400) and water system having different S_{mix} ratio.

It can be seen that these phase diagrams contained different areas of clear and isotropic microemulsion region. It can be also seen that microemulsion region exists at S_{mix} ratio 1:1. Increasing the concentration of surfactant (2:1) resulted in even larger area of microemulsion region. Further increasing surfactant concentration from 2:1 to 3:1 resulted in slight influence on microemulsion region . The influence of concentration of co-surfactant on the microemulsion region was also seen by constructing the phase diagram in ratio of 1:2. It was seen that the region of microemulsion was decreased with increase in concentration of co-surfactant.

The existence of large or small microemulsion region depends on the capability of a particular surfactant or surfactant mixture to solubilize the oil phase. The extent of solubilization resulted in a greater area with clearer and homogenous solution. It was seen that when the surfactant (Tween 80) was used alone, the oil phase was solubilized to a lesser extent at higher concentration of surfactant implying that surfactant alone was not able to reduce the interfacial tension of oil droplet to a sufficiently low level and thus was not able to reduce the free energy of the system to an ultra-low level desired to produce microemulsions. When a co-surfactant was added, the interfacial tension was reduced to a very low level and very small free energy was achieved which helps in larger microemulsion region. With further increase in surfactant from 1:1 to 2:1 and 3:1 further drop in interfacial tension and free energy was achieved resulting in maximum region of microemulsion formation. Thus, pseudo-ternary phase diagram for **S_{mix} 1:1, 2:1 and 3:1** were selected for the formation of drug loaded self micro-emulsifying drug delivery system.

3.4. Selection of Formulation from Pseudo ternary Phase Diagram

It is well known that large amounts of surfactants cause GI irritation; therefore, it is important to determine the surfactant concentration properly and use minimum concentration in the formulation. S. Shafiq et al. reported the basis of selecting different microemulsion formulations from the phase diagram, as hundreds of formulations can be prepared from microemulsion region of the diagram. From the data shown in different pseudo-ternary phase diagrams ,it was understood that oil could be solubilized up to the extent of 50% w/w. Therefore, from phase diagram with different concentrations of oil, which formed microemulsions, were selected at a difference of 10% (10, 20, 30, 40%) so that maximum formulations could be prepared covering the microemulsion/ self emulsification area of the phase diagram. For each percentage of oil selected, only those formulations were taken from the phase diagram, which needed minimum concentration of S_{mix}. There was no sign of change in the phase behaviour and microemulsion area of phase diagrams when Olmesartan medoxomil was incorporated in the formulations, which indicated the formation and stability of microemulsions consisting of non-ionic components is not affected by the pH and or ionic strength.

3.4.1. Thermodynamic stability studies

Microemulsions are thermodynamically stable systems and are formed at a particular concentration of oil, surfactant and water, with no phase separation, creaming or cracking. It is the thermostability which differentiates nano- or microemulsion from emulsions that have kinetic stability and will eventually phase separate. Thus, the selected formulations were subjected to different thermodynamic stability testing by using heating cooling cycle,

centrifugation and freeze thaw cycle stress tests. Those formulations, which passed thermodynamic stability tests, were taken for dispersibility test. Thus, it was concluded that the efficiency of surfactant and co-surfactant mixture was unaffected after exposing to extreme conditions.

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3.4.2. Dispersibility test

When infinite dilution is done to micro-emulsion formulation, there is every possibility of phase separation, leading to precipitation of a poorly soluble drug as micro-emulsions are formed at a particular concentration of oil, surfactant and water. For oral micro-emulsions the process of dilution by the GI fluids will result in the gradual desorption of surfactant located at the globule interface. The process is thermodynamically driven by the requirement of the surfactant to maintain an aqueous phase concentration equivalent to its CMC.

In the present study, we used distilled water as a dispersion medium because it is well reported that there is no significant difference in the micro-emulsion prepared using non-ionic surfactants, dispersed in either water or simulated gastric or intestinal fluid Formulations in **Group I** ,**Group II**, **Group III** that passed dispersibility test in Grade A, B and C were taken for further study, as Grade A and B formulations will remain as nano emulsions when dispersed in GIT. Formulation falling in Grade C could be recommended for self-emulsifying drug delivery formulation.

So, from the study, total 4 formulations were selected for further study 2 each from **Group II**, **Group III.**

Table 3.6: Data for Thermodynamic stability test and Dispersibility of different formulations from Group 1

Group I S_{mix} ratio 1:1	**Percentage w/w of different components in formulation**		**Observations based on the preparation, thermodynamic stability studies and dispersibility tests**				**Inference**
Formulations	**Oil**	**S_{mix}**	**H/C**	**Cent.**	**Freez. Tha.**	**Dispers e.**	
F1	20	80	√	√	X	Grade B	Rejected
F2	25	75	√	X	X	Grade C	Rejected
F3	30	70	√	X	X	Grade C	Rejected
F4	35	65	X	X	X	Grade D	Rejected

Table 3.7: Data for Thermodynamic stability test and Dispersibility of different formulations from Group 2

Group I S_{mix} ratio 2:1	**Percentage w/w of different components in formulation**		**Observations based on the preparation, thermodynamic stability studies and dispersibility tests**				**Inference**
Formulations	**Oil**	**S_{mix}**	**H/C**	**Cent.**	**Freez. Tha.**	**Dispers e.**	
F5	20	80	√	√	√	Grade A	Selected
F6	25	75	√	√	√	Grade A	Selected
F7	30	70	√	X	X	Grade C	Rejected
F8	35	65	X	X	X	Grade C	Rejected

Table 3.8: Data for Thermodynamic stability test and Dispersibility of different formulations from Group 3

Group I S_{mix} ratio 3:1	**Percentage w/w of different components in formulation**		**Observations based on the preparation, thermodynamic stability studies and dispersibility tests**				**Inference**
Formulations	**Oil**	**S_{mix}**	**H/C**	**Cent.**	**Freez. Tha.**	**Disper se.**	
F9	20	80	√	√	√	Grade A	Selected
F10	25	75	√	√	√	Grade B	Selected
F11	30	70	√	X	X	Grade C	Rejected
F12	35	65	X	X	X	Grade C	Rejected

Where, Heating cooling cycle (H/C).

Freeze-thaw cycle (Freez. Tha.).

Centrifugation (Cent.).

Dispersibility test (Disperse.)

3.5. Preparation of Liquid SMEDDS Formulations

Formulations selected in section 2.6 were prepared as per the composition reported in Table 2.1 and found to be thermodynamically stable even after addition of a drug.

3.6. Evaluation of Liquid SMEDDS Formulations

1. Appearance

The prepared SMEDDS were inspected visually and found to be clear without presence of any particulate matter.

2. Determination of Self emulsification time

The rate of emulsification is an important index for the assessment of the efficiency of emulsification that is the SMEDDS should disperse completely and quickly when subjected to aqueous dilution under mild agitation. The emulsification time of liquid SMEDDS are presented in Table 3.9. Emulsification time study showed that all the formulations emulsified within 30 s. Among the tested formulations, F6 and F9 showed shortest emulsification time than others.

3. FT-IR of liquid SMEDDS

FT-IR spectrum is reported in Fig 3.2. The scanning range was 400 to 4000 cm-1 and resolution was 1cm-1 . So, from the spectra of pure drug OLM and the Liquid SMEDDS it can be concluded that all the characteristic peaks of OLM were present in the Liquid SMEDDS spectrum.

4. Determination of Cloud point

Cloud points of all formulation are given in Table 3.9 Knowing the cloud point is important for:

- Determining storage stability, storing formulations at temperatures significantly higher than the cloud point may result in phase separation and instability.
- Generally, non-ionic surfactants show optimal effectiveness when used near or below their cloud point.
- Wetting, cleaning and foaming characteristics can be different above and below the cloud point.
- Its gives information about stability of microemulsion at body temperature.

5. Determination of Viscosity

The rheological properties of the SMEDDS are evaluated by Brookfield viscometer. These viscosities determination confirm whether the system is w/o or o/w. If system has low viscosity, then it is o/w type of the system and if high viscosities then it are w/o type of the system. The F6 formulation shows the lowest viscosity. The results of all formulation are given in table 3.9.

6. Robustness to Dilution

In order to simulate in vivo dilution behavior, effect of dilution on emulsion characteristics was studied. 1 mL of each formulation was diluted with 10, 100 and 1000 times with distilled water, 0.1 N HCl and phosphate buffer pH 6.8. These systems were stored at an ambient temperature for 24 h then visually observed for any signs of phase separation. The results of all formulation are given in table 3.10.

Table 3.9 - Data for Evaluation of Liquid SMEDDS formulations

Evaluation Parameters	Group 2 (S_{mix} 2:1)		Group 3 (S_{mix} 3:1)	
	F5	F6	F9	F10
Self-Emulsification time[a] (sec)	18.66±1.24	13.66±1.24	15.33±1.24	16.66±1.24
Cloud point (^{0} c)	79	87	85	82
Viscosity (cps)[a]	53.39±0.54	46.23±0.77	49.06±0.62	53.76±0.61

[a]Mean ± SD, n = 3

Table 3.10. Data for Robustness to dilution of liquid SMEDDS

Sr No	Groups	Formulations	Distill water			0.1 N HCl			6.8 pH Buffer		
			10	100	1000	10	100	1000	10	100	1000
1	Group 2 Smix (2:1)	F5	√	√	√	√	√	√	√	√	√
2		F6	√	√	√	√	√	√	√	√	√

3	**Group 3 Smix (3:1)**	**F9**	√	√	√	√	√	√	√	√	√
4		**F10**	√	√	√	√	√	√	√	√	√

7. Determination of Refractive Index and Percent Transmittance

The refractive index measured using Abbes refractometer. Refractive indexes and % transmission of all formulations are shown in following table 3.11.

8. Determination of Drug Content

The drug content of all formulations ranged between 98.61±0.12 to 96.57±0.27 % and passed uniformity of content. The drugs content of F6 formulation was found to be 98.61% while other formulation drug content found less than 98% so it was concluded that F6 formulation have more drug content as compare to others. The results are reported in table 3.11.

Table 3.11 - Data for Evaluation of Liquid SMEDDS formulations

Evaluation Parameters	**Group 2 (S_{mix} 2:1)**		**Group 3 (S_{mix} 3:1)**	
	F5	**F6**	**F9**	**F10**
Refractive Index [a]	1.43±0.01	1.46±0.02	1.44±0.01	1.46±0.007
% Transmittance [a]	93.32±0.17	98.39±0.18	97.56±0.27	95.34±0.27
Drug Content [a] %	96.57±0.27	98.61±0.12	97.90±0.13	97.81±0.13

[a]Mean ± SD, n = 3

9. Determination of Droplet size, PDI & Zeta-potential

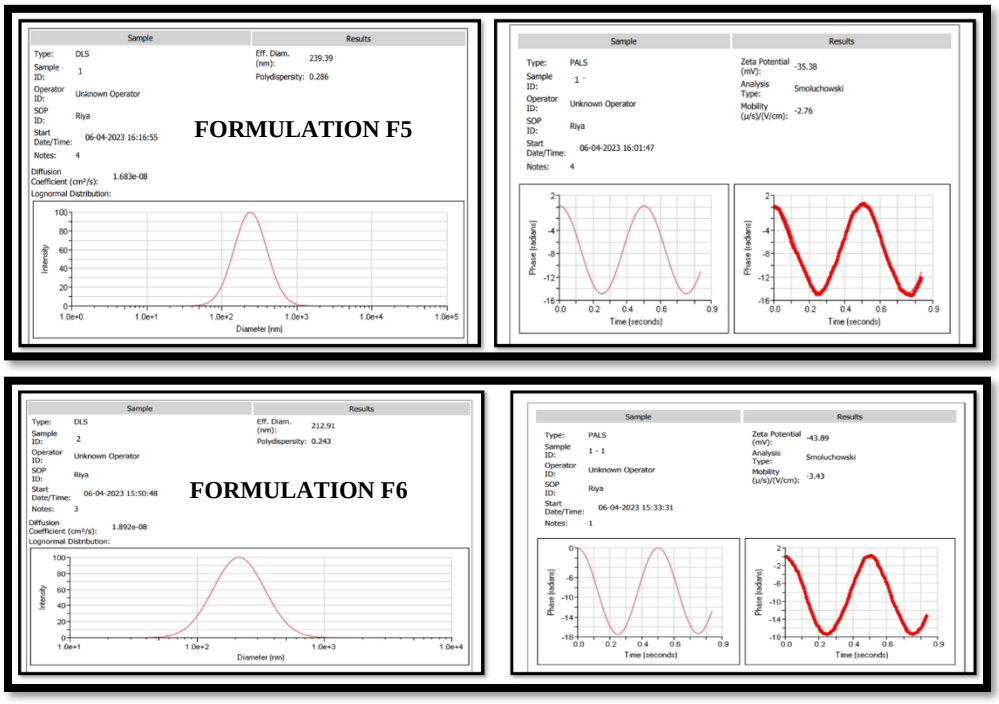
The droplet size of the emulsion is a crucial factor in self-emulsification process because it determines the rate and extent of drug release as well as drug absorption. Also, it has been reported that the smaller particle size of the emulsion dro1plets may lead to more rapid absorption as well as enhance the bioavailability of the formulation. The batch F6 was with mean particle size 212.91 nm in water. The resulting microemulsion produced was with a small mean size and a narrow particle size distribution regardless of the dispersion medium. The charge of SMEDDS is another important property that should be assessed. All formulations were diluted with purified water to avoid error caused by the dispersion medium and the zeta-

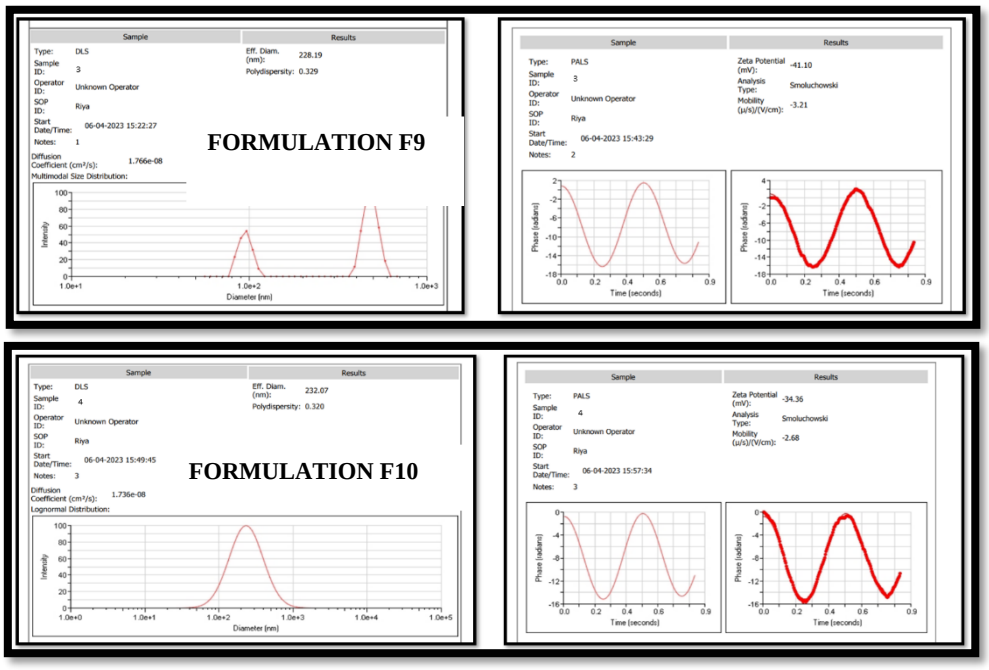
potential of the resulting emulsions was measured. The blank SMEDDS formulation exhibited almost no charged emulsion whereas a negatively charged emulsion was obtained with drug-loaded SMEDDS. This may be because the emulsifier used in the formulation was a non-ionic-surfactant. The batch F6 had the zeta potential i.e. -43.89 mV with highest zeta potential towards negative side. The zeta potential governs the stability of microemulsion, it is important to measure its value for stability samples. The high value of zeta potential indicates electrostatic repulsion between two droplets. DLVO theory states that electric double layer repulsion will stabilize microemulsion where electrolyte concentration in the continuous phase is less than a certain value.

Table 3.12. Data for Droplet size, PDI & Zeta-potential of liquid SMEDDS

Evaluation Parameters	Group 2 (S_{mix} 2:1)		Group 3 (S_{mix} 3:1)	
	F5	F6	F9	F10
Droplet size (nm)	239.39 nm	212.91 nm	228.19 nm	232.07 nm
PDI	0.286	0.243	0.329	0.320
Zeta-potential (mV)	-35.38 mV	-43.89 mV	-41.10 mV	-34.36 mV

Figure 3.4 - Results of Droplet size distributions and zeta potential of Liquid SMEDDS





10. In-vitro Drug Release Study

The *in- vitro* drug release study of liquid SMEDDS were performed in 0.1N HCl. The percent drug release for different formulations is shown in **Table 3.13**. In the self-micro emulsifying systems, the free energy required to form an emulsion was very low, thereby allowing spontaneous formation of an interface between the oil droplets and water. It is suggested that the oil/surfactant/co-surfactant and water phases effectively swell and eventually there was increase in the release rate. The in-vitro release was examined for optimized formulation F6 and F9 was performed. It was clear from the **Figure 3.5** that the maximum percentage of the drug released within 15min because of fast emulsification.

The SMEDDS represented in solubilized form in gastric fluids after ingestion and hence provided large interfacial area for Olmesartan medoxomil. Therefore, the optimized formulations (F6 and F9), had higher drug release than Plain drug OLM. **Among F6 and F9, the F6 formulation showed highest drug release with least particle size, so F6 is considered to be the best formulation and thus will be converted into solid SMEDDS.**

Table 3.13 Dissolution data for Drug and Liquid SMEDDS formulations in 0.1N HCl

Time (min)	Percent drug released [a]		
	Pure Drug	F6	F9
00	00	00	00

05	6.27±0.31	82.31±0.61	81.64±0.31
15	17.89±0.82	87.40±0.40	84.91±0.20
30	30.11±0.51	92.61±0.51	90.77±0.40
45	37±0.40	97.40±0.20	92.20±0.31
60	42.63±0.71	98.60±0.31	95.36±0.35

[a] Represents mean ± S.D. (n = 3)

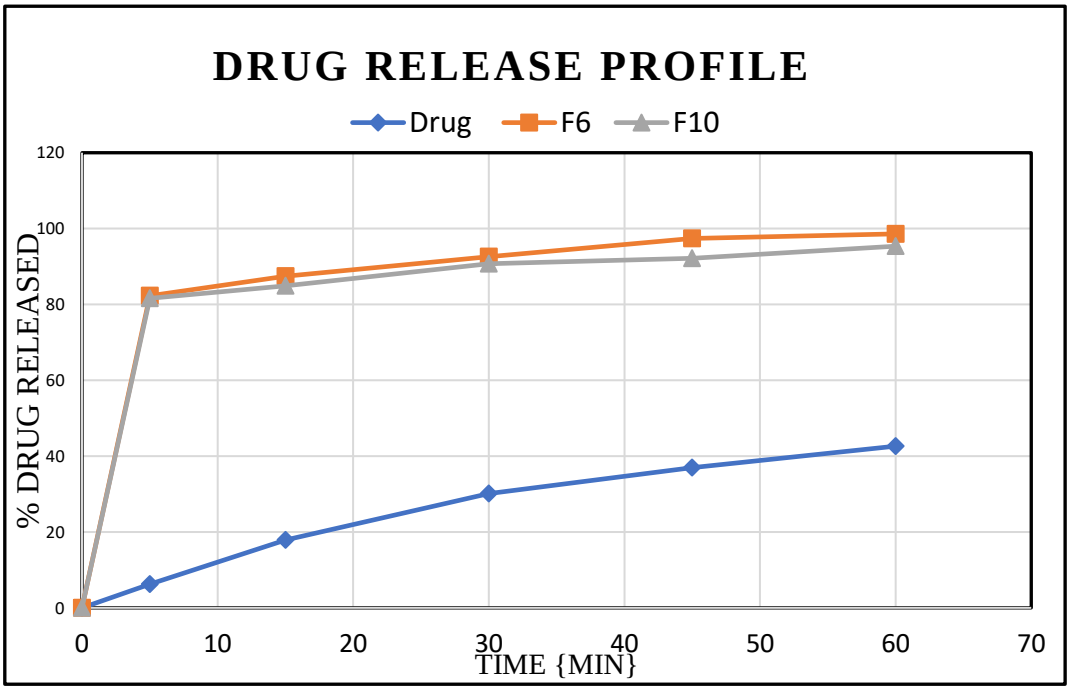


Figure 3.5. In- vitro drug release profile of Pure drug [OLM] and Liquid SMEDDS Formulations in 0.1N HCl

3.7. Conversion of liquid SMEDDS into Solid SMEDDS

Solid SMEDDS were prepared as per the composition reported in **Table 3.14.** Formulation 6 was selected for converting into S-SMEDDS**.**

Table 3.14: Data for Preparation of Solid SMEDDS Formulation

Adsorbent	Amount of Liquid SMEDDS (ml)	Amount of adsorbent required to get free flow powder (g)
Aerosil 200	10 ml	2.5 g

3.8 Characterization of S-SMEDDS

1. Determination of Micromeritic properties

Results of powder characteristics are given below, in **Table 3.15**

Table 3.15. Data for micromeritic properties of S-SMEDDS

Formulation Code	Angle of Repose (degree)	Bulk Density (gm/ml)	Tap Density (gm/ml)	Carr's Index (%)	Hausner's Ratio
SF6	27.9 degree	0.5 g/ml	0.55g/ml	9.09	1.111

2. Determination of Emulsification time

S-SMEDDS should disperse completely and quickly when subjected to aqueous dilution under mild agitation. The emulsification time of S-SMEDDS are presented in **Table 3.16**

Table 3.16. Data for Emulsification time of S-SMEDDS

Evaluation Parameters	SF6
Self Emulsification Time (sec) [a]	19±0.8

[a] Represents mean ± S.D. (n = 3)

3. FT-IR of Solid SMEDDS

FT-IR spectrum is reported in Fig 3.2. The scanning range was 400 to 4000 cm-1 and resolution was 1cm-1 . So, from the spectra of pure drug OLM and the Solid SMEDDS it can be concluded that all the characteristic peaks of OLM were present in the Solid SMEDDS spectrum.

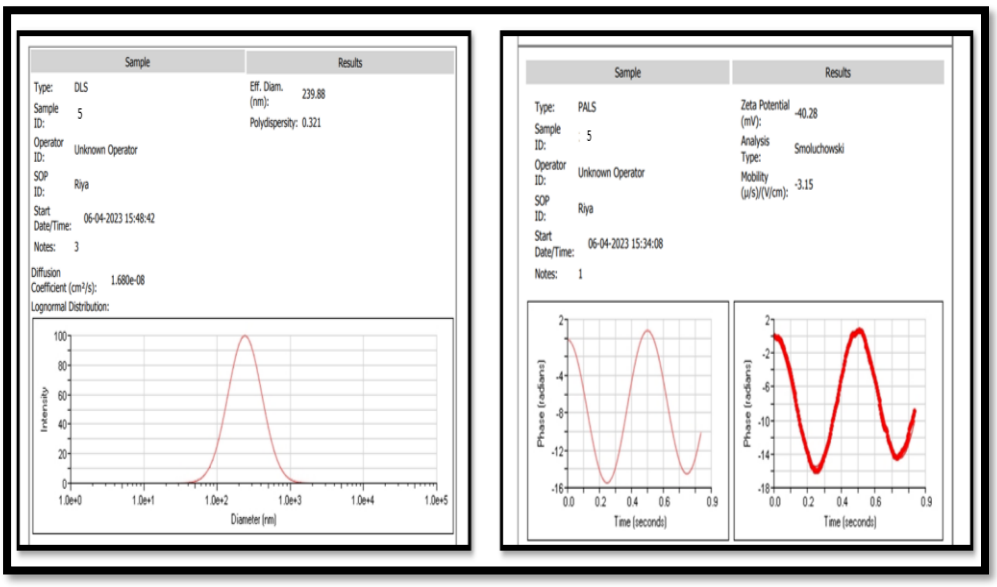
4. Determination of Droplet size, PDI & Zeta-potential

Globule size, PDI and Zeta potential was determined by photon correlation spectroscopy using Zetasizer. The results are reported below

Table 3.17. Data for Droplet size, PDI & Zeta-potential of liquid SMEDDS

Evaluation Parameters	SF6
Droplet size (nm)	239.88 nm
PDI	0.321
Zeta-potential (mV)	-40.28 mV

Figure 3.6 - Results of Droplet size distributions and zeta potential of S-SMEDDS



5. Determination of Drug Content

The drug content of SF6 formulation is given below,

Table 3.18. Data for Drug Content of S-SMEDDS

Evaluation Parameters	SF6
Drug Content %	98.23±0.11

[a]Represents mean ± S.D. (n = 3)

6. In-vitro drug release study of S-SMEDDS

The *in-vitro* drug release studies were performed in order to ensure the quick release of the drug in the dissolution medium. *In-vitro* dissolution studies also give an idea about the self-emulsification efficiency of the developed system. The *in-vitro* drug release profile of was evaluated in 0.1N HCl (n = 3). It was observed that both the solid SMEDDS formulations SF6 released more than 90% of Olmesartan medoxomil within 60 min. The formulation dispersed almost instantaneously indicating the high self-emulsion efficiency of the developed formulations.

The graph of the drug release profile is shown in **Figure 3.6**. OLM from the solid SMEDDS was completely and rapidly dissolved in medium without affecting the dissolution pattern also.

Table 3.19: Dissolution data for S-SMEDDS in 0.1N HCl

Time (Minute)	Percent drug dissolved [a]	
	Pure Drug	**SF6**
00	00	00
05	6.27±0.31	75.29±0.4
15	17.89±0.82	78.54±0.5
30	30.11±0.51	82.89±0.3
45	37±0.40	86.99±0.3
60	42.63±0.71	96.70±0.3

[a] Represents mean ± S.D. (n = 3)

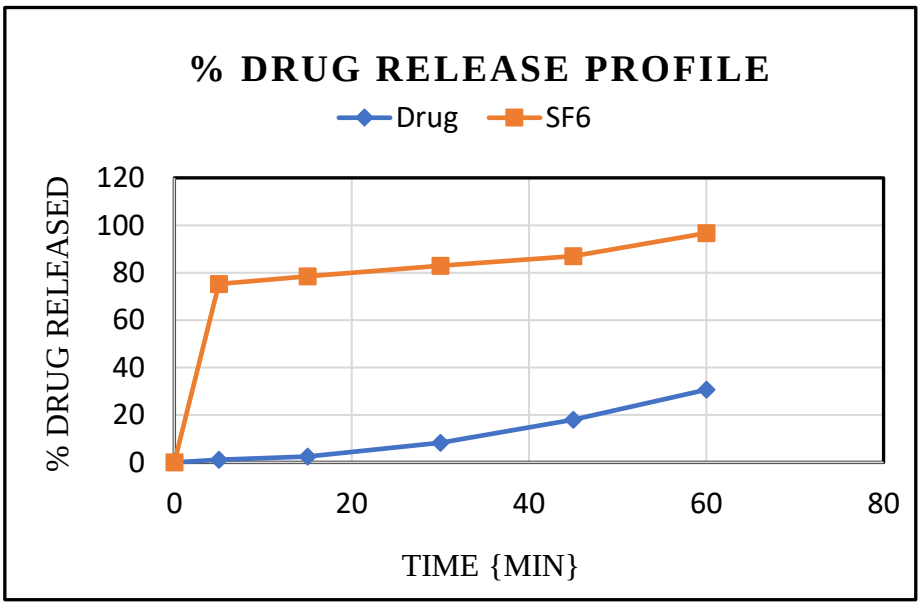


Figure 3.6:- *In- vitro* drug release profile of Solid SMEDDS

10.10.6 X-ray Powder Diffraction (XRPD) Study

The PXRD patterns of pure drug (OLM) and S-SMEDDS (SF6) were presented in **Figure 10.31 and 10.32**. The XRPD patterns of pure drug OLM showed numerous sharp peaks which are the characteristic of a crystalline compound. And these peaks are absent in the PXRD pattern of S-SMEDDS indicating the transformation crystalline nature to Amorphous nature.

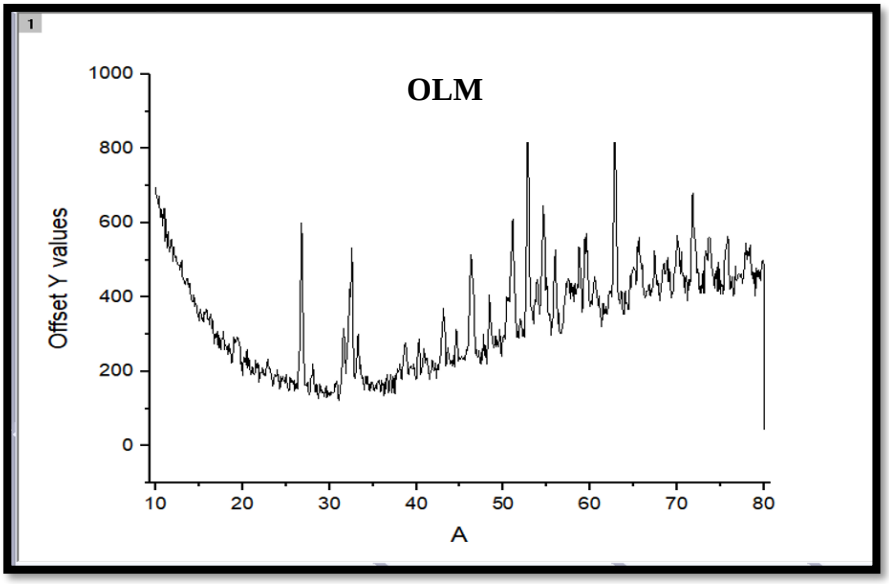


Figure 3.7 XRPD of Olmesartan medoxomil

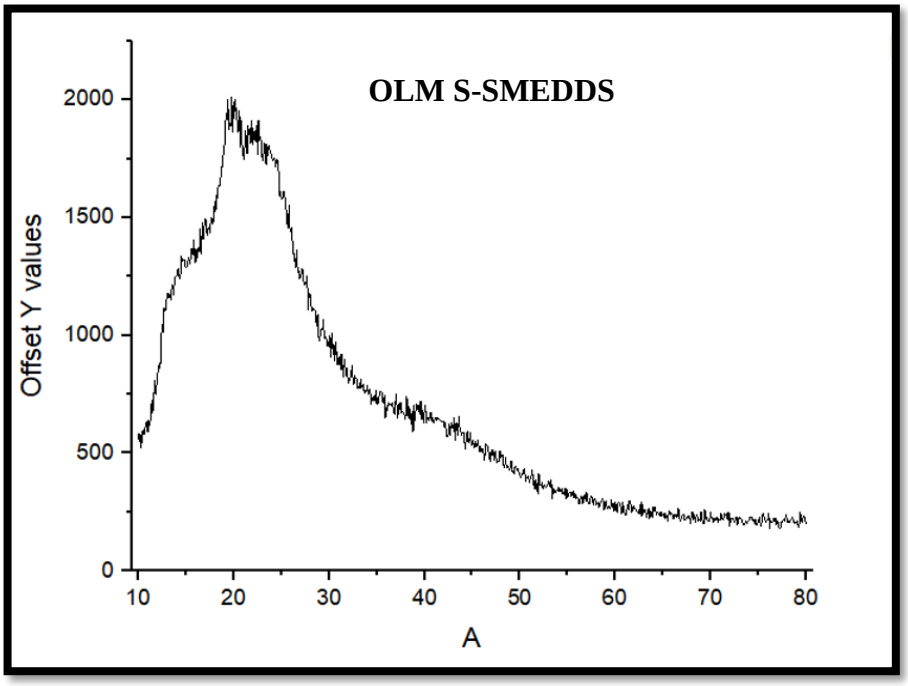


Figure 3.8 XRPD of S-SMEDDS (SF6)

10.10.7 Morphological analysis {Scanning Electron Microscopy}

The surface morphology of the pure drug and solid SMEDDS were examined by the SEM and the images are represented in **figure 10.33 and 10.34.** SEM revealed OLM as crystalline powder with irregular shaped crystals. The typical crystalline structure of Olmesartan medoxomil was absent in S-SMEDDS of OLM, which indicates the transformation of the drug from crystalline state to amorphous state i.e. the drug is completely solubilised in oil phase of L-SMEDDS. The S-SMEDDS appeared as smooth surfaced particles with no evidence of precipitation of the drug on the surfaces of the carriers indicating that the liquid SMEDDS was absorbed or coated inside the pores of Aerosil 200. The figure clearly illustrates that there are no signs of coalescence, indicating thereby the enhanced physical stability of the formulation.

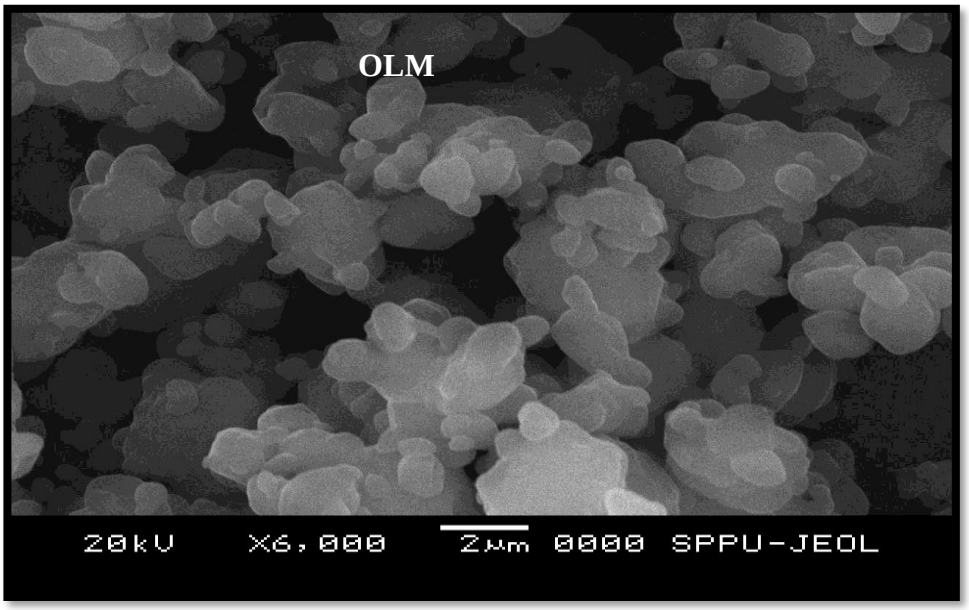


Figure 3.9 SEM of Olmesartan medoxomil

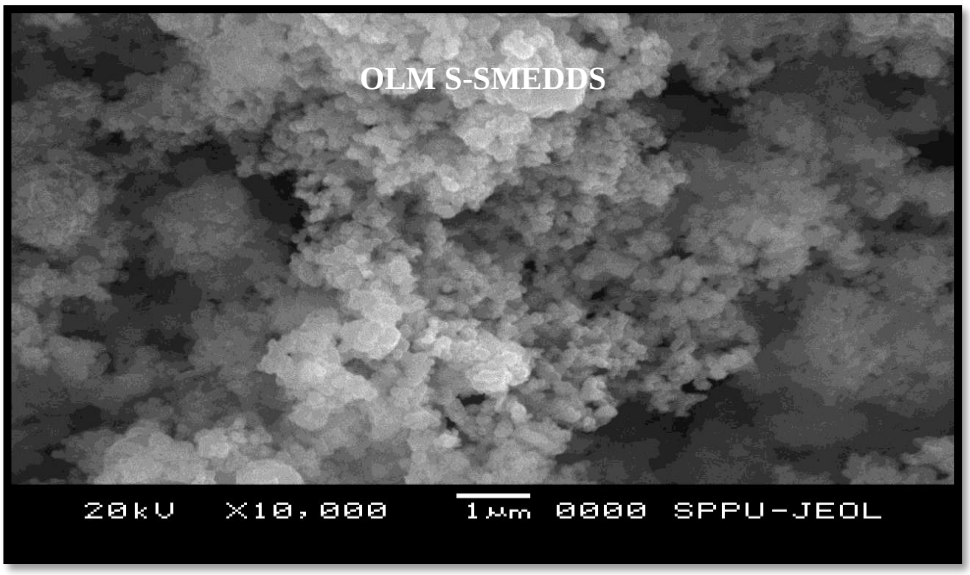


Figure 3.10 SEM of S-SMEDDS (SF6)

3.9 Stability Study

The real time stability study ($25^0C \pm 2^0C$ /60% ± 5% RH) was performed on batch SF6 for a period of three months. No significant changes were observed in appearance, Emulsification

time and Drug content. This indicated that formulation was stable at this condition. Results are shown below;

Table 3.20: Stability study data for S-SMEDDS

Evaluation Parameters	Observations			
	Initial	**30th day**	**60th day**	**90th day**
Appearance	White Amorphous Powder	No change	No change	No change
Self-emulsification time [a] (sec)	19±0.80	19.66±1.24	20±0.81	20.33±1.24
% Drug Content [a]	98.23±0.11	98.07±0.15	98.01±0.14	97.95±0.15

[a]Represents mean ± S.D. (n = 3)

CONCLUSION

Olmesartan medoxomil is orally administered novel selective angiotensin II receptor blocker for the treatment of hypertension. But its solubility and oral bioavailability are poor. The objective of our investigation was to formulate a self-micro-emulsifying drug delivery system (SMEDDS) of Olmesartan medoxomil using minimum surfactant concentration that could improve is solubility of drug without causing GI irritation. The composition of optimized formulation, consisted of Oleic acid as oil, Tween 80 as surfactant and PEG-400 as cosurfactant ,scontaining 10 mg of Olmesartan medoxomil. The formulation F6 showed drug release (98.60±0.3%), droplet size (212.91 nm). Zeta potential (-43.89 mV) and infinite dilution capability. In-vitro drug release of the F6, was highly significant. The F6 was further used for the preparation of Solid-SMEDDS(S-SMEDDS) formulations (powder). The powder was prepared via adsorption to solid carrier technique Aerosil 200 as adsorbent. The in vitro release for the S-SMEDDS was 96.70±0.3%. In conclusion, the study illustrated that adsorption to solid carrier technique could be a useful method to prepare the solid SMEDDS powder from liquid SMEDDS, which can improve aqueous Solubility and oral absorption of Olmesartan medoxomil nearly equivalent to the liquid SMEDDS, but better in the formulation stability, drug leakage, precipitation, patient compliant etc. Hence, it was concluded that Solid Self-

Micro Emulsifying drug delivery system is a good approach to enhance the solubility and dissolution property of Olmesartan medoxomil.

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CONFLICT OF INTEREST

All authors declared no conflicts of interest.

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Section: Research Paper

FORMULATION, EVALUATION AND OPTIMIZATION OF B –CYCLODEXTRIN BASED NANOSPONGES OF CLARITHROMYCIN

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ABSTRACT

The aim of present study was to develop an optimized gastric floating controlled drug delivery system of Clarithromycin (CLA). The Clarithromycin is poorly water soluble drug and gastric irritant. To overcome these problems attempt was made in present study to form inclusion complex of Clarithromycin with Nanosponges. β-Cyclodextrin (CD) based Nanosponges (NS) are novel class of cross-linked derivatives of Cyclodextrin. The Nanosponges were synthesized by carbonylation of β-Cyclodextrin to exploit its porous structure for drug entrapment. A better alternative to β-CD is it's Nanosponges due to low solubility & toxicity of β-CD. The final Nanosponges structure contains both lipophilic cavities of CD and carbonate bridges leading to a network of more hydrophilic channels. NS are solid, insoluble in water, crystalline in nature and thermally stable compounds. They have been used to increase the solubility of poorly water soluble actives, to avoid gastric irritation and control the release of drug. Present study aimed at formulating complex of CLA with NS by solid dispersion technique and absence of interaction of CLA with NS was confirmed by XRPD, DSC and FTIR studies. The result of XRPD results showed that the crystallinity of CLA was decreased after loading into NS. The 3^2 full factorial experimental designs were applied for tablet formulation. The *in vitro* dissolution studies indicated a slow and prolonged release of drug over the period of 12 h. Histopathological study revealed non irritancy of drug-NS complex to gastric mucosa (of rat). Hence drug-NS complex found to be suitable for designing into unit dosage forms. The release study of drug from tablet as well as capsule as unit dosage forms indicated controlled release of a drug when compared with marketed preparation.

Keywords: Nanosponges, β-Cyclodextrin, Clarithromycin, Solubility, Controlled drug delivery System.

1. INTRODUCTION

The objective of any drug delivery system is to provide therapeutic amount of drug to targeted site in body to achieve the desired therapeutic effect (1). For curing of disease, it is necessary to achieve and maintain the concentration of administered drug within the therapeutically effective range for this drug dosage must be taken several times which results in fluctuating drug levels in plasma. This drawback of conventional dosage form can be overcome by formulation of controlled release dosage forms which provides drug release in an

amount sufficient to maintain the therapeutic drug level over extended period of time, with release profiles controlled by the special technological construction and design of the system (2). The primary objectives of controlled drug delivery are to ensure safety and enhancement of efficacy of drug with improved patient compliance. So the use of these dosage forms is increasing in treatment of acute and chronic diseases as they maintain the concentration of drug in plasma above minimum effective concentration and below the minimum toxic level for extended period of time. Thus, controlled drug delivery results in optimum drug therapy with reduced frequency of dosing and side effects (3).

Effective ness can also can be enhanced by Gastro retentive systems. These are hydro dynamically balanced systems. In these systems dosage form have the specific gravity less than gastric juice, so they float in stomach and retain the drug over for extended period of time. Thus, total residence time in stomach is increased. Also these systems are relatively large in size and passing from pyloric opening is prohibited. This system is useful for drugs which are absorbed in stomach and also for local action of drug (4). Floating Drug Delivery is one of the method to enhance Gastric retention. The drug is released progressively from the swollen matrix, as in the case of conventional hydrophilic matrices (5, 6).

Another most effective method to deliver the insoluble drug at the targeted site is to preparation of Complexation with Nanosponges. A complex is a species of definite substrate-to-ligand stoichiometry that can exist both in solution and in solid state. The distinction between substrate and ligand is arbitrary, and is made solely for experimental convenience. Based on the type of chemical bonding, complexes can be classified into coordination and molecular complexes. The first form coordinate bond then present weak intermolecular force between substrate and ligand. Generally, pharmaceutical systems belong to the second group, being small molecule small molecule complexes, and/or inclusion complexes in which one molecule (host) possess a cavity into which it can admit a guest molecule (7). Nanosponges are prepared from β -Cyclodextrin as nanoporous materials for possible use as carriers for drug delivery. The structure of β-Cyclodextrin-based Nanosponges was principally investigated analyses. Sizes, morphology and toxicity were also examined. The capacity of the Nanosponges to incorporate molecules within their structure was evaluated using drugs with different structures and solubility. The Nanosponges were found capable of carrying both lipophilic and hydrophilic drugs and of improving the solubility of poorly water-soluble molecules (8). Nanosponges are a new class of material made of microscopic particles with cavities a few nanometers wide, characterized by the capacity to encapsulate a large variety of substances that can be transported through aqueous media. The efficacy of some pharmaceuticals adsorbed in the Nanosponges showed an activity 3–4 times higher and exhibited no detrimental side effects. Cyclodextrin based Nanosponges (of dexamethasone, flurbiprofen and Doxorubicin hydrochloride) demonstrated the ability to include either lipophilic or hydrophilic drugs and to release them slowly into physiological media. Thus Nanosponges can be used as a vessel for pharmaceutical principles to improve the aqueous solubility of lipophilic drugs, to protect degradable molecules and to formulate drug delivery systems for various administration routes beside the oral one. Beta Cyclodextrin (β CDs) have been the most widely used of all the Cyclodextrin (9, 10).

2. MATERIALS AND METHODS

2.1 Materials - Clarithromycin was gifted by Cipla pvt Ltd, Kurkumbh, India. Hydroxypropyl Methyl Cellulose K100 was also received as Gift Sample from Colorcon, Goa. Beta – Cyclodextrin was obtained from Gangwal Chemicals, Mumbai. TLC Plate and Diphenyl Carbonate was purchased from S.K. Enterprises. Dimethyl Sulfoxide, Dichloromethane, Acetone, Methanol, Benzene, Chloroform, Hexane, and acetonitrile was purchased from Research lab, Mumbai and all the solvent used were of Analytical Grade.

2.2 Synthesis of Nanosponges

2.2.1 Reaction

The reaction is a nucleophilic substitution where Cyclodextrin is reacted with Carbonyl compound of formula X-CO-X wherein X is Imidazolyl or –OR group in which R is C_1-C_4 alkyl (11). The reaction can be represented by the following scheme:

H-O-β-CD-OH + X-CO-X ⟶ (β-CD-OCOO- β-CD-OCOO) n

Where X is the carbonyl compound and n is the integer which can range within 3 to 6 depending upon the conditions used in the reaction which is shown in Figure 3.1.

2.2.2 Procedure

A round-bottomed flask equipped with a reflux condenser with thermometer. Weighed accurate quantities of beta CD and Diphenyl Carbonate (DPC) with DMSO as a solvent. The ratio was varied with 1:2, 1:4, and 1:8 equimolar mixture of beta CD: diphenyl carbonate. The reaction time was 12 h with conventional heating continuously with temperature maintain to 90^0-100^0C.The reaction mixture then added to cold water and product obtained was filtered and washed with water to remove excess amount of the beta CD. The product was Sohxlet extracted by ethanol to remove either impurities or unreacted diphenyl carbonate (11).

2.3 Optimization of Synthesized Product (Nanosponges)

The synthesized product was optimized to cross linker used in 1:2, 1:4, 1:8 (β- CD: Diphenyl Carbonate). The optimization for percentage yield is shown in Table 3.1.

2.4 Characterization of Synthesized Product (Nanosponges) (12, 13, 14)

1) Thin Layer Chromatography (TLC)

The TLC was used to evaluate the change in the R_f value of starting and product. Both samples dissolve into appropriate solvent and used for TLC. Chloroform is used as a mobile solvent. The TLC is observed under U.V. chamber. The photograph of TLC was shown in Figure 3.2 and R_f value are shown in Table 3.2.

2) FTIR Spectra

FTIR spectrophotometer was used for recording IR Spectrum of various samples by mixing the sample with dry potassium bromide and the sample was examined at transmission mode over a range 4000-400 cm-1 for studying principle peaks using FTIR spectrophotometer (FTIR-8400, Shimadzu). The FTIR Spectrum of product obtained in synthesis and beta-Cd are shown in Table 3.3 and in Figure 3.3.

3) Differential Scanning Calorimetric analysis (DSC)

Thermogram of the NS was taken on a Mettler Toledo India Pvt. Ltd, Switzerland. (STAR^e SW 9.20). An empty aluminium pan was used as a reference. DSC measurements were performed at a heating rate of 10^0C/min from 30^0 to 400^0C using aluminium sealed pan. During the measurement, the Sample was purged with nitrogen gas. DSC thermograms of Nanosponges are shown in Figure 3.4.

4) Powder X-ray diffraction (PXRD)

The PXRD spectra of samples were recorded using high power powder x-ray diffractometer (Ru-200B, Pune, India) with Cu as target filter having a voltage/current of 40 KV/40 mA at a scan speed of 4°/min. The samples were analyzed at 2θ angle range of 5° to 50°. Step time was 0.5 seconds and time of acquisition was 1 h. The results are reported in Figure 3.5.

5) Nuclear Magnetic Resonance Spectroscopy

The C^{13} NMR of β- CD and NS were recorded in DMSO using as a solvent in NMR Varian-Mercury 30 MHz spectrometer and chemical shifts are given in Parts per million, downfield from tetramethylsilane (TMS) as an internal standard. C^{13} NMR of Nanosponges and beta- CD are shown in Figure 3.6 and Figure 3.7.

2.5 Phase solubility study

Phase solubility equilibrium plots were obtained for binary systems at 25 ^{0}C in 0.1 N HCl. The studies were performed as per the procedure of Higuchi and Connors. Studies for binary system were carried out by adding excess amount of the drug to 10 ml of 0.1 N HCl containing increasing amounts of Nanosponges (0–2% w/v).The so formed series of suspensions were equilibrated on a mechanical shaker for 48 h. The equilibrated suspensions were then filtered through a membrane filter (0.45 lm) and absorbances observed by UV-spectrophotometer (13). The phase solubility diagram was constructed by plotting the dissolved clarithromycin concentration against the respective concentration of Nanosponges. The binding constant Ka was calculated from phase solubility diagram using its slope and intercept values (15). The phase solubility graph is shown in Figure 3.8. The stability constant was calculated by using equation 8.1.

$$K_{(a)\,1:1} = \frac{\text{Slope}}{S_o\,(1-\text{Slope})}\ M^{-1} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ (2.1)$$

Where, S_o is intrinsic solubility of drug

M is molar concentration
Ka is apparent stability constant
Slope is calculated from regression equation

2.6 Preparation of binary systems

1) Drug incorporation (13)

Clarithromycin was dissolved in dichloromethane to form a solution. To this solution Nanosponges were added and triturated until the solvent evaporates. The drug and Nanosponges were added in a ratio of 1:1 by weight. The obtained solid dispersion was dried in an oven over night (at 50 ^{0}C at atmospheric pressure) to remove any traces of dichloromethane. The obtained powder was sieved through 60 mesh and used for further work.

2) Preparation of Physical mixture

Equimolar physical mixtures were prepared 1:1 by weight homogenously blending exactly weighed amounts of drug and Nanosponges mixture is obtained.

2.7 Characteristics of Complex

1) FT-IR spectroscopy study (16)

FT-IR spectra of selected inclusion complex, Nanosponges and drug were recorded on Jasco FT-IR spectrophotometer using KBr discs. The instrument was operated under dry air purge and the scans were collected at scanning speed 2 mm/sec with resolution of 4 cm^{-1} over the region 4000-400 cm^{-1}. The scans were evaluated for presence of principle peaks of drug, shifting and masking of drug peaks due to Nanosponges and appearance of new peaks due to complexation. The FT-IR spectra of pure Clarithromycin, pure Nanosponges, physical mixture, and inclusion complex are shown in Figure 3.9.

2) Differential Scanning Colorimetry (DSC) (17)

The DSC study was carried out for pure Clarithromycin, pure Nanosponges, beta-CD, complex of Nanosponges and drug. The DSC patterns were recorded on a Mettler Toledo India Pvt. Ltd, Switzerland (STAR^e SW 9.20). Each sample (2-4mg) was heated in crimped aluminum pans at a scanning rate of 10^{0}C/min in an atmosphere of nitrogen using the range of 30-400^{0}C. The temperature calibrations were performed periodically using indium as a standard. The DSC curves are shown in Figure 3.10.

3) Powder X-Ray Diffraction Study

The PXRD spectra of samples were recorded using high power powder x-ray diffractometer (Ru-200B, Pune, India) with Cu as target filter having a voltage/current of 40 KV/40 mA at a scan speed of 4°/min. The samples were analyzed at 2θ angle range of 5° to 50°. Step time was 0.5 seconds and time of acquisition was 1 h which is shown in Figure 3.11.

4) Scanning Electron Microscopy

The morphology of the surfaces of the drug loaded Nanosponges and Complex was examined by scanning electron microscopy (SEM). The dried sample was observed under different magnifications with an analytical scanning electron microscope (JEOL-JSM 6360A-Japan). SEM Images of Nanosponges and Complex is shown in the Figure 3.12 and 3.13 respectively.

2.8 Gastric Irritation Test on Rats

As Clarithromycin supposed to cause irritation to gastric mucosa. To determine whether the complex of Clarithromycin and Nanosponges causes gastric irritation or it prevents gastric irritation test was done as follows. Rats weighing about (200-250 g) are selected. They are divided into 3 groups each group contain three rats. One group is treated with control, second group is treated with standard Clarithromycin and another is treated with test i.e. complex. 75 mg of complex is given to test group by oral suspension for 15 days and to standard group is also given 37.5 mg of drug for 15 days. On 16th day all animals are fasted and their stomach is removed and examined for irritation after that histopathology was done (18). The photographs of the stomach tissue of all three groups were shown in Figure 3.14 and the histology reports were shown in Table 3.4.

2.9 Preparation of Preliminary Batches for selection of Polymer

2.9.1 Preparation of Granules

Granules required for controlled release tablet (CRT) formulations were prepared by Wet granulation technique. All the ingredients as given in Table 2.1 were weighed accurately and passed through sieve 30 mesh.

Isopropyl alcohol used as a granulating agent. Required quantity of complex, polymer and diluents were mixed thoroughly in a glass mortar. Sufficient quantity of granulating agent was sprinkled over the powder mixture to obtain enough cohesiveness. This cohesive mass was then sieved through 16 mesh to obtain granules. The granules were then dried at 60^0C for 30 min. in hot air oven. Magnesium stearate and talc were finally added as glidant and lubricant mixed well with granules for 5 minutes (19).The prepared dried granules ready for compression was then evaluated for various granule properties as discussed below.

Table 2.1: Data for Composition of Preliminary Batches

Batches		**H1**	**H2**	**H3**	**P1**	**P2**	**P3**	**P4**	**P5**
Complex		200	200	200	200	200	200	200	200
HPMC	**K4M**	100	-	-	-	-	-	-	-
	K15M	-	100	-	-	-	-	-	-
	K100M	-		100	30	40	50	60	70
Lactose		50	50	50	120	110	100	90	80
Magnesium Stearate		5	5	5	5	5	5	5	5
Talc		5	5	5	5	5	5	5	5
NaHCo3		60	60	60	60	60	60	60	60
Citric Acid		15	15	15	15	15	15	15	15
Total		**435**	**435**	**435**	**435**	**435**	**435**	**435**	**435**

*All quantities in mg / tablet

2.9.2 Preparation of Control Release Tablet (CRT)

Different control release tablet (CRT) formulations were prepared by procedure reported in preparation of granules (section 2.9.1) using wet granulation technique. All the batches of tablets were prepared using rotary punch tablet compression machine (Karnavati Rimek minipress II) using 12 mm size punch. Prepared tablets were evaluated for various tablet properties.

2.9.3 Evaluation of CRT (Preliminary Batches)

1) *In vitro* dissolution study for Preliminary batches

In vitro dissolution study was performed using USP Dissolution Testing Apparatus II (Disso TDT 08L, Electrolab). The dissolution test was performed using 900 ml of 0.1 N HCL, at 37 ± 0.5^0C and paddle speed was rotated at 50 rpm. A sample (5 ml) of the solution was withdrawn from the dissolution apparatus after every 1hr. for next 12 hrs, and the samples were replaced with fresh dissolution medium equilibrated at the same temperature to maintain the volume. The samples were filtered through Whatman filter paper no. 41.The samples collected were diluted taking dilution factor as 10 i.e. 1ml sample diluted with 2ml of sodium carbonate(20%), 3ml of FCR(2:1 diluted with water) and then 4ml of 0.1N HCL. Samples were then analyzed by UV spectrophotometer at 760 nm using UV spectrophotometer Jasco V-630. The % drug release data is reported in sec 2.9.4, Table 3.6. The graphical presentation of % drug released verses time interval is shown in Figure 3.15 and 3.16. Dissolution tests were performed in duplicate (20).

2.10 Factorial Design Batches (Experimental design) (8, 21,22)

A 3^2 factorial design was implemented for optimization of oral controlled release tablet. According to the model it contains two independent variables at three levels +1, 0 and -1 (Table 2.2). The translation of coded levels in actual units is enumerated in Table 2.3. According to the model total nine formulations are possible. The composition of different formulations is shown in Table 2.4.

A. Dependent variables

Y1 - Time taken for 50% drug release (%)

Y2 - Time taken for 85% drug release (%)

Y3 - Floating lag time (Seconds)

B. Independent variables:

X1 - HPMC K100M (%)

X2 - Citric Acid

Table 2.2: Factorial Design for Preparation of Batches.

Batch Code	Variable levels in Coded form	
	X_1	X_2
F1	+1	+1
F2	+1	0
F3	+1	-1
F4	0	+1
F5	0	0
F6	0	-1
F7	-1	+1
F8	-1	0
F9	-1	-1

Table 2.3: Translation of coded values in actual unit.

Independent Variable levels	Low (-1)	Medium (0)	High (+1)
X_1 = Concentration of HPMC K100 M (%)	20	30	40
X_2 = Concentration of Citric Acid (%)	5	7.5	10

Table 2.4: Combination batches by using HPMC K100M & Citric Acid in various concentrations according to 3^2 factorial designs.

Batch code	F1	F2	F3	F4	F5	F6	F7	F8	F9
Complex	500	500	500	500	500	500	500	500	500
HPMC K100M	**100**	**100**	**100**	**150**	**150**	**150**	**200**	**200**	**200**
Citric Acid	**25**	**37.5**	**50**	**25**	**37.5**	**50**	**25**	**37.5**	**50**
PVP K-30	60	60	60	60	60	60	60	60	60
Mg. Stearate	5	5	5	5	5	5	5	5	5
$NaHCo_3$	80	80	80	80	80	80	80	80	80
Lactose	30	17.5	5	30	17.5	5	30	17.5	5
Total	**800**	**800**	**800**	**850**	**850**	**850**	**900**	**900**	**900**

*All quantities in mg/tablet

2.11 Preparation of Factorial Design Batches

2.11.1 Preparation of Granules

Preparation of Granules was done by Wet Granulation Technique using composition mention in Table 2.4. Procedure is mention in the section 2.9.1 was used (19).

2.11.2 Evaluation of Granules

The granule properties include bulk density; tap density, Hausner ratio, and Carr's index were determined using Tap density tester (TD 1025, Lab India).

1) Angle of Repose

Angle of repose has been defined as the maximum angle possible between the surface of pile of powder and horizontal plane. The angle of repose for the granules of each formulation was determined by the funnel method. The granules mass was allowed to flow out of the funnel orifice on a plane paper kept on the horizontal surface. This forms a pile of angle of granules on the paper. The angle of repose was calculated with the help of values of the base radius 'R' and pile height 'H' (23,24).

$$\tan \Theta = h / r \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (2.2)$$

Where, Θ = angle of repose

h = height of the cone

r = Radius of the cone

Table 2.5: Relationship between angle of repose (Ɵ) and Flowability

Angle of Repose (Ɵ)	Flowability
< 20	Excellent
20 – 30	Good
30 – 34	Passable
> 40	Very Poor

2) Bulk Density

The bulk density was obtained by dividing the mass of a powder by the bulk volume in cm^3 (23,24). It was calculated by using equation given below:

$$\rho_b = M / V_0 \text{..... (2.3)}$$

Where, ρ_b = bulk density

M = weight of sample in grams

V_0 = Apparent unstirred volume

3) Tapped Density

The tapped density was obtained by dividing the mass of a powder by the tapped volume in cm^3 (23,24).It was calculated by using equation given below:

$$\rho_t = M / V_f \text{..... (2.4)}$$

Where, ρ_t = Tap density

M = weight of sample in grams

V_f = final Tap volume

4) Carr's Index

The Carr's index is determined from the tapped density and poured density (bulk density) as per the formula (Eq. (2.4)) given below (23,24).

$$\text{Carr's index (\%)} = \frac{\text{Tapped density- bulk density}}{\text{Tapped density}} \times 100 \text{..... (2.5)}$$

Table 2.6: Relationship between % compressibility and flowability

% Compressibility	Flowability
5 – 15	Excellent
12 – 16	Good
18 – 21	Fair to Passable
23 – 35	Poor
33 – 38	Very Poor
> 40	Extremely Poor

5) Hausner ratio

Hausner ratio is determined from the ratio of tapped density to poured density using formula given below (23,24).

$$\text{Hausner ratio} = \frac{\text{Tapped density}}{\text{Poured density}} \text{..... (2.6)}$$

The Angle of repose, Bulk density, Tap density, Carr's index and Hausner ratio are reported in sec 3.9.2, Table 3.6.

2.11.3 Preparation of Control Release Tablet (CRT)

Different control release tablet (CRT) formulations were prepared by wet granulation technique. All the batches of tablets were prepared using rotary punch tablet compression machine (Karnavati Rimek minipress II) using 12 mm size punch. Prepared tablets were evaluated for various tablet properties.

2.11.4 Evaluation of Control Release Tablet (CRT)

1) Weight Variation Test

I. P. procedure for uniformity of weight was followed. Twenty tablets were randomly selected from each batch and individually weighed. By using Electronic balance (Shimatzu). The average weight and standard deviation of twenty tablets were calculated .The average weight of tablet and its allowed percent deviation were shown in Table 2.7. Result for Weight Variation test is reported in section 3.9.4, Table 3.7 (25,26).

Table 2.7: Allowable limit for weight variation

Average weight of tablet (X mg)	Percentage deviation
X ≤ 80 mg	10 %
80 < X <250 mg	7.5 %
X ≥ 250 mg	5 %

2) Tablet hardness

The resistance of tablet to shipping or breakage, under conditions of storage, transportation and handling before usage depend on its hardness. The hardness of tablet of each formulation was measured by Pfizer hardness tester. The hardness was measured in terms of kg/cm^2. For each batch three tablets were tested. The average hardness and standard deviation is reported in section 3.9.4, Table 3.7 (25,26).

3) Friability

Friability is the measure of tablet strength. Roche friabilator (FT1020, Labindia) was used for testing the friability. Twenty tablets were weighed accurately and placed in the tumbling apparatus that revolves at 25 rpm dropping the tablets through a distance of six inches with each revolution. After 100 revolutions, the tablets were weighed and the % friability was calculated measured using the formula (Eq. (8.7)). The friability of different formulations is reported section 3.9.4, Table 3.7 (25,26).

$$\text{Friability} = \frac{\text{Initial weight of tablets- Final weight of tablets}}{\text{Initial weight of tablets}} \times 100 \quad \ldots\ldots (2.7)$$

4) Thickness

Thickness of tablet is important for uniformity of tablet size. Thickness was measured using Vernier Calliper. It was determined by checking ten tablets from each formulation. Results for thickness are reported in section3.9.4, Table 3.7 (25,26).

5) Drug Content

Five tablets were weighed individually, crushed to fine powder and about 100 mg of drug was dissolved in 0.1N HCl, the solution was filtered through 0.45µ membrane filter. The absorbance was measured at 760 nm after suitable dilution using F. C. Phenol reagent as a colour forming agent. Results for drug content section 3.9.4, Table 3.7 (25,26).

6) *In vitro* dissolution study for Factorial batches

In vitro dissolution study was performed using USP Dissolution Testing Apparatus II (Disso TDT 08L, Electrolab). The dissolution test was performed using 900 ml of 0.1 N HCL, at 37 ± 0.5^0C and paddle speed was rotated at 100 rpm. A sample (5 ml) of the solution was withdrawn from the dissolution apparatus after every 1hr.for next 12 hrs, and the samples were replaced with fresh dissolution medium equilibrated at the same temperature to maintain the volume. The samples were filtered through Whatman filter paper no. 41.The samples collected were diluted taking dilution factor as 10 i.e. 1ml sample diluted with 2ml of sodium carbonate(20%), 3ml of FCR(2:1 diluted with water) and then 4ml of 0.1N HCL . Samples were then analyzed at 760 nm using UV spectrophotometer (Jasco V-630). The % drug release was calculated using disso software (PCP V3) and is reported in section 3.9.4, Table 3.8 and 3.9. The graphical presentation of % drug released verses time interval is shown in Figure 3.17, 3.18, and 3.19. The FLT for all factorial batches is shown in Figure 3.20. Dissolution tests were performed in triplicate (25,26).

2.12 Curve fitting

Release data were fitted to various mathematical models for describing the release mechanism from controlled release zero-order (Eq.2.8) [Lee, 1984] and Hixon Crowell.

$$\mathbf{Mt/M\infty = k_K P t^n} \quad \ldots\ldots\ldots\ldots\ldots\ldots (2.8)$$

Where, $Mt/M\infty$ = fraction of drug released at time‘t’;

$k_K P$ = release rate constant;

n = the release exponent.

$$\mathbf{M_t = M_0 + k_0} \ldots\ldots\ldots\ldots\ldots\ldots (2.9)$$

Where, M_t = the amount of drug released at time‘t’;

M_0 = the concentration of drug in the solution at t=0;

k_0 = the zero-order release constant.

$$\mathbf{M_t = k_H\, t^{1/2}} \text{…….. (2.10)}$$

Where, Mt = the amount of drug release at time '√t';

k_H = the Higuchi release constant.

All curve fitting, simulation and plotting was carried out by using PCP disso software. The parameters for both zero order and Hixon Crowell models were shown in Table 3.11 (27,28,29).

2.13 Optimization of Factorial Design Batches

2.13.1 Regression analysis

The effect of formulation variables on the response variables were statistically evaluated by applying one way ANOVA at P< 0.05 level using a commercially available software package Design-Expert® version 7.1.6 (Stat-Ease Inc.) (8,22). To describe the response surface curvature, the design was evaluated by quadratic model, which bears the form of equation (Eq. 2.11).

$$\mathbf{Y = b_0 + b_1X_1 + b_2X_2 + b_3\, X_1X_2 + b_4X_1^2 + b_5X_2^2} \text{…….. (2.11)}$$

Where, Y is the response variable,

b_0 the constant,

b_1, b_2... b5 the regression coefficient,

X_1 and X_2 stand for the main effect,

X_1X_2 are the interaction terms, show how response changes when

Two factors are simultaneously changed.

1) Regression analysis for response Y_1

The effect of formulation variables on the response variables were statistically evaluated by applying one way ANOVA at P< 0.05 level using a commercially available software package Design-Expert® version 7.1.6 (Stat-Ease Inc.)(8, 22).

2) Regression analysis for response Y_2

The effect of formulation variables on the response variables were statistically evaluated by applying one way ANOVA at P< 0.05 level using a commercially available software package Design-Expert® version 7.1.6 (Stat-Ease Inc.) (8, 22).

3) Regression analysis for response Y_3

The effect of formulation variables on the response variables were statistically evaluated by applying one way ANOVA at P< 0.05 level using a commercially available software package Design-Expert® version 7.1.6 (Stat-Ease Inc.) (8, 22).

4) ANOVA, Pure Error and Lack of Fit

The results for ANOVA, pure error and lack of fit were discussed in section 3.11.1.

2.14 Studies on Final Formulation

1) Water uptake studies

The rate of test medium uptake by the polymer was determined by equilibrium weight gain method similar to Fantasies and Vlachos (2000). The study was carried out in the USP dissolution apparatus II. The FCRT Tablet was accurately weighed, placed in dissolution baskets, and immersed in 0.1 N HCl solution maintained at 37 ±0.5 ^{0}C in the dissolution vessel. At regular intervals, the pre-weighed basket–matrix system was withdrawn from the dissolution vessel, lightly blotted with a tissue paper to remove excess test liquid and re-weighed. The percent water uptake, i.e., degree of swelling due to absorbed test liquid, was estimated at each time point using formula given below:

$$\% \text{ Water uptake} = \frac{(Wt - Wi)}{Wi} \times 100 \text{…….. (2.12).}$$

Where, *Wt* is the weight of the swollen matrix at time, *t*, *Wi* is the initial weight of the tablet. The % swelling or water uptake data is reported in section 3.12 in Table 3.13 (26).

2) Comparison of In vitro release study of marketed formulation with capsule fills with complex and formulated tablet

The in vitro profile of optimized formulation and complex were compared with marketed SR tablet (Biaxin-500). *In vitro* dissolution study was performed using USP Dissolution Testing Apparatus II (Disso TDT 08L, Electrolab). The dissolution test was performed using 900 ml of 0.1 N HCL, at 37 ± 0.5^0C and paddle speed was rotated at 100 rpm. A sample (5 ml) of the solution was withdrawn from the dissolution apparatus after every 1hr.for next 12 hrs, and the samples were replaced with fresh dissolution medium equilibrated at the same temperature to maintain the volume. The samples were filtered through Whatman filter paper no. 41.The samples collected were diluted taking dilution factor as 10 i.e. 1ml sample diluted with 2ml of sodium carbonate(20%), 3ml of FCR(2:1 diluted with water) and then 4ml of 0.1N HCL . Samples were then analysed at 760 nm using UV spectrophotometer (Jasco V-630). The % drug release was calculated using disso software (PCP V3) and is reported in section 3.12 (Table 3.14). The graphical presentation of % drug released verses time interval is shown in Figure 3.27 (25,26).

3) Optimization

A numerical optimization technique by the desirability approach was used to generate the optimum settings for formulation. The process was optimized for dependent variables Y_1-Y_3. The optimized formula arrived by targeting the Y_2 at 650 minute, Y_1 was kept at range 360-400 min. ,Y_3 also kept at range 16-62 sec. Results were discussed in section 3.12 (30)

3. RESULTS AND DISCUSSIONS

3.1 Synthesis of Nanosponges

3.1.1 Reaction

Nanosponges was synthesized and purified by ethanol in Sohxlet apparatus. The carbonylation of β-CD and DPC occurred and characterised by various techniques. The reaction is represented in Figure 3.1.

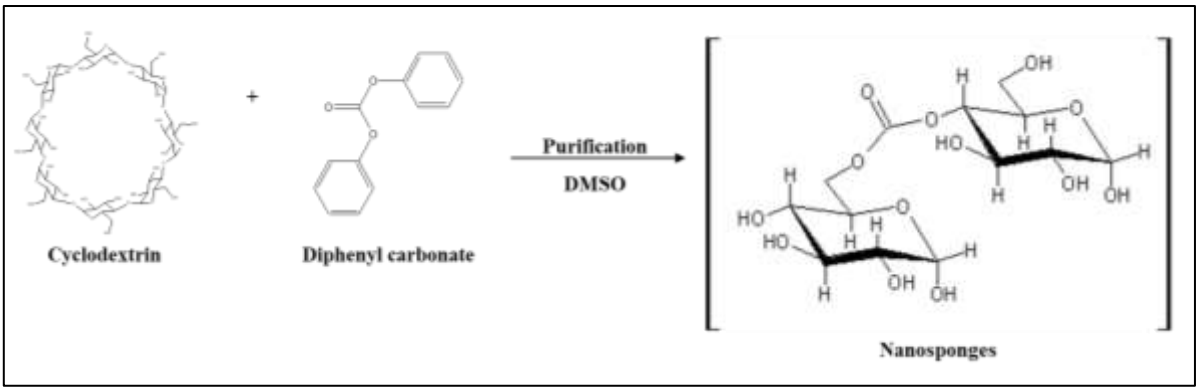

Figure 3.1: Nucleophilic reactions of beta-CD and Diphenyl Carbonate.

3.1.2 Preparation of Nanosponges

Preparation of Nanosponges was carried out according to the procedure mention in the section 2.2.2.

3.2 Optimization of Synthesized Product (Nanosponges)

Reaction was optimised to various concentrations of cross linker. This reaction was optimised in terms of percentage yield. The obtained yield was 55%, 69% and 70% by keeping β- CD: DPC in the ratio 1:2, 1:4 and 1:8. The reactions were carried out batch 1, 2 & 3 for these proportions respectively. The yield obtained in batch 2 and 3 were almost same so combination used in the batch number 2 was finally selected for Nanosponges synthesis.

Table 3.1: Optimization of Synthetic procedure

Batches	BCD:DPC ratio	Energy type	Yield %
1	1:2	Conventional Heating	55
2	1:4	Conventional Heating	69
3	1:8	Conventional Heating	70

3.3 Characterization of Synthesized Product (Nanosponges)

Table 3.2: Characteristics of Nanosponges

Parameters	Characteristics
Colour, State	White, Solid
TLC	Chloroform; R_f = 0.23
IR (KBr) cm^{-1}	1775 / cm (C=O group)
DSC	Degradation occurs after 300^0c

1) Thin Layer Chromatography (TLC)

TLC showed clear separation between starting material and product. As there was complete consumption of starting material β- CD absence of spot in product the formation of product was confirmed.

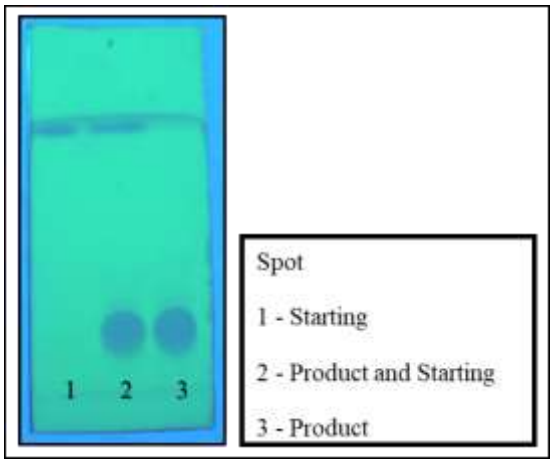

Figure 3.2: TLC Photograph

2) FTIR Spectra

The FTIR spectra of Nanosponges and β- CD were portrayed in Figure 3.3. FTIR spectra of β- CD was characterized by 2925 cm^{-1}(C-H asym./sym. stretch), peak at 1646 cm^{-1} (C=C stretching), 1415(C-H bend)cm^{-1} and a band with distinct peaks in the region between 1200 and 1000cm^{-1}. The FTIR spectra of Nanosponges exhibited distinct peaks at 2926 cm^{-1} (C-H asym. /sym. stretch), 1638 cm^{-1}(C=C stretching), 1775 cm^{-1} (Aryl Carbonate), 1026 cm^{-1} (Primary alcohol, C-O stretch), 1413cm^{-1}(C-H bend) confirming the earlier report. The appearance of peak at 1775 cm^{-1} clearly indicated the carbonylation of β- CD which is shown in Table 3.3. The peak at 1775 /cm confirmed presence of the carbonyl group in the structure of Nanosponges.

Table 3.3: IR peak of β-CD and NS

Group	Beta-CD	Nanosponges
C-H asym./sym. stretch	2925	2926
C=C stretching	1646	1638
Aryl Carbonate	absent	1775
Primary alcohol, C-O stretch	1027	1026
C-H bend	1415	1413

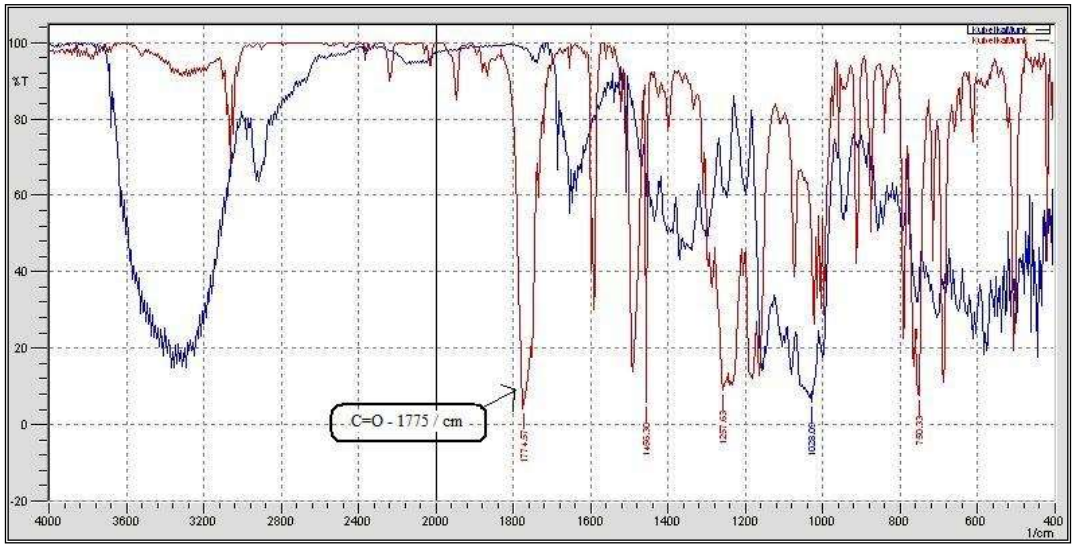

Figure 3.3: FTIR spectra of Nanosponges and Beta-CD

3) DSC Graph

Thermal degradation of Nanosponges is reported after 300^{0}C. The absence of endotherm below 300^{0}C in the present study it was confirmed that the Nanosponges was synthesized. The graph of β- CD and Nanosponges are shown in Figure 3.4.

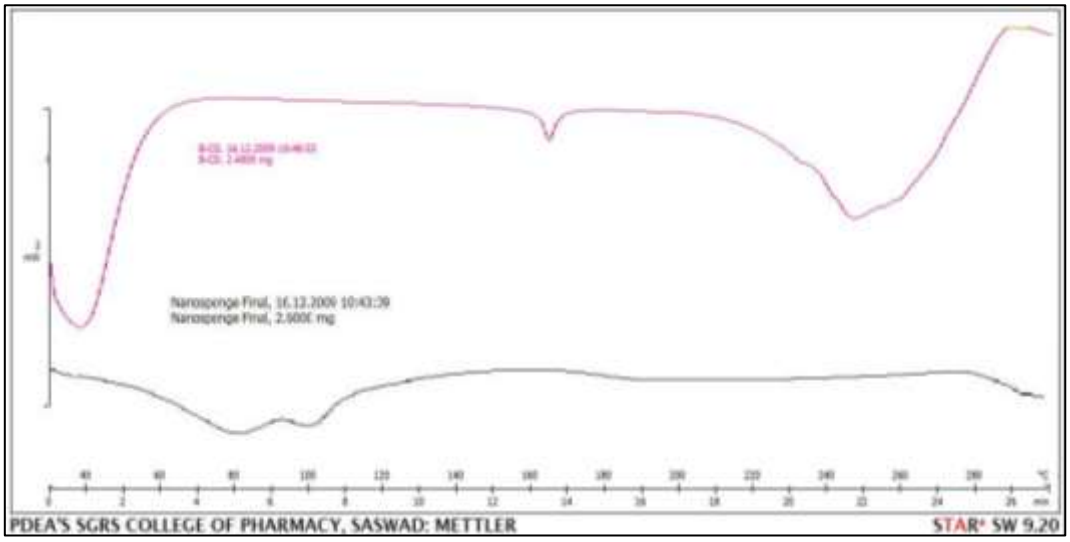

Figure 3.4: DSC graph of Nanosponges and Beta-CD.

4) X-Ray Powder Diffraction (XRPD) Analysis

Formation of Nanosponges was confirmed by XRPD spectra. As shown in Figure 3.5, the number of peaks reduced in Nanosponges as compared to β-CD with peak broadening. This clearly indicated formation of poorly crystalline Nanosponges.

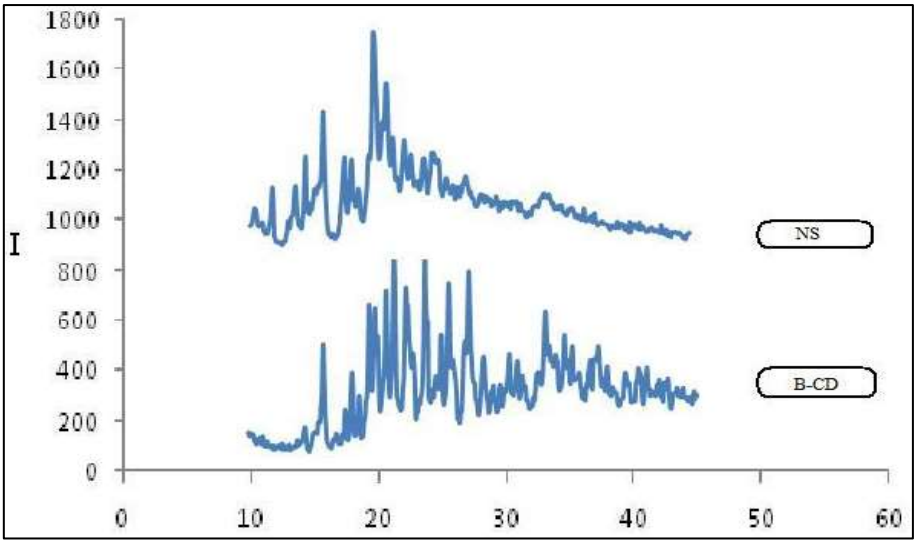

Figure 3.5: PXRD of Nanosponges and Beta-CD

5) NMR

The C^{13} NMR of Nanosponges and β -CD were shown in Figure 3.6 and 3.7 respectively. NMR of NS shows various peaks at different δ- values. The carbonyl bridge between two β –CD showed the peak at 155.5 δ value which confirmed the Nanosponges was synthesized.

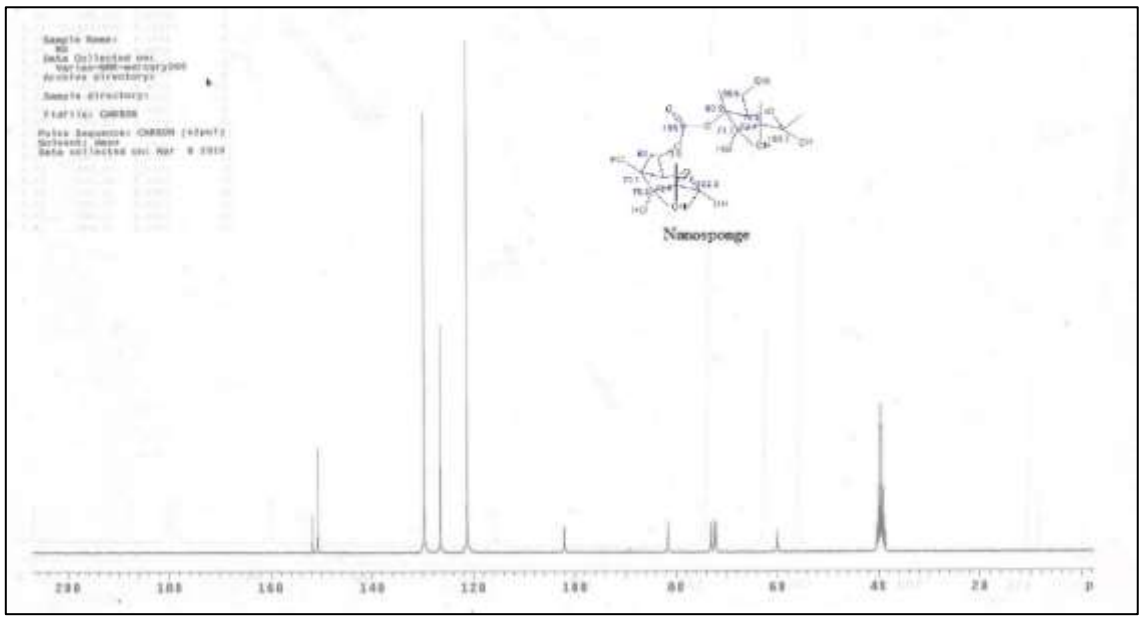

Figure 3.6: NMR of Nanosponges

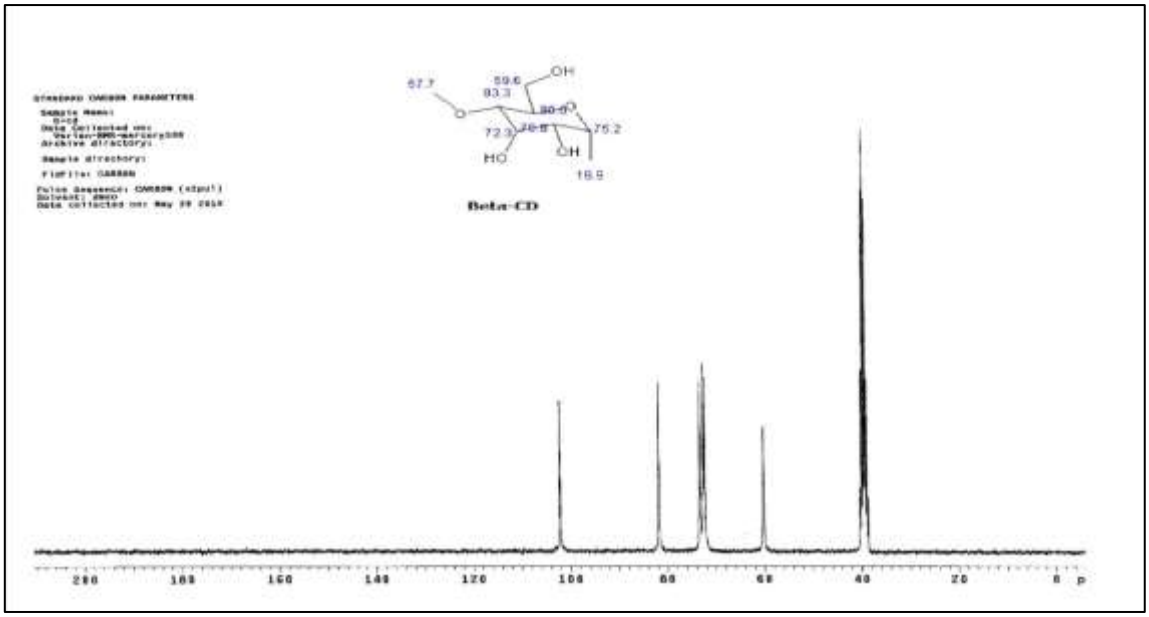

Figure 3.7: NMR of Beta-CD

3.4 Phase solubility studies

The phase solubility studies conducted at 25^0C indicated that, solubility of Clarithromycin increased linearly (R^2=0.961) as a function of Nanosponges concentration, as shown in Figure 3.8. As apparent solubility of Clarithromycin increased linearly with Nanosponges concentration over the entire concentration range studied; the phase solubility diagram was classified as A_L type. The slope and intercept of the curve were found to be 0.00003047 and 28.21×10^{-8} M, respectively. The stability constant computed from the slope and intercept of the phase solubility diagram was found to be 1080.12 M^{-1}. The value of stability constant obtained indicated a labile association of Clarithromycin and Nanosponges. The solubility of clarithromycin was significantly increased with Nanosponges.

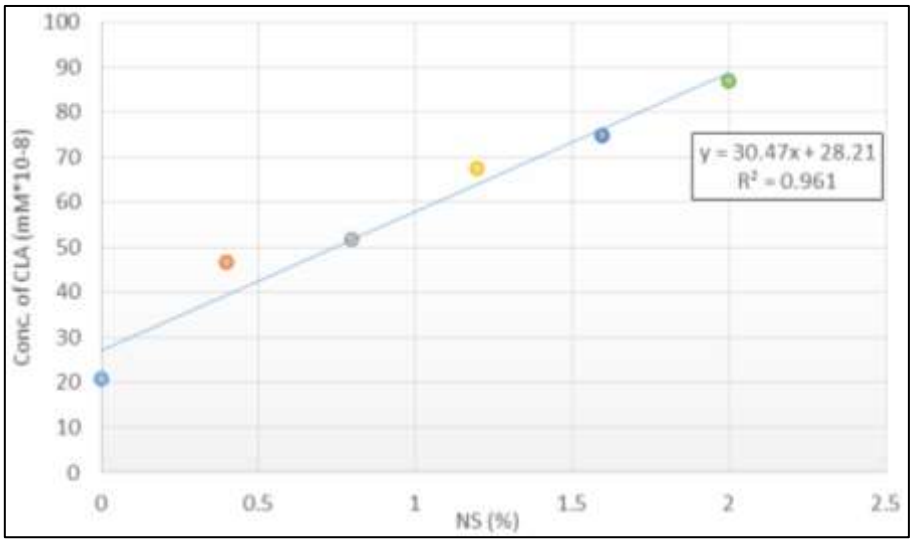

Figure 3.8: Phase solubility study of drug and Nanosponges in 0.1 N HCL

3.5 Preparation of binary systems

Drug Incorporation and Preparation of Physical Mixture was done using the procedure mention in the section 2.6.

3.6 Characteristics of Complex

1) FT-IR spectroscopy study

The FTIR spectra of Nanosponges, Clarithromycin and complex were portrayed in Figure 3.9. The FTIR studies showed that there are weak interactions between NS and CLA that were evident from broadenings and disappearance of the drug peak in case of complexes.

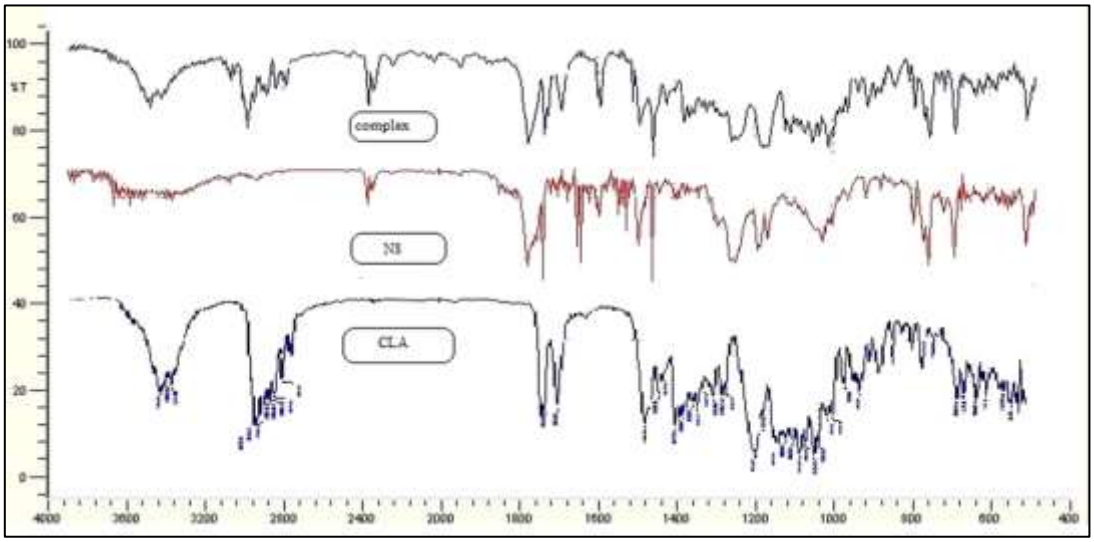

Figure 3.9: FTIR spectra of Nanosponges, Clarithromycin and complex

2) DSC study

The thermal analysis graphs of pure Clarithromycin, complex and Nanosponges are shown in Figure 3.10. Area of enthalpies of the drug progressively decreased in following order Plain drug, Nanosponges, drug Nanosponges PM, and drug Nanosponges solid dispersions.

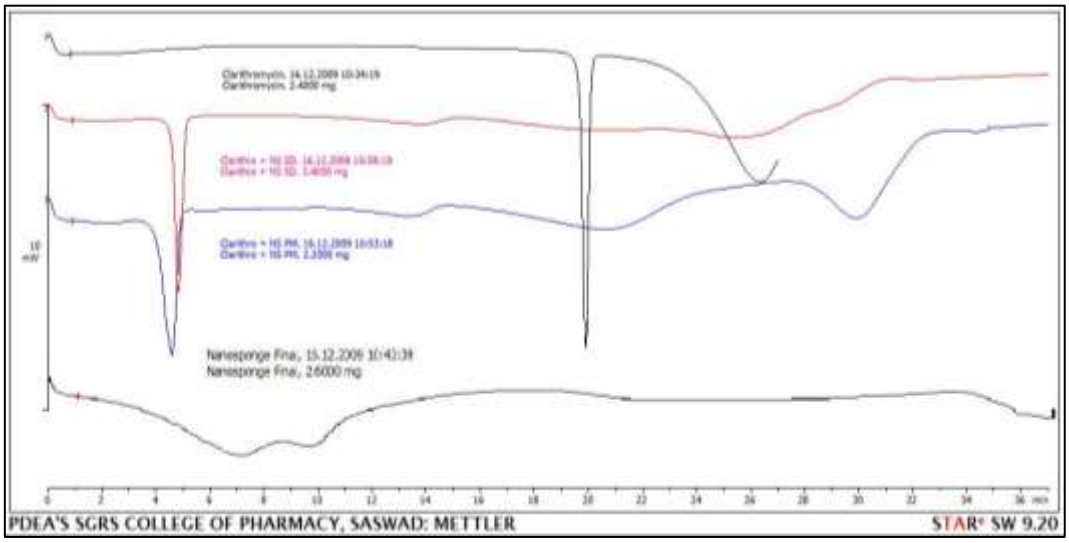

Figure 3.10: DSC graph of pure CLA, complex and Nanosponges

These could be due to change in the state of the drug from crystalline to amorphous. Thus the energy required to melt the drug is reduced i.e. enthalpy reduced. DSC thermograms of the complexes did not show the melting peak corresponding to drug fusion. This indicates that the drug is no longer crystalline and confirms its interaction with NS structure. On the contrary, the binary P.M. presented the melting peak of the drug indicating that CLA maintained its original crystallinity in the P.M. due to a lack of interaction.

3) PXRD Study

The complexation between Clarithromycin and Nanosponges was also confirmed by PXRD. As shown in the PXRD pattern of drug loaded Nanosponges (Figure 3.11), number of peaks of Clarithromycin were reduced. Also, no perfect coincidence was found in PXRD patterns of CLA and CLA- NS complex indicating

the formation of a new ordered phase which might be responsible for increase in the solubility of CLA. Thus it can be predicted that the solubility of CLA is due to its molecular dispersion i.e. complexation with Nanosponges.

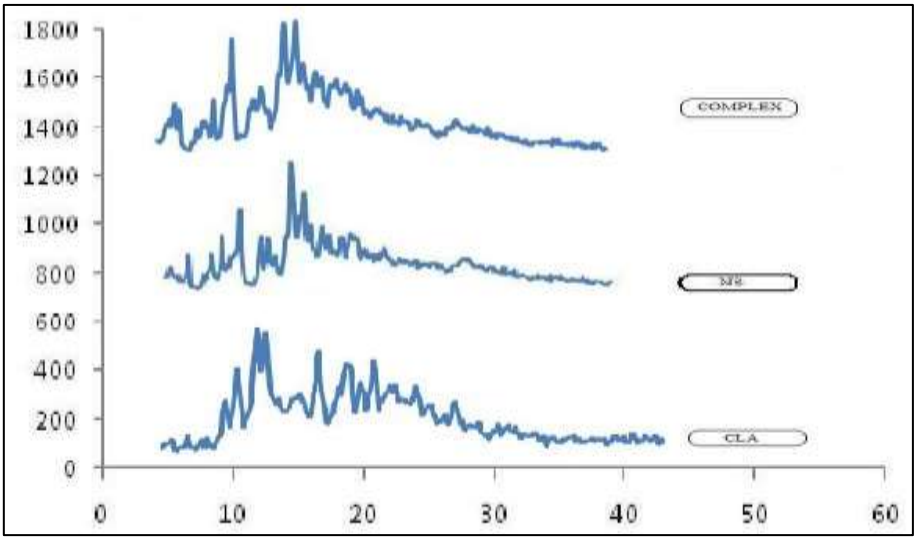

Figure 3.11: PXRD of CLA, NS and Complex

4) Scanning Electron Microscopy

SEM images of NS and complex were shown in Figure 3.12 and 3.13. These images revealed striking difference between the microstructure of plain NS and complex of NS and CLA. Plain NS exhibited highly porous structure while complex was compacted. The SEM of complex confirmed drug loading in the NS as the surface is smooth as compared to porous surface of plain NS.

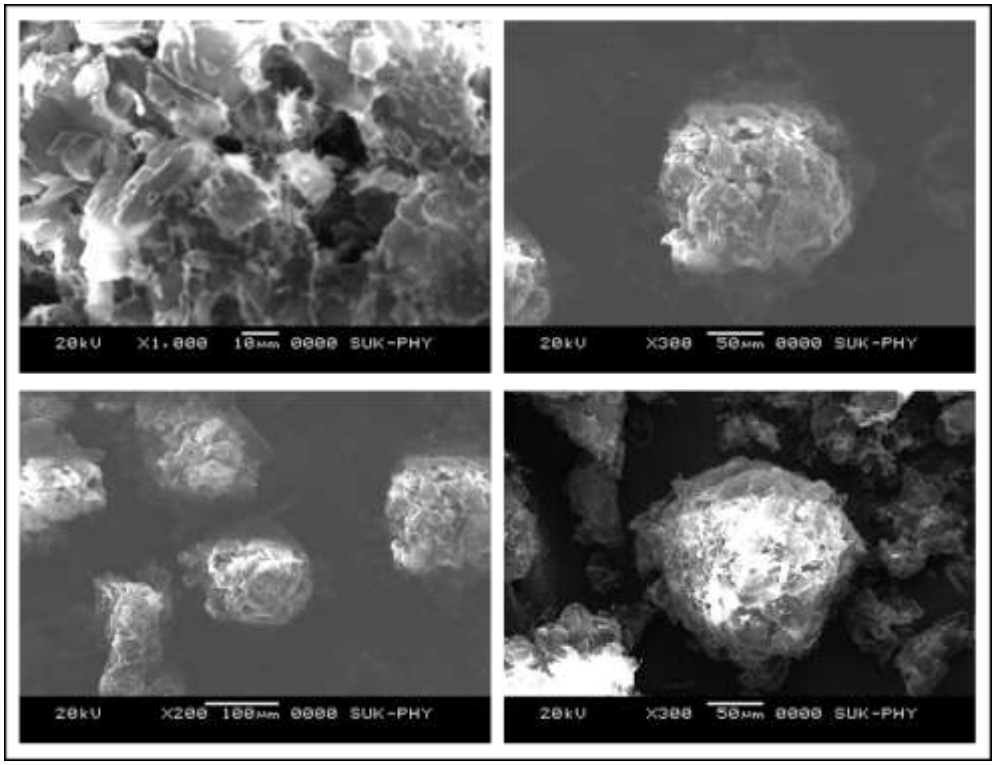

Figure 3.12: SEM Images of NS

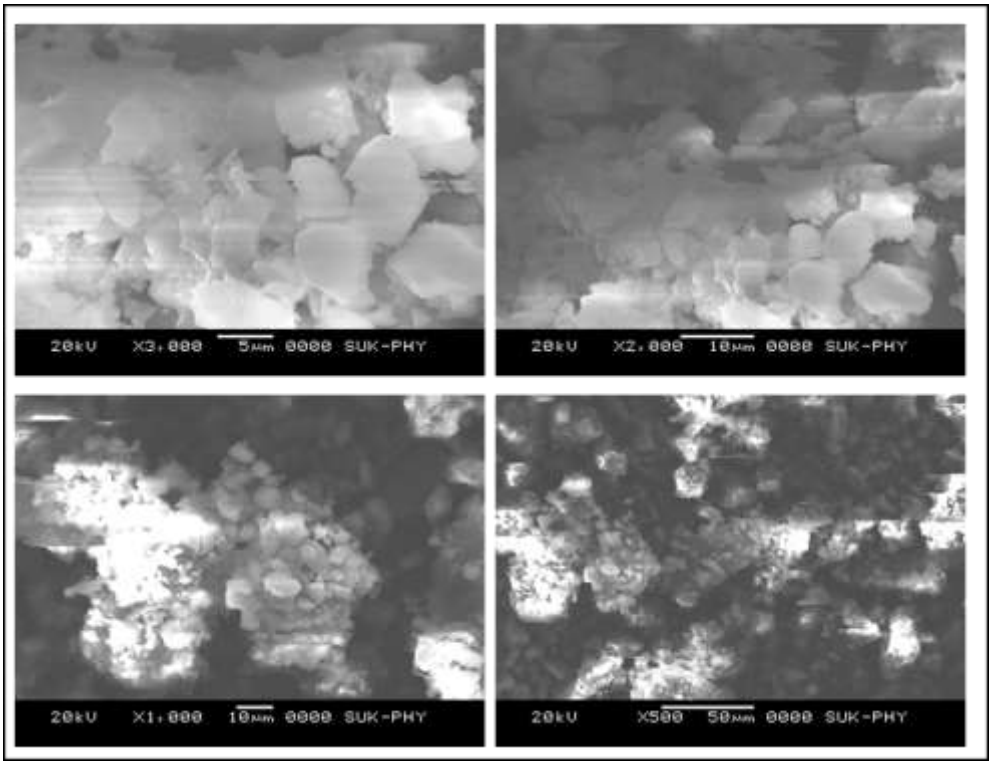

Figure 3.13: SEM Images of Complex

3.7 Gastric Irritation Test

Histopathological examination - Rats treated with Plain drug showed marked mucosal damage. Lesion formation was found to be 75% in these cases whereas in rats which received complex showed reduced gastric lesions as compared with plain drug photographs are shown in Figure 3.14. The results are shown in Table 3.4. From the results it was concluded that severity of ulceration was lowered in test groups than standard group.

Table 3.4: Histopathological report of stomach tissues

Group	Control (A)	Standard(B)	Test (C)
Congestion	00	++	+
Necrosis	00	+++	+
Cellular infiltration	00	++	+
Edema	00	++	+
Ulceration	00	+++	+
Hemorrhages	00	+++	+
Note: 0 indicates no abnormality detected, + indicates pathological changes up to less than 25 %, ++ indicates Pathological changes up to less than 50 % , +++ indicates Pathological changes up to less 75 %, ++++ indicates Pathological changes up to more than 75 %			

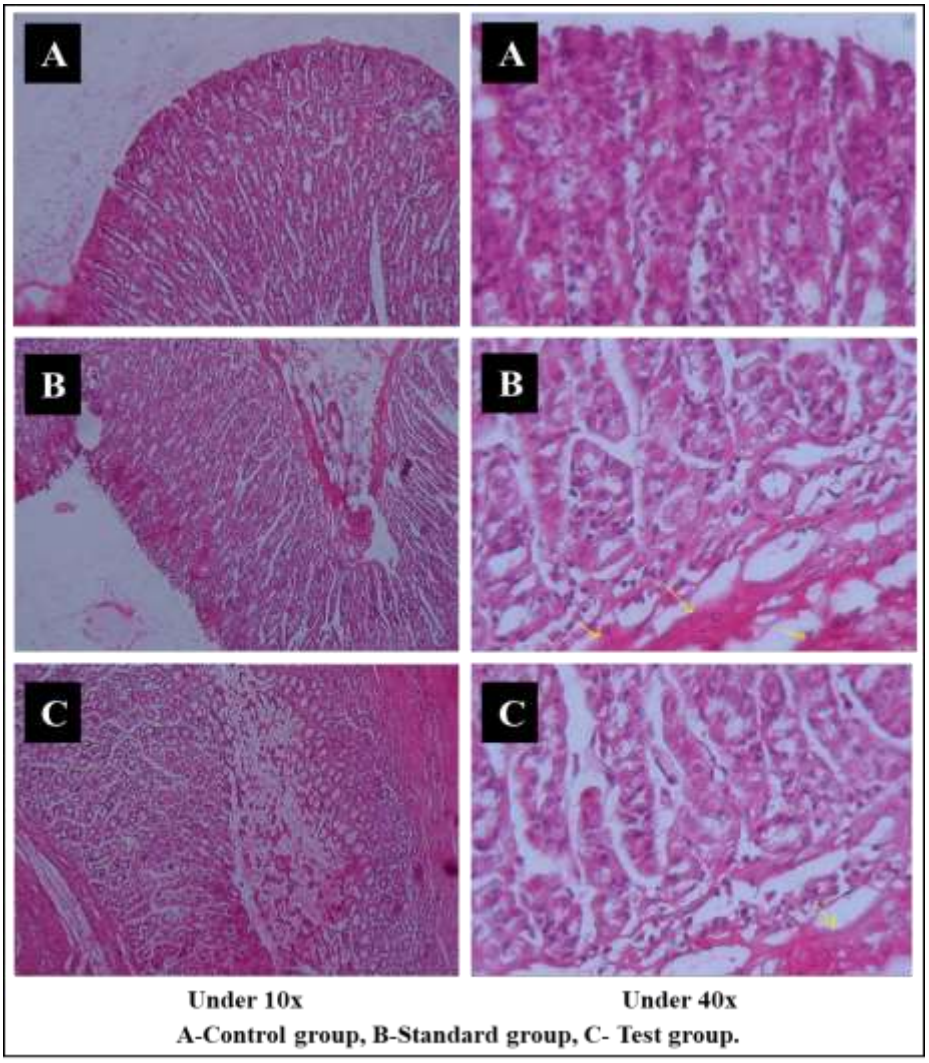

Figure 3.14: Photographs of stomach Tissue

3.8 Preparation of Preliminary Batches for selection of Polymer

3.8.1 Preparation of Granules

Granules were prepared according to the Procedure mentioned in the section 2.9.1.

3.8.2 Preparation of Control Release Tablet (CRT)

Control release tablet of clarithromycin was prepared according to the procedure mentioned in the section 2.9.2.

3.8.3 Evaluation of CRT (Preliminary Batches)

1) *In vitro* dissolution study for Preliminary batches

In vitro dissolution study was performed using USP Dissolution Testing procedure and the result are mention in the Table 3.5. Figure 3.15 shows % Drug Release of Clarithromycin from batches H1-H3. H1 contains HPMC K4M 100 mg alone and released 50% of the drug in 3 hrs. H2 contains HPMC K15M 100 mg alone and released 50% of the drug in 5 hrs. H3 contains HPMC K100M 100 mg alone and released 50% of the drug in 9 hrs which was attributed to its high viscosity as compared to K4M & K15M (Table 3.5). Hence HPMC K100M was used in further studies of preliminary formulations. From the discussion data for batches H1-H3 it was concluded that HPMC K100M showed highest release retarding property.

Table 3.5: *In vitro* dissolution study of preliminary batches in 0.1N HCL

Time (h)	Drug Release %							
	H1	**H2**	**H3**	**P1**	**P2**	**P3**	**P4**	**P5**
0	0.000	0.000	0.00	0.00	0.00	0.00	0.00	0.00
1	21.76	13.46	01.32	23.12	16.64	08.97	07.54	05.06

2	44.54	23.12	11.65	32.63	22.87	16.86	14.63	18.47
3	**57.89**	39.54	19.87	43.78	39.56	23.83	21.36	27.90
4	65.43	42.45	24.75	**51.75**	45.21	35.19	33.69	33.17
5	78.63	**58.37**	31.21	63.71	**53.87**	41.09	42.14	35.68
6	89.12	67.84	37.43	72.05	63.97	**50.59**	47.96	44.01
7	95.27	75.28	41.65	79.24	73.56	59.93	**52.41**	**53.14**
8	99.12	82.45	49.08	87.41	81.43	68.48	61.37	58.45
9	99.13	85.63	**55.60**	94.83	89.54	78.12	68.57	61.45
10	99.19	89.91	57.98	98.67	94.67	82.63	79.63	62.98
11	99.20	92.59	59.12	99.32	99.67	96.94	89.31	75.32
12	99.20	97.61	61.78	99.32	99.68	99.72	95.78	79.92

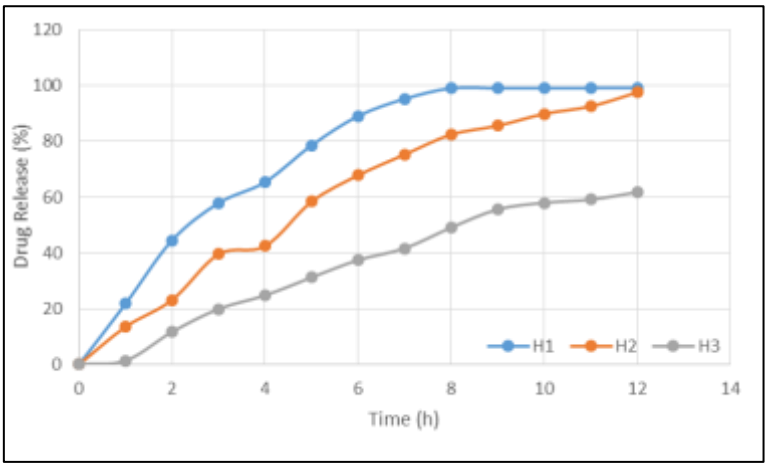

Figure 3.15: % Cumulative Drug release from preliminary Batches H1-H3 in 0.1N HCL

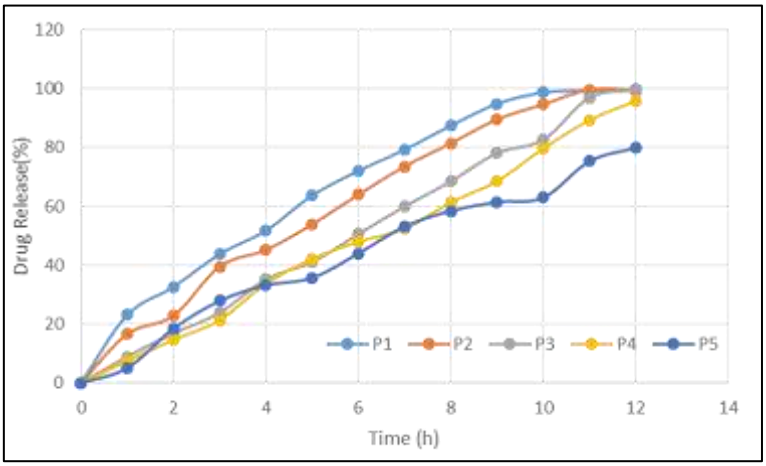

Figure 3.16: % Cumulative Drug release from preliminary Batches P1-P5 in 0.1N HCL

Figure 3.16 shows, Clarithromycin release from batches P1, P2, P3, P4 and P5 contained HPMC K100M alone in increasing concentration from 30, 40, 50, 60 and 70mg /tablet respectively. Preliminary Batches P1, P2, P3, P4 and P5 released its 50% drug content in 230 min., 282min., 355min., 409min. and 407min respectively. As the concentration of HPMC K100M increased the release rate decreased. From the results it was clear that optimized release was from batch P3 and P4 containing 50mg & 60 mg of HPMC K100M per tablet (25% w/w & 30% w/w per tablet). To evaluate the effect of concentration of HPMC K100M and citric acid on in vitro dissolution pattern of drug a statistical model of 3^2 full factorial designs was applied. Hence for further study 20, 30 and 40 % of the HPMC and Citric acid used in 5, 7.5 and 10% used in 3^2 Factorial design.

3.9 Preparation of Factorial Design Batches

3.9.1 Preparation of Granules

Preparation of Granules was done by Wet Granulation Technique using composition mention in Table 2.4.

3.9.2 Evaluation of Granules

The dried granules were evaluated for Angle of repose, Bulk Density, Tapped Density, Carr's index and Hausner's Ratio and the data is shown in Table 3.6.

Table 3.6: Data for Granules properties prepared for Factorial Design Batches

Batch	Angle of Repose	Tapped Density (g/ml)	Bulk Density (g/ml)	Carr's Index %	Hausner ratio
F1	32.80±0.11	0.878±0.05	0.754±0.07	15.09±0.06	0.858±0.05
F2	30.06±0.08	0.899±0.09	0.781±0.09	15.10±0.05	0.86±0.07
F3	31.33±0.16	0.930±0.11	0.784±0.09	15.68±0.09	0.843±0.05
F4	32.97±0.12	0.836±0.08	0.735±0.12	14.52±0.06	0.879±0.09
F5	30.68±0.09	0.891±0.09	0.764±0.14	16.62±0.13	0.857±0.06
F6	32.16±0.11	0.902±0.08	0.782±0.08	15.34±0.08	0.866±0.09
F7	31.83±0.12	0.883±0.13	0.767±0.09	15.12±0.11	0.868±0.07
F8	31.62±0.09	0.895±0.09	0.781±0.12	14.59±0.05	0.872±0.05
F9	30.85±0.13	0.910±0.11	0.792±0.15	14.89±0.05	0.8703±0.07

3.9.3 Preparation of Control Release Tablet (CRT)

Different control release tablet (CRT) formulations were prepared by wet granulation technique. All the batches of tablets were prepared using rotary punch tablet compression machine (Karnavati Rimek minipress II) using 12 mm size punch. Prepared tablets were evaluated for various tablet properties.

3.9.4 Evaluation of compressed tablets:

The Tablets from each batch of factorial design were evaluated for Uniformity in Average weight, Thickness, Hardness, Friability, Drug content and result are reported in Table 3.7.

1) Weight Variation Test

The results indicated was no weight variation as per I.P limit.The average weight of the tablet was found to be in range.

2) Tablet Hardness

The hardness of the tablets was found in the range of 5.2 to 5.8 kg/cm^2. The results indicated that the tablets having enough hardness and sufficient strength.

3) Friability

Percentage weight loss was measured and found to be less than 1%. As all the batches were within the pharmacopoeial limit (F< 1%).

4) Thickness

Size of tablets was found to be 12 mm in diameter and thickness of tablet was found to range from 3.8 to 4.9 mm.

5) Drug Content

All the formulations complied with the uniformity of drug content test for tablets. The drug content in all the batches of Clarithromycin floating tablets was in the range of 95 to 105%. This ensured good uniformity of the drug content in the tablets

Table 3.7: Data for Tablet properties from Factorial Batches.

Formulation	Average Weight in mg (n=5)	Hardness in Kg/cm^2 (n=2)	Thickness in mm (n=2)	Friability in %	Drug Content in % (n=3)
F1	800.03 ±0.64	5.5 ± 0.3	3.9 ± 0.07	0.28	103.03 ±0.31
F2	800.14 ±0.91	5.2 ± 0.6	3.8 ± 0.05	0.32	97.86 ±0.70

F3	800.06 ±1.02	5.5 ± 0.2	3.9 ± 0.11	0.23	96.27 ±1.02
F4	850.52 ±0.83	5.8 ± 0.2	4.5 ± 0.08	0.22	99.61 ±0.73
F5	850.05 ±0.61	5.5 ± 0.4	4.4 ± 0.27	0.33	98.83 ±0.41
F6	850.12 ±0.90	5.8 ± 0.3	4.4 ± 0.13	0.31	104.83 ±1.13
F7	900.05 ±1.24	5.2 ± 0.2	4.9 ± 0.15	0.29	99.94 ±0.42
F8	900.79 ±1.61	5.5 ± 0.2	4.8 ± 0.09	0.33	102.02 ±1.1
F9	900.02 ±1.02	5.8 ± 0.4	4.9 ± 0.07	0.28	99.57 ±0.7

6) In-Vitro Drug release for Factorial batches F1-F9

The matrix tablets displayed a controlled drug release that depended on the total polymer level and citric acid level as well as presence of the drug either in the free or the complexes form. The actual values of % cumulative Drug release of factorial batches F1- F9 are reported in Table 3.8 and Drug release profile of factorial batches F1- F9 are shown in Figure 3.17, 3.18 and 3.19. The values of the release at of T_{50}, T_{85} and floating lag time are shown in Table 3.9. At lower concentration of polymer % release was more. As concentration of polymer increases the release rate was retarded. The drug release at the end of 12h from the matrix tablets containing Clarithromycin was found to range from 68.15 ± 1.56 to 98.90 ± 1.09 %.

Table 3.8: Dissolution data for Factorial Batches F1-F9 in 0.1N HCL

Time (h)	**% Drug Release (n=3)**								
	F1	**F2**	**F3**	**F4**	**F5**	**F6**	**F7**	**F8**	**F9**
0	0	0	0	0	0	0	0	0	0
1	15.73± 1.47	18.68± 1.21	21.12± 0.52	8.93± 1.25	9.85± 2.09	11.25± 0.37	4.32± 1.23	3.95± 1.05	4.06± 2.09
2	22.15± 1.21	29.48± 1.93	31.24± 1.45	17.84± 1.82	11.52± 1.27	18.03± 1.29	12.49± 1.82	16.74± 1.25	17.47± 2.64
3	34.12± 2.67	41.34± 1.37	43.49± 1.96	23.71± 1.57	29.45± 1.23	26.87± 1.42	17.89± 1.62	23.51± 2.34	26.9± 2.09
4	43.47± 1.82	**54.93**± 2.35	**57.85**± 2.31	31.24± 2.09	33.81± 1.85	37.09± 1.65	27.84± 1.07	29.21± 1.21	32.17± 1.84
5	**55.42**± 0.89	58.71± 1.83	62.37± 1.21	39.71± 2.26	42.9± 1.07	41.87± 1.97	34.16± 2.48	30.42± 2.14	35.68± 1.96
6	61.53± 2.41	67.43± 1.02	71.81± 2.94	43.88± 2.67	49.85± 1.63	**51.74**± 1.21	38.12± 2.09	35.2± 1.34	44.03± 1.41
7	69.85± 1.79	73.84± 2.19	79.8± 1.57	**52.79**± 1.21	**54.61**± 1.83	56.41± 1.32	48.02± 2.15	39.41± 1.82	**52.14**± 1.82
8	77.6± 1.25	79.3± 1.07	**87.92**± 1.89	61.82± 1.05	63.89± 2.09	66.83± 1.82	**54.56**± 1.54	48.19± 1.52	59.45± 1.09
9	**85.87**± 2.09	**87.85**± 2.26	89.8± 1.62	69.84± 0.59	71.33± 1.78	78.41± 2.58	62.14± 1.71	**51.54**± 1.45	62.64± 1.07
10	91.93± 1.19	92.73± 2.09	94.37± 1.97	78.73± 0.54	79.84± 1.27	81.3± 1.67	63.48± 1.21	57.58± 2.09	63.78± 1.48
11	97.3± 2.36	97.8± 1.27	98.3± 1.58	**84.67**± 1.93	**87.02**± 1.82	**89.56**± 2.09	67.73± 2.69	63.58± 1.26	74.62± 1.21
12	98.41± 1.63	98.81± 2.51	98.9± 1.09	94.79± 1.37	95.82± 2.48	95.87± 2.50	68.15± 1.56	72.8± 1.17	79.5± 1.86

The Factorial batches F1, F2 & F3 which had lower total polymer level, were found to release 98.41 ± 1.63 %, 98.81±2.51% and 98.9±1.09 of the drug by the end of 12 h respectively which is shown in Figure 3.17. The Factorial batches F4, F5 & F6 which had medium level of polymer exhibited better drug release as they released 94.79 ± 1.37% ,95.82 ± 2.48 % & 95.87 ± 2.50 % respectively of the drug at the end of 12 h of dissolution , which is shown in Figure 3.18.

The Factorial batches F7, F8 & F9 which had higher polymer level, exhibited an impeded drug release as they released 68.15 ± 1.56 %, 72.8 ± 1.17 % & 79.5± 1.86 % respectively of the drug at the end of 12 h of dissolution which is shown in Figure 3.19. An increase in the polymer i.e. HPMC K100M concentration caused the increase in viscosity of diffusion layer and also the formation of gel layer serve as longer diffusional path for drug this might had decreased the effective diffusion coefficient of drug and therefore there was reduction in drug release rate.

Formulation F4, F5 & F6 containing medium polymer level exhibit better drug release in 12 h. So by considering release profile from all factorial batches batch F5 which containing 30% of HPMC K100M and7.5% citric acid. Formulation F4 & F6 also release nearly same but medium level concentration of citric acid containing F5 was selected. These formulations contain drug in the complexes form exhibited a controlled and complete drug release during the dissolution period due to improved drug solubility.

The 3^2 factorial designs, preliminary trials were carried out to obtain the optimized concentration of polymer. The second variable citric acid was chosen because of its significant effect on the FLT and the drug release profile. All the nine batches showed variable release profile. The polymer concentration being constant and an increase in the concentration of citric acid the dissolution profile was improved significantly. The 3^2 full factorial design was selected to study the effect of independent variables HPMC K100M (X1) and Citric Acid (X2) on dependent variables t50%, t85% and floating lag time (Figure 3.20).

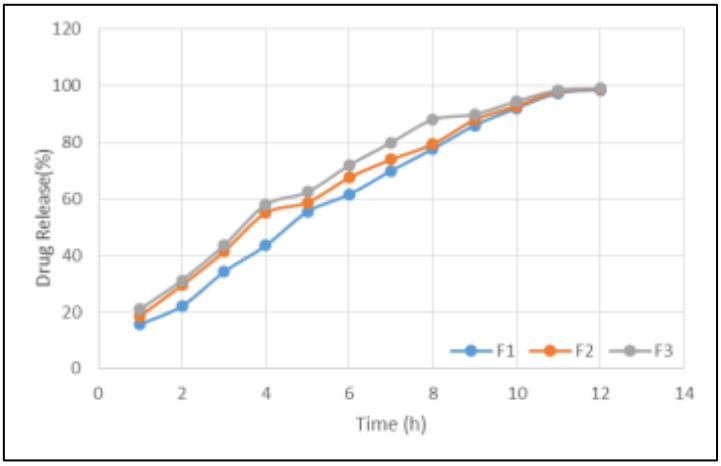

Figure 3.17: % Cumulative Drug release from factorial batches F1-F3

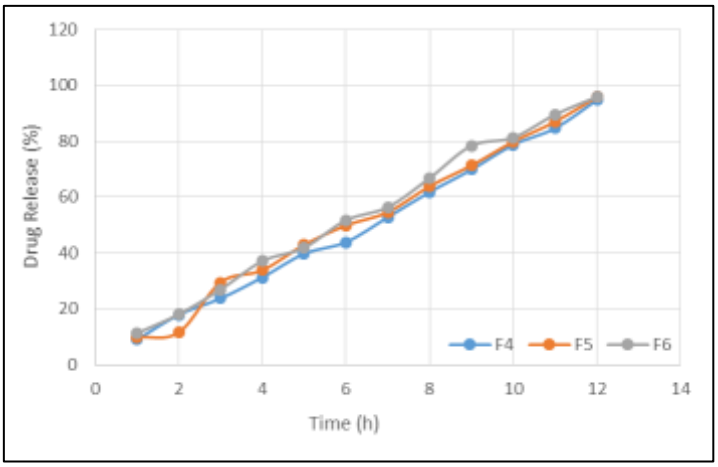

Figure 3.18: % Cumulative Drug release from factorial batches F4-F6

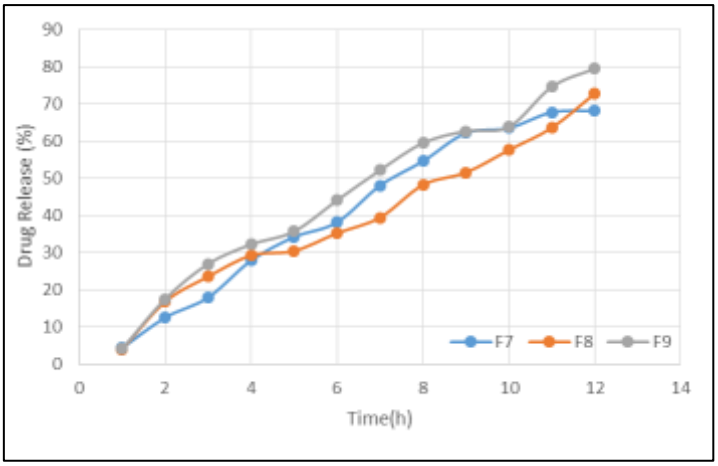

Figure 3.19: % Cumulative Drug release from factorial batches F7-F9

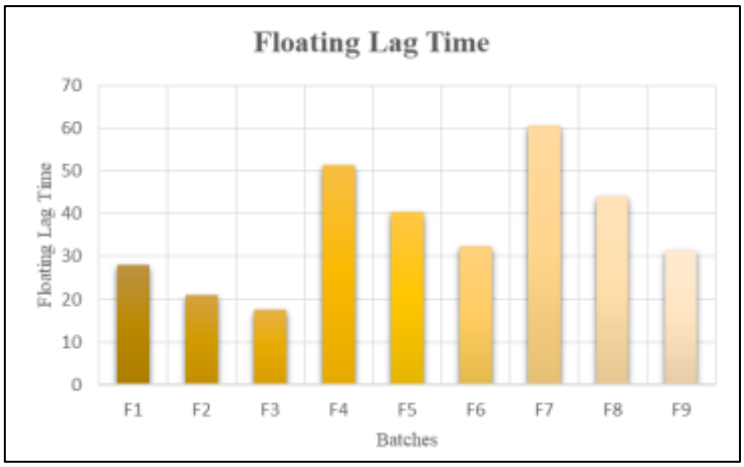

Figure 3.20: Floating Lag Time of Factorial Batches

Table 3.9: Data for Response parameter of Tablet

Batch Code	Clarithromycin release at 12 h (%)	$T_{50\%}$ (minutes)	$T_{85\%}$ (minutes)	Floating Lag Time (seconds)	Tablet Integrity
F1	98.41±1.63	281.4±1.04	539.5±1.23	28±1	+
F2	98.81±2.51	223.42±1.21	518.41±0.57	21±1	+
F3	98.9±1.09	208.14±1.43	403.83±1.09	17.66±1.52	+
F4	94.79±1.37	394.9±1.62	671.47±1.32	51.33±1.52	+
F5	95.82±2.48	381.2±0.79	648.13±1.54	40.33±2.08	+
F6	95.87±2.5	365.4±1.97	613.40±1.93	32.33±1.53	+
F7	68.15±1.56	444.0±1.34	1008.8±1.46	60.66±1.52	+
F8	72.8±1.17	414.6±1.51	889.5±1.75	44±1	+
F9	79.5±1.86	391.7±1.47	859.9±1.39	31.33±1.52	+
*The values represent the average of three determinations (n=3) + = Good Integrity for 12 h, - =No Integrity.					

3.10 Curve fitting (Release mechanism)

The response Parameter and curve-fitting data of matrix tablet prepared as per 3^2 Factorial designs are summarized in Table 3.9 and 3.10 respectively, indicated that the possible mechanism of drug release. As most of the batches produced yielded quality adjustment with the Hixon Crowell (average R2=0.9832). However, the best fit model was found to be the Zero order (average R^2 =0.9942) suggesting that the mechanism of drug release was

combination of diffusion and erosion. Different values for diffusion exponent n in equation represent different drug release mechanisms. When the n value is around 0.45, the Fickian diffusion phenomenon dominates, and when n ranges between 0.45 and 0.89 it is anomalous or non-Fickian release that is, the drug release proceeded by diffusion as well as erosion of the polymer. When the n value exceeds 0.89, the release can be characterized by case II and super case II, which illustrate a zero-order release. The values of the diffusion exponent, as shown in Table 3.10 were found to range from 0.77- 1.0239. Formulations F1, F2 and F3 showed non Fickian type drug release as values of 'n' that is diffusional exponent is lies between 0.45 to o.89 remaining formulations (F4 – F9) shows Class 2 drug release as the value of 'n' is greater than 0.89. The hydrophilic matrix tablets exhibited R^2= (0.9942) when analyzed using the Zero-order equation, suggesting that the drug release from most of the batches followed zero-order kinetics.

Table 3.10: Data for study of release mechanism by curve fitting analysis.

Batch code	Zero order			Hixon Crowell		
	K	R^2	n	K	R^2	n
F1	0.64±0.017	0.9946±0.003	o.77±0.01	0.62± 0.033	0.9858±0.005	0.65±0.02
F2	0.65±0.023	0.9933±0.004	0.78±0.01	0.60 ±0.034	0.9887±0.006	0.68±0.02
F3	0.64±0.013	0.9907±0.002	0.78±0.02	0.64±0.024	0.9953±0.004	0.69±0.01
F4	0.15±0.017	0.9983±0.005	0.97±0.03	0.12± 0.017	0.9610±0.008	0.94±0.03
F5	0.16±0.021	0.9971±0.006	0.97±0.02	0.26± 0.021	0.9768±0.007	0.91±0.02
F6	0.14±0.025	0.9964±0.004	0.96±0.02	0.13± 0.034	0.9648±0.003	0.93±0.01
F7	0.11±0.014	0.9940±0.05	1.02±0.03	0.59± 0.016	0.9843±0.008	1.09±0.03
F8	0.10±0.016	0.9910±0.004	1.01±0.02	0.37± 0.021	0.9900±0.007	1.03±0.02
F9	0.11±0.015	0.9927±0.002	1.07±0.02	0.54± 0.021	0.9776±0.005	1.02±0.01

3.11 Optimization of Factorial Design Batches

3.11.1 Regression analysis

1) Effect of formulation variables on $T_{50\%}$ Clarithromycin release

The Quadratic model for **$T_{50\%}$ (Y1)** was found to be significant with an F value 361.54 (P<0.0001). In this case X_1, X_2, X_2^2 was found to be significant and the model describes the $T_{50\%}$ release. The factorial equation for $T_{50\%}$ (Y_1) can be written as:

$$T_{50\%} = + 375.40 - 25.50\ A + 88.78\ B + 6.06\ A\ B + 7.58\ A^2 - 52.61 B^2 \ldots\ldots\ldots (3.1)$$

As the concentration of HPMC K100M increased it causes an increase in viscosity of swollen gel matrix, which contributes more hindrance for drug diffusion and thus decreases the release rate whereas Citric acid increase the solubilization increase the release rate. The combined effect of X_1 & X_2 shown in response surface plot Figure 3.22 While the increasing amount of HPMC K100M causes the decreases in the drug release, due to formation of high viscous gel matrix. HPMC K100M is swellable polymer which causes a gel layer. The Figure 3.21 shows a graph of observed verses predicted values. The HPMC K100M (X_1) have negative effect on Y1 & Citric acid (X_2) have positive effect on Y_1, means if we increasing the concentration of X1 $T_{50\%}$ decreases & increase in X2 the $T_{50\%}$ increases due to increased solubilization of drug.

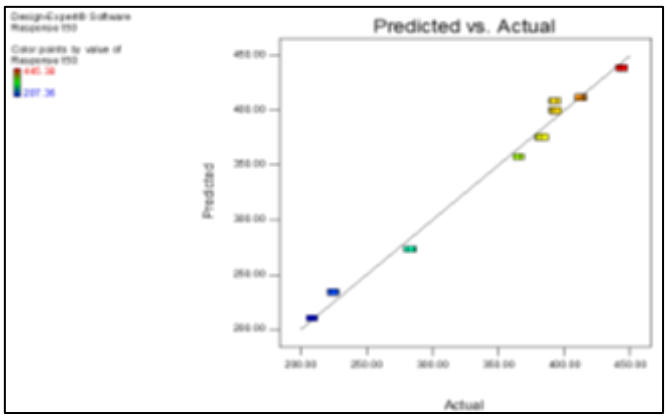

Figure 3.21: Correlation between actual and predicted values for $T_{50\%}$ (Y_1)

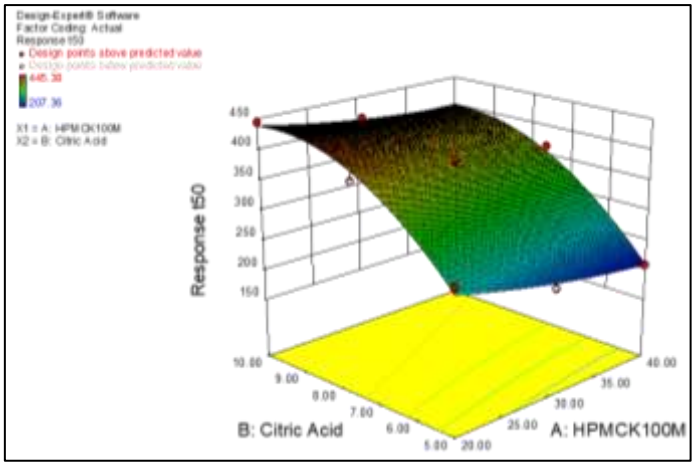

Figure 3.22: Response surface plot showing effect of formulation variables on $T_{50\%}$ (Y_1)

2) Effect of formulation variables on $T_{85\%}$ (Y_2)

The Quadratic model terms for response Y_2 ($T_{85\%}$) were found to be significant with F value of 229.56 ($p<0.0001$). In this case all the factors except $X_1.X_2$ and X_1^2were found to be significant and the factorial equation for response Y_2 ($T_{85\%}$) can be written as:

$$T_{85}= +645.90 -56.81\ A +214.75\ B -3.64\ A\ B -1.16\ A^2 +56.47\ B^2 \ldots\ldots\ldots\ldots (3.2)$$

As the amount of X_1 increases the corresponding $T_{85\%}$ (time required to release 85% of the drug) also increases. The Figure 3.24 shows the response surface plot. It indicates at all the high levels of X_1 the $T_{85\%}$ value is high. As discussed above this behavior is due to increase in amount of HPMC K100M forms a high viscous gel matrix and thus decreases the drug release and hence $T_{85\%}$ value increases. Whereas X_2 increases the release rate also increases. The Figure 3.23 shows the graph of predicted verses actual data. The HPMC K100M (X_1) has positive effect on $T_{85\%}$ Y_2 and Citric acid (X_2) has negative effect on Y_2 means if we increasing the concentration of X_1 then Y_2 of the drug also increases due to increased viscosity and gel strength and increase in X_2 then decrease in Y_2 means decrease in time require for release.

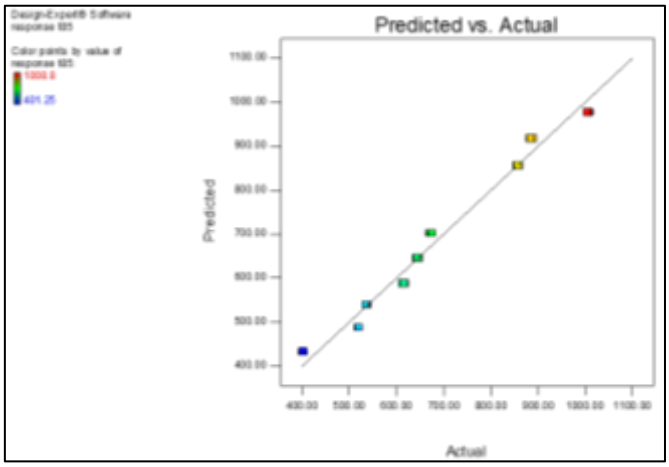

Figure 3.23: Correlation between actual and predicted values for $T_{85\%}$ (Y_2)

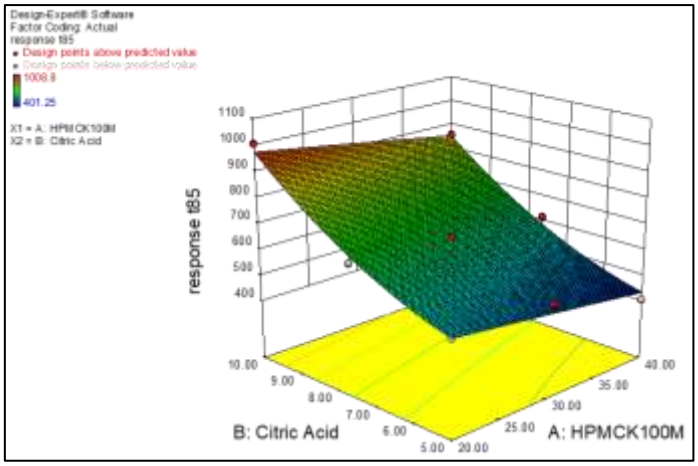

Figure 3.24: Response surface plot showing effect of formulation variables on $T_{85\%}$ (Y_2)

3) Effect of formulation variables on Floating Lag Time (FLT, Y3)

The Quadratic model terms for response Y_3 (FLT) were found to be significant with F value of 229.56 ($p<0.0001$). In this case all the factors except X_1^2 were found to be significant and the factorial equation for response Y_3 (FLT) can be written as:

$$\mathbf{FLT = +40.15 - 9.78\ A + 11.56\ B - 4.75\ A\ B + 1.78\ A^2 - 7.56\ B^2 \ldots\ldots\ldots} (3.3)$$

As the amount of X_1 increases the corresponding FLT (time required to float the tablet) also increases. The Figure 3.26 shows the response surface plot. It indicates at all the high levels of X_1 the FLT value is high. On the contrary X_2 increases the FLT decreases respectively. The Figure 3.25 shows the graph of predicted verses actual data. The HPMC K100M (X_1) has negative effect on Y_3 and Citric acid (X_2) has positive effect on Y_3 means if we increasing the concentration of X_1 then Y_3 of the drug also increases due to increased viscosity and gel strength and increase in X_2 then decrease in Y_3 means decrease in time require for float.

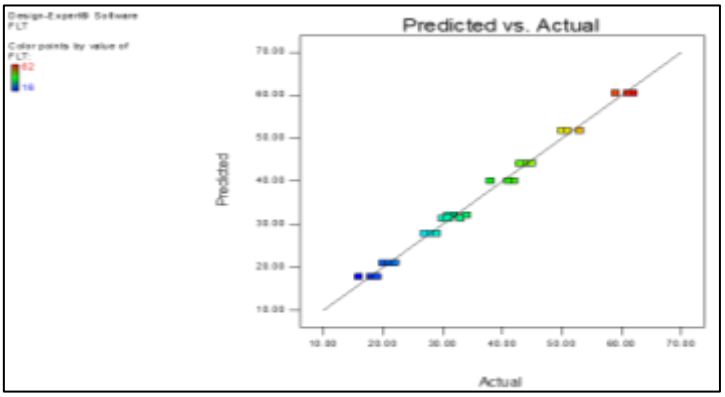

Figure 3.25: Correlation between actual and predicted values for FLT (Y_3)

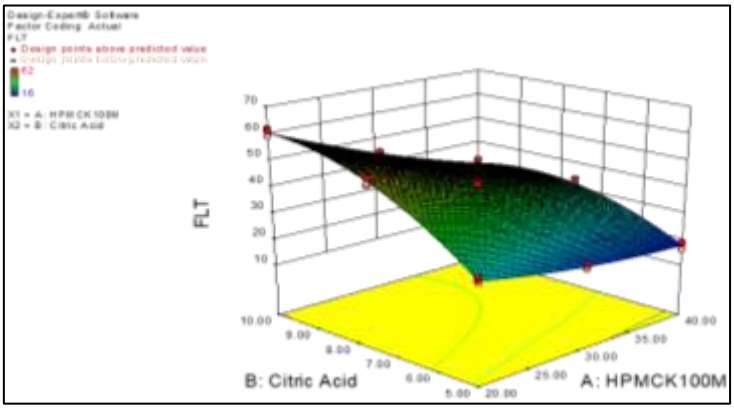

Figure 3.26: Response surface plot showing effect of formulation variables on FLT (Y_3)

4) ANOVA, Pure error, Lack of fit

The results of ANOVA for dependent variables from 3^2 factorial designs shown in Table 3.11 demonstrate that the model was significant for all response variables. Regression analysis was carried out to obtain the regression coefficient shown in Table 3.11 and effects as follows; all factors other than $X_1.X_2$ and X_1^2 found significant for response Y_1 and Y_2 whereas for response Y3 except X_1^2 all other factors found significant. The above results conveyed us that the amount of HPMC K100M & Citric acid plays important role in formulation of Oral Controlled Release matrix tablets of Clarithromycin. The data of pure error and lack of fit are summarized in Table 3.12. The residuals are the difference in the observed and predicted value. Since computed F values were respectively less than critical F values, denotes non-significance of lack of fit.

Table 3.11: Data of ANOVA study for dependent variables from 3^2 factorial designs

Source	d.f.	Sum square	Mean square	F value	Probability
Response (Y_1) = $T_{50\%}$ (h)					
X_1	1	11705.52	11705.52	127.77	< 0.0001*
X_2	1	1.419E+005	1.419E+005	1500.03	< 0.0001*
X_1X_2	1	440.08	440.08	4.65	0.0427
X_1^2	1	345.14	345.14	3.65	0.0698
X_2^2	1	16607.22	16607.22	175.60	< 0.0001*
Response (Y_2) = $T_{85\%}$ (h)					
X_1	1	58084.82	58084.82	73.47	< 0.0001*
X_2	1	8.301E+005	8.301E+005	1049.91	< 0.0001*
X_1X_2	1	158.92	158.92	0.20	0.6585
X_1^2	1	8.13	8.13	0.010	0.9202
X_2^2	1	19130.53	19130.53	24.20	< 0.0001*
Response (Y3) =FLT(Sec)					
X_1	1	1720.89	1720.89	927.73	< 0.0001*
X_2	1	2403.56	2403.56	1295.76	< 0.0001*
X_1X_2	1	270.75	270.75	145.96	< 0.0001*
X_1^2	1	18.96	18.96	10.22	0.0043
X_2^2	1	342.52	342.52	184.65	< 0.0001*

* -Indicates significant

Table 3.12: Data of ANOVA study for results in analysing lack of fit and pure error

For T_{50}						
Source	**Sum of Squares**	**Degrees of Freedom**	**Mean Square**	**F Value**	**P Value**	**Model Significant/ Non-significant Relative to Noise**
Model	1.710E+05	5	34191.91	361.54	0.0001	Significant
Residual	1986.02	21	94.57	-	-	-
Core Total	1.729E+05	26	-	-	-	-
Lack of fit	1931.25	3	643.75	211.56	0.0001	Significant
Pure Error	54.77	18	3.04	-	-	-
For T_{85}						
Model	9.075E+05	5	1.815E+05	229.56	0.0001	Significant
Residual	16603.09	21	790.62	-	-	-
Core Total	9.241E+05	26	-	-	-	-
Lack of fit	16495.40	3	5498.47	919.08	0.0001	Significant
Pure Error	107.69	18	5.98	-	-	-
For FLT						
Model	4756.68	5	951.34	512.87	0.0001	Significant
Residual	38.95	21	1.85	-	-	-

Core Total	4795.63	26	-	-	-	-
Lack of fit	0.95	3	0.32	0.15	0.9280	Not Significant
Pure Error	38.0	18	2.11	-	-	-

3.12 Studies on Final Formulation

1) Water Uptake Study

The water uptake was determined of F5 batch. It was observed that Water uptake was increase with respect to time. Data for water uptake study is given in the Table 3.13.

Table 3.13: Water Uptake Study of F5 Batch

Time (h)	Water Uptake (%)
1	133.8
2	136.5
3	137.4
4	139.2
5	140.1
6	142.2
7	143.3
8	143.8
9	144.8
10	145.1
11	146.3
12	147.9

2) In-Vitro Drug release for Marketed Tablet, Complex and F5

In vitro release profile of optimized formulation F5 compared with marketed SR tablet (Biaxin-500) and complex. The time for drug release t50% of F5, Biaxin and complex were found to 381.2, 315.36 and 354 minutes respectively. The percentage drug release after 12 hour for F5, Biaxin and complex were found to 95.82, 86.32 and 87.62 respectively shown in Table 3.14, so the release from the optimized formulation and complex were higher compared to marketed product. Release of drug from complex was approximately same so it was concluded that without any polymer drug release is controlled which is shown in Figure 3.27.

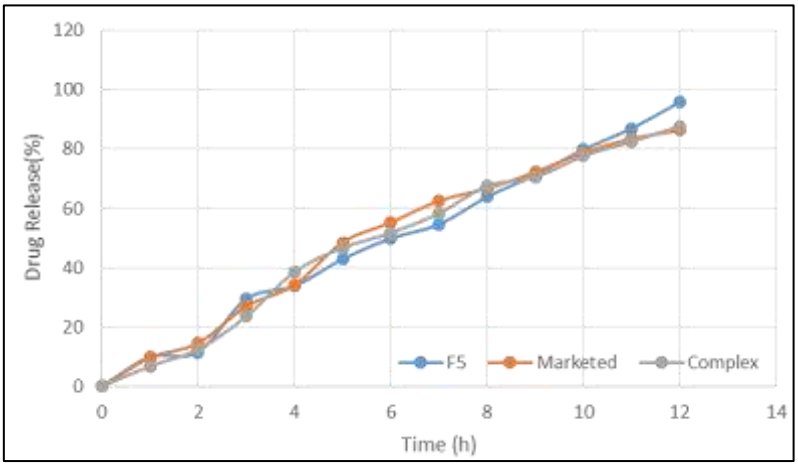

Figure 3.27: Dissolution Profile of marketed formulation with capsule fills with complex and formulated tablet in 0.1N HCL.

Table 3.14: Dissolution data of Marketed Tablet (Biaxin), Complex and F5 in 0.1N HCL

Time (h)	% Drug Release		
	F5	**MARKETED TAB.**	**COMPLEX**
0	0.00±0.00	0.00±0.00	0.00±0.00
1	09.85±0. 92	09.59±0.36	03.76±0.65
2	11.52±1.32	14.58±0.63	12.45±1.28
3	29.45±2.03	26.94±1.89	23.56±2.43
4	33.81±2.32	34.18±2.92	38.54±1.45
5	42.90±1.45	48.51±1.61	46.68±1.87
6	49.85±1.93	**55.26±0.85**	**51.76±0.69**
7	**54.61±1.23**	62.52±1.44	58.32±0.78
8	63.89±2.04	66.74±0.31	67.54±2.58
9	71.33±0.78	72.23±2.45	70.56±2.65
10	79.84±1.12	78.83±2.68	77.65±1.95
11	**87.02±1.67**	**83.45±1.56**	**82.56±2.08**
12	95.82±2.04	86.32±1.30	87.65±1.71

n=2 (±SD)

3) Optimization

A numerical optimization technique by the desirability approach was used to generate the optimum settings for formulation. The process was optimized for dependent variables Y_1-Y_3. The optimized formula arrived by targeting the Y_2 at 650 minute, Y_1 was kept at range 360-400 min. ,Y_3 also kept at range 16-62 sec. The optimized results obtained to give 13 results out of that one formula is shown in Table 3.14. The results of optimized formula were compared with the predicted values (Table 3.16), which showed good relationship between experimented and predicted values, which confirms the practicability and validity of the model. The value of n was found to be 0.991.

Table 3.15: Composition of optimized formulation

Ingredients	Quantities (mg)
Complex	500
HPMC K100M	163.5
Citric Acid	38.6
$NaHCO_3$	80
PVP K30	60
Mg stearate	5
Lactose	12.9
Total weight	860

Table 3.16: Comparison between the experimented and predicted Values for most probable optimal formulation

Dependent variables	Optimized formulation	
	Experimented value	**Predicted value**
$T_{50\%}(Y_1)$	381.2	376.71
$T_{85\%}$ (Y_2)	648.13	649.99
FLT	40.3	38.49

CONCLUSION

The Clarithromycin is poorly water soluble drug and gastric irritant. To overcome these problems attempt was made in present study to form inclusion complex of Clarithromycin with Nanosponges. β-Cyclodextrin (CD) based Nanosponges (NS) are novel class of cross-linked derivatives of Cyclodextrin. The Nanosponges were synthesized by carbonylation of β -Cyclodextrin to exploit its porous structure for drug entrapment. After synthesis of Nanosponges, Drug Clarithromycin was entrapped in it. The Charateristics of Complex was studied by FTIR, DSC, PXRD and SEM. The result of XRPD results showed that the crystallinity of CLA was decreased after loading into Nanosponges. Histopathological study was carried out and it revealed non irritancy of drug-NS complex to gastric mucosa (of rat). Hence drug-NS complex found to be suitable for designing into unit dosage forms. Preliminary Batch were prepared and Evaluated (*in vitro* dissolution) for selection of Polymer. HPMC K100M showed highest release retarding property so it was selected as the polymer for further study. The 3^2 full factorial experimental design was applied and 9 Factorial Design Batches were obtained. Granules were prepared using Wet granulation method and evaluated for their properties. All the batches of tablets were prepared using rotary punch tablet compression machine using 12 mm size punch. Prepared tablets of Batch F1-F9 were evaluated for various tablet properties. Regression analysis was carried out and F5 Batch was found to be optimized Batch. F5 batch showed 95.82±2.48 % Drug Release in 12 hours further it was evaluated for water uptake and compared with the Marketed formulation for % Drug Release.

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CONFLICT OF INTEREST

All authors declared no conflicts of interest.

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Formulation and evaluation mouth dissolving tablets of solid dispersion of fenofibrate

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ABSTRACT

Fenofibrate is a drug of the fibrate class. It is a widely used hypolipidemic drug. The poor aqueous solubility of the drug leads to variable dissolution rates. It is slightly soluble in water. The present investigation was to develop and characterize mouth dissolving tablets of fenofibrate using solid dispersion technique. Mouth dissolving tablets of solid dispersion of Fenofibrate were prepared using different superdisintegrating agents like Cross Carmellose Sodium, Sodium Starch Glycolate and Cross povidone in different concentrations using direct compression method.

The formulation of solid dispersion prepared by solvent evaporation technique by using polymers like PEG 4000 and PEG 6000 respectively in various ratios such as Fenofibrate and PEG 4000 (1:1, 1:2, 1:3, 1:4, 1:5); Fenofibrate and PEG 6000 (1:1, 1:2, 1:3, 1:4, 1:5). Solid dispersion prepared by using PEG 6000 improved solubility & dissolution rate of Fenofibrate as compared to pure drug. Hence F10 formulation of PEG 6000 is selected for further formulation of mouth dissolving tablets. Then, nine batches of mouth dissolving tablets of optimized solid dispersion of fenofibrate are prepared with different concentrations of superdisintegrants of cross carmellose, sodium starch glycolate, cross povidone. The wetting time was observed to be very fast with batch F9 tablets which contain cross povidone. The total drug from the optimized batch was found to be released within the first ten minutes of dissolution study. These tablets rapidly dissolved (within 60-70 sec) in saliva. The prepared tablet gives benefit in terms of patient compliance, low dosing, rapid onset of action, increased bio-availability, low side effect and good stability which make these tablets popular as a dosage form for the treatment of hyperlipidemia.

KEYWORDS

Mouth dissolving tablet, direct compression, Fenofibrate, super disintegrants, cross povidone, wetting time.

INTRODUCTION

The oral route remains the favored route for administration of therapeutic agents thanks to accurate dosage, low cost therapy, self medication, non invasive method and straightforward administration leading to high level of patient compliance. MDTs are known by various names such as "fast-melting, fast-dissolving, oral disintegrating or orodisperse". The European Pharmacopoeia defines the term "orodisperse" as a tablet that can be placed in the mouth where it disperses rapidly before swallowing. Suitable drug candidates for such systems include neuroleptics, cardiovascular agents, analgesics, antiallergics and drugs for erectile dysfunction. In the present study, an attempt was made to develop mouth dissolving tablets of fenofibrate and to investigate the effect of various superdisintegrants on the disintegration time, wetting time and release profile of the drug in the tablets.[4,5]

Mouth dissolving tablets are dosage form, which disintegrate in patient's mouth within a few seconds without the need of water, or chewing, providing best remedy for the patient suffering from dysphasia. Some drugs are absorbed from the mouth, pharynx and esophagus as the saliva passes down the stomach. In such cases the bioavailability is greater than those observed for conventional dosage form. The advantages of mouth dissolving dosage form are increasingly being recognized in both industry and academia. The basic approach used in the development of the mouth dissolving tablet is the use of superdisintegrants. Croscarmellose sodium, sodium starch glycolate, and cross povidone were screened in the present study, and the best one was used for further studies.

Fenofibrate (FFA) (isopropyl ester of 2-[4-(4- chlorobenzoyl) phenoxy]-2-methylpropanoic acid) is a widely used hypolipidemic drug available as tablets for oral administration. Each tablet contains 50 mg fenofibrate. The empirical formula is C20H21O4Cl (figure 1) and the molecular weight is 360.83; fenofibrate is insoluble in water. The melting point is 79° to 82°C.Fenofibrate is a white solid which is stable under ordinary conditions. Its pharmacological activity consists in reducing triglyceride and cholesterol concentration in plasma. Solubility and permeability are the fundamental parameters controlling the rate and extent of drug absorption. According to the Biopharmaceutics Classification System (BCS), FFA is a Class II having low solubility and high permeability. Bioavailability of FFA solely depends on dissolution rate in the gastrointestinal tract. This drug is used mostly in lipid regulation as it decreases low-density lipoprotein (LDL) and very-low density lipoprotein (VLDL) levels, and increases high density lipoprotein (HDL) level.[7-10]

MATERIALS AND METHODS

MATERIALS

Fenofibrate was supplied as a gift sample from Mylan Laboratories Limited, Sinnar,India.PEG 6000, Cross Carmellose, Sodium Starch Glycolate, Cross Povidone, Mannitol, Saccharin Sodium, Talc, Magnesium Stearate, Vaniline Research lab fine chemical Industry, Mumbai, India. All other chemicals, solvents and reagents were used of either pharmacopoeial or analytical grade.

METHODS

Preformulation Studies of Fenofibrate

Identification and Characterization of Fenofibrate

1. Organoleptic Evaluation

The drug sample as evaluated for its physical properties such as colour, odour, taste, appearance.

2. Melting point

The melting point of drug can be determined by introducing a tiny amount into a small capillary tube, attaching this to the stem of a thermometer centred in a heating bath, heating the bath slowly, and observing the temperature at which drug melted was recorded and compared with the standard.[1-3]

3. Solubility profile

Solubility determination can be done by ultraviolet absorption, nephlometry, Nuclear magnetic resonance and Potentiometric in drug discovery. A drug is considered highly soluble when the highest dose strength is soluble in 250 mL or less of aqueous media over the pH range of 1 to 7.5.[5,7]

4. Loss on drying

Loss on Drying is a back-weighing application used to determine the amount of volatile matter present in tablets, capsules or bulky material. Samples are weighed before and after treatment, and the weight difference is measured. It mainly depends on API and it varies from ingredient to ingredient. But it is preferred to be < 1%. [12,15]

$$\% \text{ LOD} = \frac{\text{Weight of sample before dry} - \text{weight of sample after dry}}{\text{Weight of sample before dry}} \times 100$$

UV Spectroscopic analysis of fenofibrate

Scanning of Fenofibrate in Phosphate Buffer p 6.8 solution

The standard solution (10µg/mL) was scanned from 200-400 nm on UV spectrophotometer (Shimadzu UV–1800). The absorption maxima were found to be at 280 nm. In phosphate buffer Ph 6.8.[13,14]

Preparation of phosphate buffer pH 6.8

The phosphate buffer pH 6.8 was prepared by mixing the 28.20 gm of disodium hydrogen phosphate and 11.45 gm of potassium dihydrogen in sufficient distilled water to produce 1000ml. [9,13]

Procedure

An accurately weighed quantity of fenofibrate was transferred into a 50 mL volumetric flask, diluted up to the mark with phosphate buffer pH 6.8 to get a standard stock solution of 0.5 mg/mL. Aliquot portions of standard stock solution was appropriately diluted to get the concentration of 10µg/mL and scanned in the range 400-200 nm. The zero order spectrum and its first order derivative spectrum were recorded. The calibration curve is shown in figure (10.2) and absorbances of different concentration of fenofibrate are reported in table (10.4)[18-21]

Drug Excipient Compatibility Studies

FTIR Spectroscopy of Fenofibrate, Fenofirate and PEG 6000

FTIR in drug analysis makes an important contribution to the fight against drug-related deaths. Special testing centers, offer drug addicts the possibility to have their drugs checked by FTIR, minimizing the drugs potential damage and preventing overdosing. Identification of Unknown Illegal Substances by FT-IR Spectroscopy. Infrared spectroscopy quickly and reliably identifies legal and illegal substances in-house or on the road. It is ideal for law enforcement, security and safety organizations to save time and money by conducting their own analysis.[22,24]

Formulation and developement

Preparation of solid Dispersion

Solid dispersion of drug and polymer was prepared by using solvent evaporation method with the help of PEG 6000 & PEG 4000 in various ratios.[24]

Solvent Evaporation Method

Table 1. Formulation of drug and polymer

In this method, preparation of solid dispersions fenofibrate with all carriers took place. The ratio of drug and carriers were1: 1, 1: 2, 1: 3, 1:4 and 1:5. The drug and the carriers were dissolved in methanol. The solution was stirred for 1 hour on magnetic stirr. The solvent was evaporated at 40°C in vacuum dryer. After solvent removal, the solid dispersion products were kept in desiccators for 48 hours. The product samples were pulverized using a glass mortar and pestle, sieved through 120 meshes and kept the powder of solid dispersion in desiccators throughout the experimental period. The ratio of drug and carriers were shown in table No.1.[17-20]

Formulation /Batches	Composition	Method	Ratio
F1	Fenofibrate + PEG 4000	Solvent Evaporation method	1:1
F2	Fenofibrate + PEG 4000	Solvent Evaporation method	1:2
F3	Fenofibrate + PEG 4000	Solvent Evaporation method	1:3
F4	Fenofibrate + PEG 4000	Solvent Evaporation method	1:4
F5	Fenofibrate + PEG 4000	Solvent Evaporation method	1:5
F6	Fenofibrate + PEG 6000	Solvent Evaporation method	1:1
F7	Fenofibrate + PEG 6000	Solvent Evaporation method	1:2
F8	Fenofibrate + PEG 6000	Solvent Evaporation method	1:3
F9	Fenofibrate + PEG 6000	Solvent Evaporation method	1:4
F10	Fenofibrate + PEG 6000	Solvent Evaporation method	1:5

Comparative Solubility and Dissolution study to select the optimum batch of solid dispersion

1. Solubility study of solid dispersion and fenofibrate

Solubility measurement of fenofibrate were performed according to published method. The amount of solid dispersion powder containing 2.5 mg equivalents fenofibrate was weighed accurately in volumetric flask was dissolved 5ml distilled water by sonication for 15min, the solutions were filtered through a whatman filter pper no.1. Filtered solution was diluted properly with distilled water. The diluted solution was analysed for fenofibrate in UV at 375 nm. [25]

2. In vitro Dissolution study of Pure Drug and Solid Dispersion

- **Dissolution study of Pure Drug**

The release rate of fenofibrate from fast dissolving tablets was determined using USP Dissolution Testing Apparatus II (Paddle type). The dissolution test was performed using 900 ml of 0.1 M SLS, at 37 ± 0.50C and 50 rpm. A sample (5 ml) of the solution was withdrawn from the dissolution apparatus every 2 min. for 30 min, and the samples were replaced with fresh dissolution medium. The samples were filtered through Whatmann filter paper no. 41. Absorbance of these solutions was measured at 290 nm using UV spectrophotometer Shimadzu 1601. To increase the reliability of the observations, the dissolution studies were performed in triplicate.[23,24]

- **Dissolution study of Solid Dispersions prepared by Solvent Evaporation Method**

Dissolution test apparatus type II (Paddle) at rotation speed of 50 rpm was used for the study of dissolution of the drug and solid dispersion was carried out the prepare solid dispersion accurately weight equivalent to the 200mg of drug. The solid dispersion were filled in the empty capsule and analyzed for the drug in the 900 ml dissolution media and 37 ± 0.2 0C. The dissolution media in which performed test ware performed was 0.1 N HCl. solutions. The sample was withdrawn in automatically at time interval 10 min, 20 min, 30 min, 40 min, 50 min, and 60 min. the sample analyzed by UV spectrophotometer at 286 nm. Maximum wavelength of drug against the blank.

- **Comparative Dissolution study of Fenofibrate and solid dispersion**

The dissolution of pure drug is compare with solid dispersions by solvent evaporation method. And graph was plotted to show % drug release which was represented.

Selection of Optimum Batch of Solid Dispersion for formulation of Mouth Dissolving Tablet

For selection of the optimum batch of solid dispersion technique, the dissolution of the pure drug fenofibrate and its solid dispersion by solvent evaporation method with the help of PEG 4000 & PEG 6000 is compare.[16,17]

Evaluation of Solid Dispersion

1. Physical appearance

The prepared solid dispersion was evaluated for visual inspection of all batches of solid dispersion such as colour and appearance.

2. Percentage yield study of solid dispersion

Yield was calculated with respect to dry product. Based on the practical yield (P.Y.) obtained and the calculated theoretical yield (T.Y), % yield was calculated by using the following formula :

P.Y (%) = [Practical weight / Theoretical weight (Drug + Carrier)] ×100

Where,

a = Practical weight of solid dispersion obtained

b = Theoretical weight of solid dispersion prepared

3. Drug Content

Drug content analysis was done by preparing 1 mg/ml solution of the solid dispersions samples in methanol. Samples equivalent to 40 mg of fenofibrate was dissolved in 40ml of methanol. This solution was then kept for 24 h complete extraction of the drug. After 24 hrs, the solution was filtered and a 40 ug/ml solution was prepared with this solution by dilution with methanol. The solution was assayed through UV spectrophotometric method. [19]

$$\% \text{ drug content} = X/Y \times 100$$

X =concentration obtained from spectrophotometer analysis.

Y =Theoretical concentration

4. Different scanning calorimetery studies

DSC thermo gram showed of drug at 82.4°C corresponding to the melting of drug .the physical mixture of endotherm broad and slightly shifted to lower temperature. There was no peak obsevered in the thermo gram of solid dispersions and indicating amorphous form of drug. The DSC of pure fenofibrate, physical mixture and solid dispersion.[10,12]

Formulation of Mouth Dissolving Tablets of Solid Dispersion of Fenofibrate

After evaluation of solid dispersion of fenofibrate preprared by solvent evaluation, mouth dissolving tablets were prepared according to formula given in table 2.

Table 2. Formulation of Mouth Dissolving Tablets

Ingredients	**F1**	**F2**	**F3**	**F4**	**F5**	**F6**	**F7**	**F8**
Solid Dispersion of Fenofibrate (equivalent to 50 mg of drug)	300	300	300	300	300	300	300	300
Cross Carmellose	5	10	15					
Sodium Starch Glycolate				5	10	15		
Cross Povidon							5	10
Magnesium Stearate	1	1	1	1	1	1	1	1
Talc	2	2	2	2	2	2	2	2
Aspartame	2	2	2	2	2	2	2	2
Peppermint	2	2	2	2	2	2	2	2
Mannitol	38	33	28	38	33	28	38	33
Total	350	350	350	350	350	350	350	350

Evaluation of Powder blend for Mouth Dissolving Tablets

Method : Accurate quantity of drug and all ingredients were weighed according to formula and powder except mannitol and magnesium stearate was blended homogeneously in mortar and pestle for 15minutes. Prepared powder blend was passed through sieve No. #60. Finally, mannitol and magnesium stearate passed through sieve No.#30 was added and further mixed for 10 minutes.

The powder blend was evaluated for angle of repose, bulk density, tapped density, compressibility index and hausner's ratio.[9,13]

1.Angle of Repose

Angle of repose was determined by using funnel method. Powder was poured from a funnel that can be raised vertically until a maximum cone height, h, was obtained. Diameter of heap, D, was measured. The angle of repose, Ɵ, was calculated by formula

$$\text{Ɵ} = \tan^{-1} (h / r)$$

Where, Ɵ is the angle of repose, h is the height in cm and r is the radius.

2. Bulk Density

Apparent bulk density was determined by pouring presieved drug excipient blend into a graduated cylinder and measuring the volume and weight "as it is". It is expressed in g/ml and is given by

BD = Weight of the powder / Volume of the powder

3. Tapped Density

The tapped volume was measured by tapping the powder to constant volume. It is expressed in g/ml and is given by

TD = Weight of the powder / Tapped of the powder

4. Compressibility Index

It is expressed in percentage and is expressed by

$$\text{Carr's compressibility index (\%)} = [(TD - BD) / TD] \times 100$$

5.Hausner's ratio

It is expressed in percentage and is expressed by

$$H = TD / BD$$

Manufacturing of Mouth Dissolving Tablet of Fenofibrate containing solid dispersion by direct compression method

The SD of Fenofibrate with PEG 6000 simultaneously in 1:4 ratio was prepared by solvent evaporation technique, The drug and the carriers were dissolved in methanol. The solution was stirred for 1 hour on magnetic stirr. The solvent was evaporated at 40°C in vacuum dryer. After solvent removal, the solid dispersion products were kept in desiccators for 48 hours. The product samples were pulverized using a glass mortar and pestle, sieved through 120 meshes and kept the powder of solid dispersion in desiccators throughout the experimental period.[8-11]

The amount of Solid Dispersion complex equivalent to 50mg of drug were taken and then mixed with directly compressible diluents and superdisintegrants in a plastic container. Magnesium stearate and talc were passed through sieve no.60, mixed and blended with the initial mixture in the plastic container followed by compression of the blend. Compression was performed on a tablet compression machine using 8mm punches.[25]

Before tablet preparation, the flow properties and other derived properties evaluated for all the 9 formulations were proved to be within limits showing good flow properties. The physical properties like bulk density, tapped density, angle of repose, compressibility index, and Hausner's ratio were calculated.

Evaluation of Mouth Dissolving Tablets

1. Apperance

The tablets were visually observed for capping, chipping, and lamination.

2. Thickness

Three tablets were selected randomly from each batch and thickness was measured by using Vernier Caliper.

3. Hardness

For each formulation, the hardness of five tablets was determined using the Monsanto hardness tester (Cadmach). These tablet hardness tests provide a meaningful picture as to the amount of force required to fracture the solid-dose tablet. This knowledge will be useful in gauging the tablet's resistance to damage that might occur during production handling, packaging, and storage.

4.Friability

The friability of a sample of 10 tablets was measured using a Friability tester (Electro Lab). Ten tablets were weighed, rotated at 25 rpm for 4 minutes. Tablets were reweighed after removal of fines (dedusted) and the percentage of weight loss was calculated. [5]

$$\textbf{Friability (\%)} = W_1 - W_2 / W_1 \times 100.$$

Where, W_1 = Weight of Tablets (Initial / Before Tumbling)

& W_2 = Weight of Tablets (After Tumbling or friability)

Limit : Friability (%) = Not More Than 1.0 %

5.Drug Content

The Fenofibrate content was estimated as follows :

Method

Twenty tablets were taken randomly and individual tablet were crushed, an amount of the powder equivalent to 50 mg of fenofibrate was dissolved in the 50 ml of 0.1M methanol was added. Shaken for 30 min and added sufficient 0.1 M methanol to produce 100 ml and filtered, diluted suitably and analyzed for drug content at 290 nm using UV-Visible spectrophotometer (UV 1601-Shimadzu, Japan).[4-6]

6.Weight Variation

Twenty tablets were randomly selected from each batch individually weighed, the average weight and standard deviation of 20 tablets was calculated. The batch passes the test for weight variation test if not more than two of the individual tablet weight deviate from the average weight.

7. Disintegration Time

The disintegration time was measured using disintegration test apparatus. One tablet was placed in each tube of the basket. The basket with the bottom surface made of a stainless-steel screen (mesh no. 10) was immersed in water bath at 37 ± 20C. The time required for complete disintegration of the tablet in each tube was determined using a stop watch. To be complied with the pharmacopoeial standards, MDT's must disintegrate within 3 min when examined by the disintegration test for tablets.[2,3]

8. Wetting time

A piece of tissue paper (12cmx10.75cm) folded twice was placed in a Petri dish (Internal Diameter=9cm) containing 6 ml of simulated saliva pH 6.8. A tablet having amaranth powder on the upper surface was placed on the filter paper. Time required to develop red color on the upper surface of tablet was recorded as wetting time. Three tablets from each formulation were randomly selected and the average wetting time was noted. Wetting time corresponds to the time taken for the tablet to disintegrate when placed gently on the tissue paper in a petridish. This method will duplicate the in-vivo disintegration as the tablet is motionless on the tongue. Less is the wetting time indicates more porous the tablets.[3]

9. In-vitro Dissolution studies

The release rate of fenofibrate from mouth dissolving tablets was determined using USP Dissolution Testing Apparatus II (Paddle type). The dissolution test was performed using 900 ml phosphate buffer pH 6.8 , at 37 ± 0.5ºC and 50 rpm. A sample (5 ml) of the solution was withdrawn from the dissolution apparatus every 2 min. for 30 min, and the samples were replaced with fresh dissolution medium. The samples were filtered through Whatmann filter paper no. 41. Absorbance of these solutions was measured at 290 nm using UV spectrophotometer Shimadzu 1601. To increase the reliability of the observations, the dissolution studies were performed in triplicate.[5]

10. Stability studies

A series of tests designed to obtain information on the stability of a pharmaceutical product in order to define its shelf-life and utilization period under specified packaging and storage

conditions.Various stability studies like accelerated stability study, intermediate and long term stability studies were done during preformulation. The sample was subjected to higher temperature or humidity or both, to know their impact on the stability of mouth dissolving tablet. The tests that monitor the quality, purity, potency, and identity which could be expected to change upon storage are chosen as stability tests. Therefore appearance, assay, degradation products, microbiological testing, dissolution, and moisture are standard tests performed on stability test samples. The fast dissolving tablets stored under the following conditions for a period as prescribed by ICH guidelines for accelerated studies.[1-3]

i.40 ± 1°C

ii.50 ± 1°C

iii.37 ±1°C and RH 75% ± 5% T

The tablets were withdrawn after a period of 15 days and analyzed for physical characterization such as visual defects, Hardness, Friability, Disintegrations, and Dissolution etc. The data obtained is fitted into first order equations to determine the kinetics of degradation. Accelerated stability data are plotting according Arrhenius equation to determine the shelf life at 25°C.

The international Conference on Harmonization (ICH) Guidelines titled " Stability testing of new drug substance and products " (QIA) describes the stability test requirements for drug registration application in the Europeans Union, Japan and United States of America. ICH specifies the length of study and storage conditions.

Long-term testing 25ºC ± 2ºC/60 % RH ± 5% For 12 Months.

Short-term testing 30ºC ± 2ºC/65 % RH ± 5% For 1 Months.

Accelerated testing 40ºC ± 2ºC/65 % RH ± 5% For 6 Months.

Stability studies for the present work carried out at 40ºC ± 2ºC/ 75 % ± 5 % RH for the selected formulation for 3 months.

RESULT AND DISCUSSION

1. Preformulation Studies of Fenofibrate

Identification and characterization of Fenofibrate

- **Organoleptic Properties**

Organoleptic evaluation reveals that the sample of Fenofibrate obtained was complied with standards. The result is presented in the table 3.

Table 3. Identification tests of fenofibrate with the reported standards.

Sr.No.	Identification Test	Observation	Inference
1.	Apperance	Fine powder	Complies with IP
2.	Colour	White	Complies with IP
3.	Odour	Odourless	Complies with IP

- **Melting Point**

The Melting point of received drug sample of Fenofibrate was determined and it was found to be 80ºC which is in the range 79º-82ºC so, complies with standard, indicating purity of drug.

- **Solubility Study of Drug**

The descriptive form of solubility profile according to parts of solvent required for 1 parts of solute given in the table 4.

Table 4. Solubility study of Fenofibrate in the different solvents

Sr.No.	Solvent	Solubility (mg/ml)	Inference
1.	Distilled water	0.003	Practically Insoluble
2.	Chloroform	3.20	Sparingly Insoluble
3.	Methylene chloride	29.74	Freely Soluble
4.	Methanol	90.38	Freely Soluble

2. UV Spectroscopic Analysis of Fenofibrate

Scanning of Fenofibrate -The standard solution of Fenofibrate was scanned in the range of 200-400nm in Phosphate buffer 6.8 and absorbance maxima was found at 280nm.

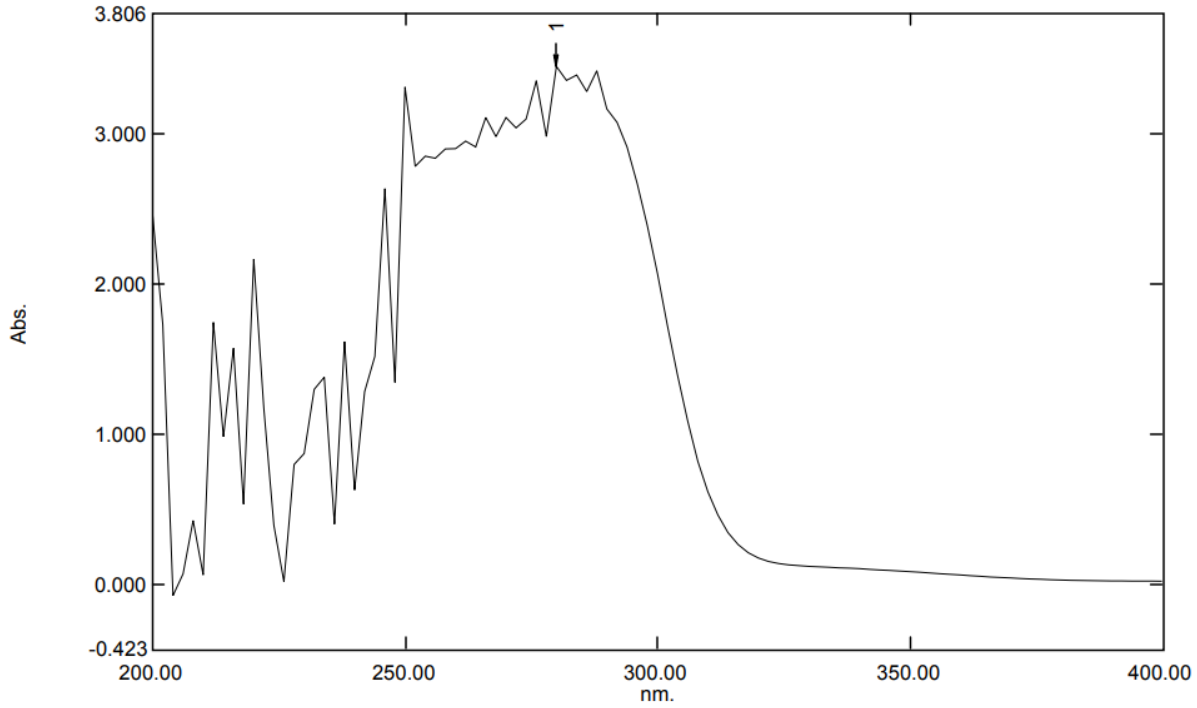


Figure 1. Scanning of Fenofibrate

Table 5. Data for Calibration Curve of Fenofibrate

Sr.No.	Concentration	Absorbance
1	10	0.037
2	20	0.056
3	30	0.083
4	40	0.11
5	50	0.147

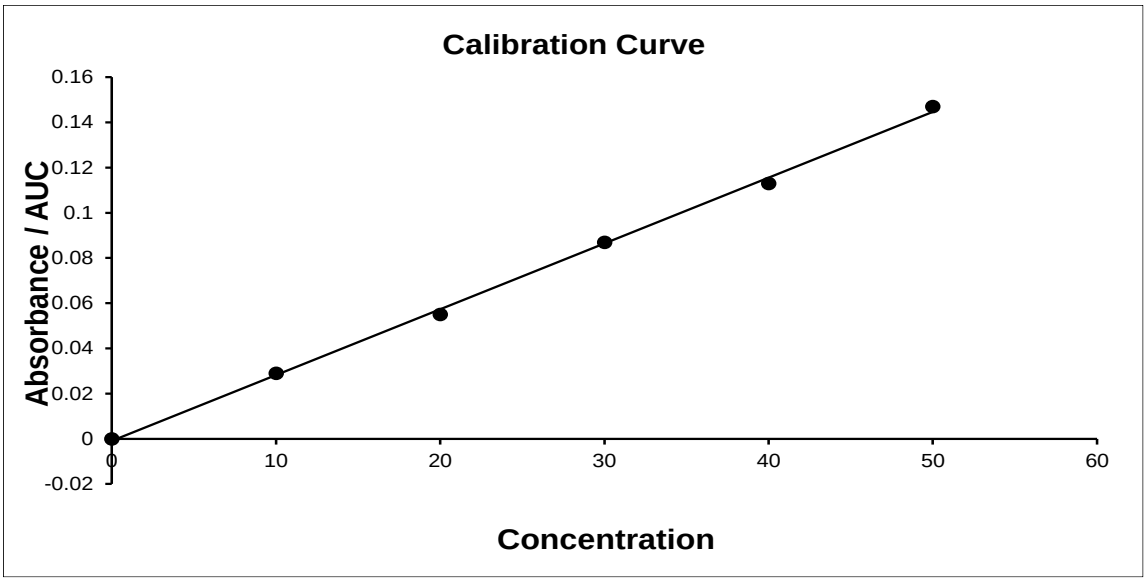
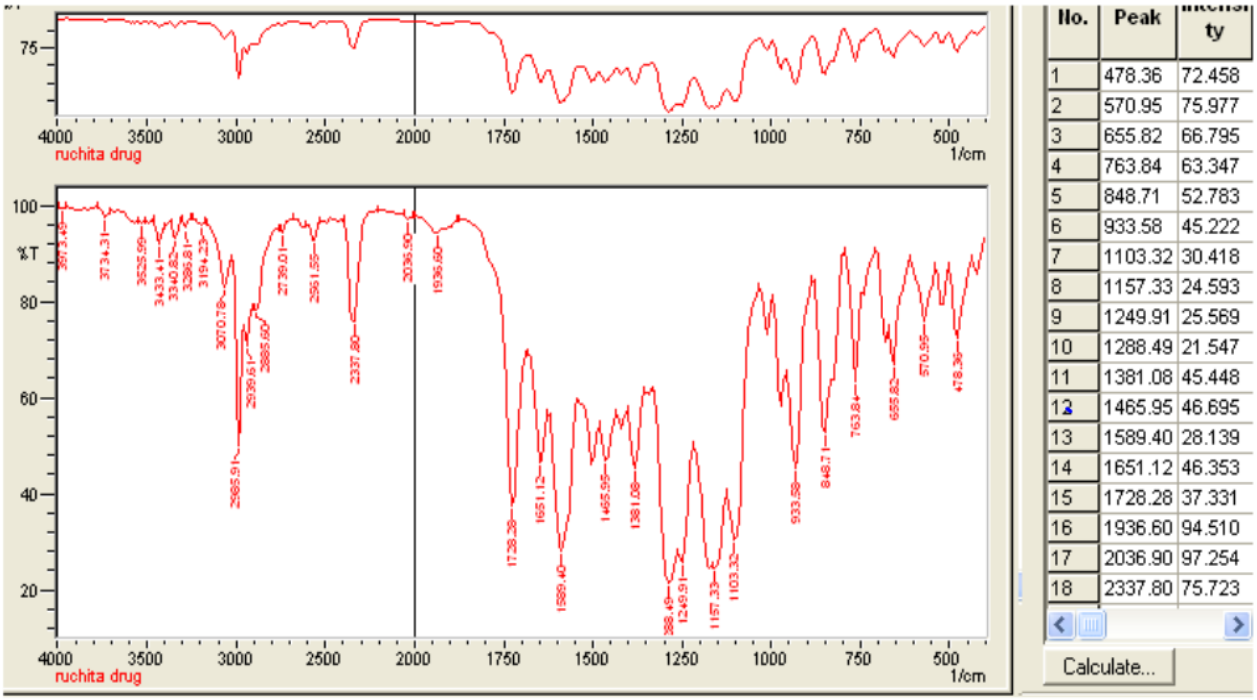


Figure 2. Calibration Curve of Fenofibrate

3. Drug Excipient Compatibility Studies

The FTIR spectrum of pure drug and drug- excipients physical mixture and its interpretation is shown below:

I. FTIR OF Fenofibrate



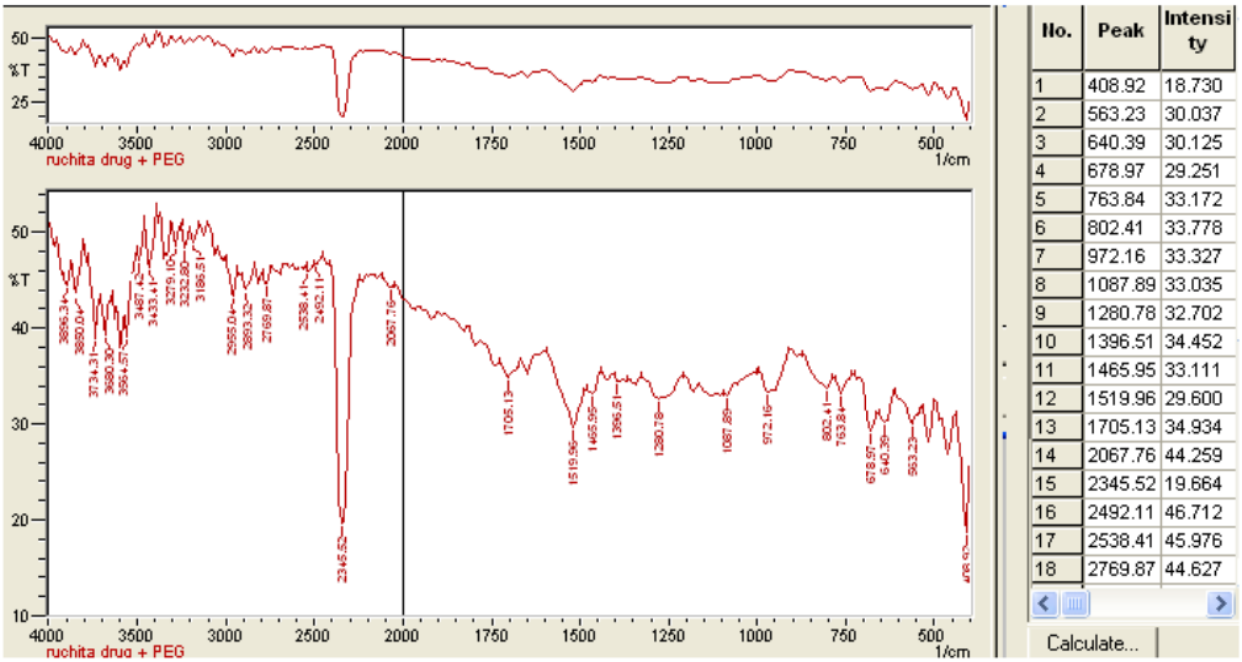
No.	Peak	Intensity
1	478.36	72.458
2	570.95	75.977
3	655.82	66.795
4	763.84	63.347
5	848.71	52.783
6	933.58	45.222
7	1103.32	30.418
8	1157.33	24.593
9	1249.91	25.569
10	1288.49	21.547
11	1381.08	45.448
12	1465.95	46.695
13	1589.40	28.139
14	1651.12	46.353
15	1728.28	37.331
16	1936.60	94.510
17	2036.90	97.254
18	2337.80	75.723

Figure 3. FTIR Spectra of Fenofibrate

Table 6. FTIR Peaks of Fenofibrate

Reference Peak Wavenumber (cm-1)	Observed Peak Wavenumber (cm-1)	Functional Group
1705-1740	1728.28	C=O Stretch
2000-2170	2036.90	C=C Stretch
2300-2500	2337.80	C-H Stretch
3130-3450	3433.41	O-H Stretch

II. FTIR of Fenofibrate + PEG 6000



No.	Peak	Intensity
1	408.92	18.730
2	563.23	30.037
3	640.39	30.125
4	678.97	29.251
5	763.84	33.172
6	802.41	33.778
7	972.16	33.327
8	1087.89	33.035
9	1280.78	32.702
10	1396.51	34.452
11	1465.95	33.111
12	1519.96	29.600
13	1705.13	34.934
14	2067.76	44.259
15	2345.52	19.664
16	2492.11	46.712
17	2538.41	45.976
18	2769.87	44.627

Figure 4. FTIR Spectra of Fenofibrate + PEG 6000

Table 7. FTIR Peaks of Fenofibrate + PEG 6000

Reference Peak Wavenumber (cm-1)	Observed Peak Wavenumber (cm-1)	Functional Group
1705-1740	1705.13	C=O Stretch
2000-2170	2067.76	C=C Stretch
2300-2500	2492.11	C-H Stretch
3130-3450	3433.41	O-H Stretch

FORMULATION AND DEVELOPMENT

PREPARATION OF FENOFIBRATE SOLID DISPERSION

The formulation of solid dispersion prepared by solvent evaporation technique by using polymers like PEG 4000 and PEG 6000 respectively in various ratios such as Fenofibrate and PEG 4000 (1:1, 1:2, 1:3, 1:4, 1:5) ; Fenofibrate and PEG 6000 (1:1, 1:2, 1:3, 1:4, 1:5). Solid dispersions

prepared by using PEG 4000 were F1, F2, F3, F4, F5 and Solid dispersions prepared by using PEG 6000 were F6, F7, F8, F9 and F10 respectively. [7-9]

Comparative Solubility and Dissolution study to select the optimum Batch of Solid Dispersion

Table 8. Solubility study of Fenofibrate and Solid Dispersion

Formulations	Drug : Carrier	Solubility (µg/ml)
Pure Drug	Pure Drug	2.385
F1	Fenofibrate + PEG 4000 (1:1)	60.80
F2	Fenofibrate + PEG 4000 (1:2)	65.96
F3	Fenofibrate + PEG 4000 (1:3)	72.50
F4	Fenofibrate + PEG 4000 (1:4)	80.76
F5	Fenofibrate + PEG 4000 (1:5)	89.54
F6	Fenofibrate + PEG 6000 (1:1)	68.55
F7	Fenofibrate + PEG 6000 (1:2)	70.97
F8	Fenofibrate + PEG 6000 (1:3)	80.56
F9	Fenofibrate + PEG 6000 (1:4)	92.43
F10	Fenofibrate + PEG 6000 (1:5)	95.34

Solubility study of various solid dispersion trial batches was performed. Solid dispersion prepared by using PEG 6000 improved solubilty of Fenofibrate as compared to pure drug. The batch F10 was more soluble than pure drug and other formulation batches.

In vitro Dissolution study of pure drug and Solid Dispersion

Dissolution study of pure drug and solid dispersion batches in phosphate buffer 6.8 was carried out and absorbance was taken in UV spectrophotometer at 280nm. is reported in table no.9.

Table 9. Dissolution profile of drug and solid dispersion

Time (min.) ⇒	**0**	**2**	**4**	**6**	**8**	**10**
Pure Drug	0	5.04±1.22	8.94±0.54	12.15±1.34	17.56±1.56	20.16±0.64

F1	0	20.19±1.13	31.05±1.37	39.98±1.93	50.46±1.94	78.87±0.26
F2	0	19.98±0.98	41.48±0.73	57.93±1.04	68.92±0.84	70.21±0.93
F3	0	32.67±0.65	50.36±1.93	61.95±0.74	71.54±1.45	75.43±0.16
F4	0	33.75±1.74	52.67±0.03	62.74±0.72	70.54±1.84	76.00±1.53
F5	0	35.76±1.84	51.95±0.86	64.50±0.46	69.87±0.56	77.90±1.73
F6	0	26.45±0.03	39.09±0.53	55.87±0.3	67.98±0.53	72.88±0.63
F7	0	31.39±0.83	50.23±1.93	65.47±1.84	71.24±0.24	75.98±0.73
F8	0	36.76±0.76	52.87±1.03	67.88±0.17	72.45±1.87	89.43±1.73
F9	0	39.73±1.20	55.93±0.97	69.93±0.37	78.73±1.95	93.96±1.84
F10	0	39.75±1.03	60.23±0.72	69.38±1.74	84.83±0.756	96.33±0.63

*Results are mean of 3 determinations

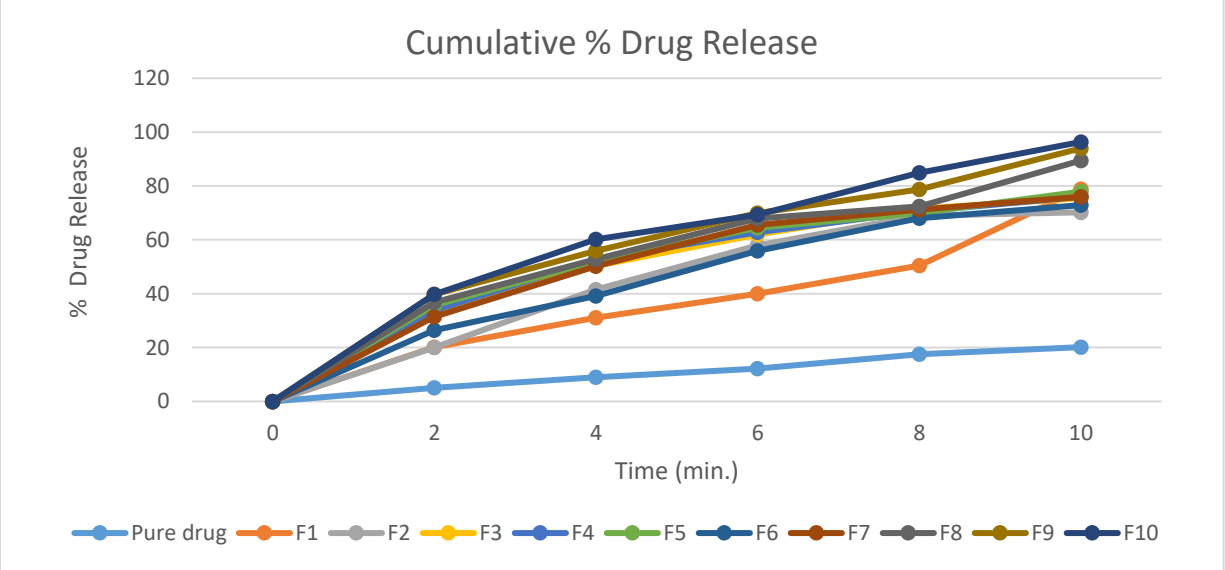


Figure 5. Dissolution profile of pure drug and solid dispersion

Selection of Optimum Batch of Solid Dispersion for formulation of Mouth Dissolving Tablets

From solubility and dissolution studies of all batches of solid dispersion by using PEG 6000 shows improved drug solubility and dissolution rate than PEG 4000, Hence F10 formulation of PEG 6000 is selected for further formulation of mouth dissolving tablets.

Evaluation of Selected batch (F10) of Solid Dispersion

1.Physical Appearance

All batches of solid dispersion were evaluated for color and appearance. The physical appearance of each formulation is shown in table 10.

Table 10. Physical Appearance of formulations Drug and Polymer

Sr.NO.	Formulation	Color	Appearance
1	**F1**	Off White	Powder
2	**F2**	Off White	Powder
3	**F3**	Off White	Powder
4	**F4**	Off White	Powder
5	**F5**	Off White	Powder
6	**F6**	Off White	Powder
7	**F7**	Off White	Powder
8	**F8**	Off White	Powder
9	**F9**	Off White	Powder
10	**F`0**	Off White	Powder

2. Percentage Practical Yield of Solid Dispersion

Table 11. Practical Yield of Solid Dispersion

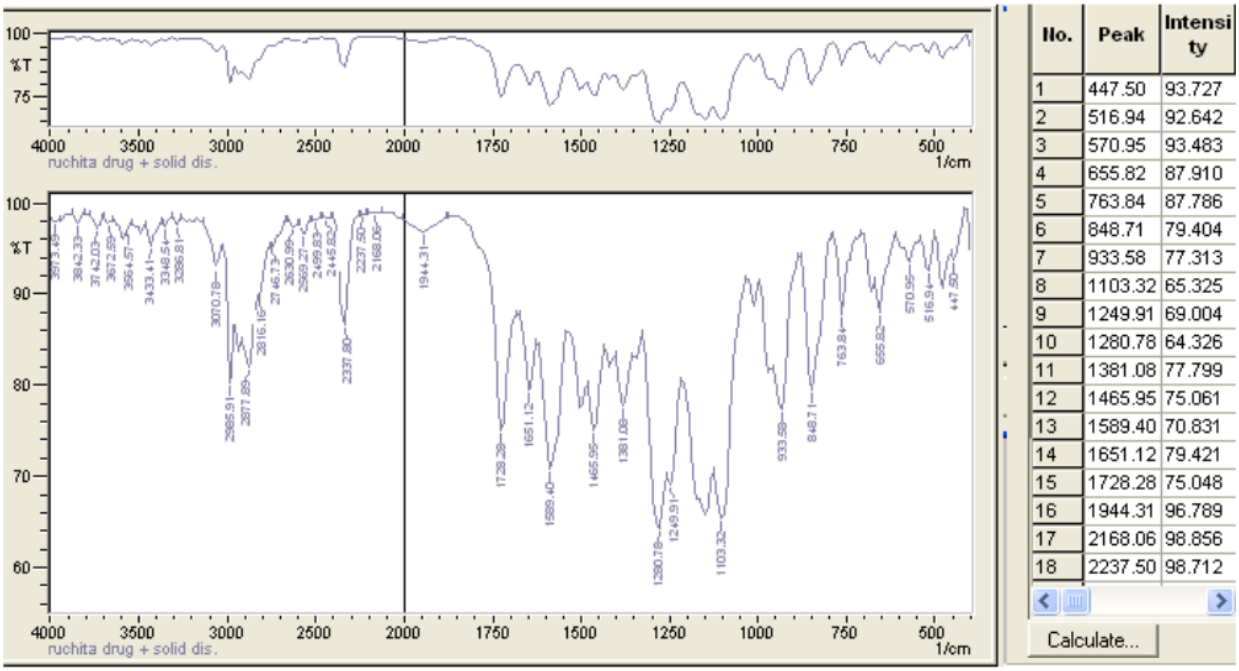
Formulation	Initial weight (mg)	Final weight (mg)	% Practical Yield
F1	2000	1.899	94.96
F2	2750	26367	95.88
F3	3000	2883	96.11
F4	3750	3517	93.80
F5	5000	4722	94.44
F6	4000	3873	96.83
F7	3750	3646	97.23
F8	4750	4689	97.89
F9	5000	4868	97.36
F10	5750	5529	98.16

3. Drug Content of Solid Dispersion of Optimized Formulation F10

The Drug Content of Optimized formulation of Solid Dispersion of Fenofibrate was found to be 99.25% indicating good content in solid dispersion.

4. Characterization of Solid Dispersion

FTIR studies of solid dispersion



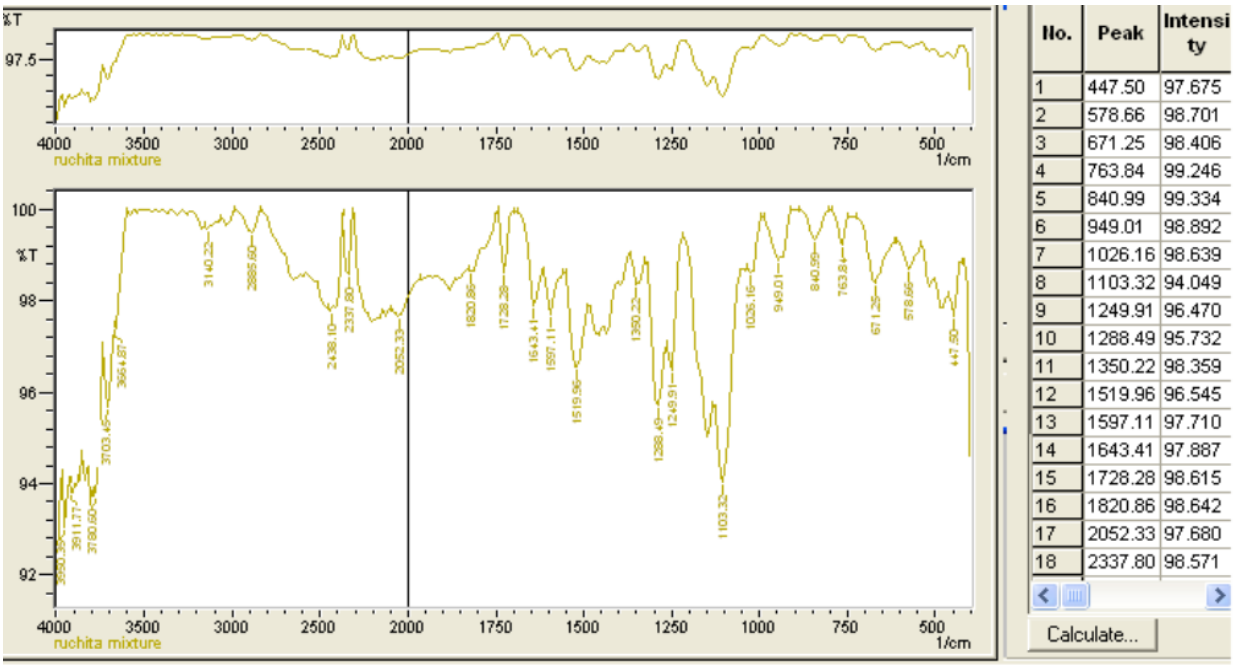
No.	Peak	Intensity
1	447.50	93.727
2	516.94	92.642
3	570.95	93.483
4	655.82	87.910
5	763.84	87.786
6	848.71	79.404
7	933.58	77.313
8	1103.32	65.325
9	1249.91	69.004
10	1280.78	64.326
11	1381.08	77.799
12	1465.95	75.061
13	1589.40	70.831
14	1651.12	79.421
15	1728.28	75.048
16	1944.31	96.789
17	2168.06	98.856
18	2237.50	98.712

Figure 6. FTIR Spectra of Solid Dispersion

Table 12. FTIR Peaks of Solid Dispersion

Reference Peak Wavenumber (cm-1)	Observed Peak Wavenumber (cm-1)	Functional Group
1705-1740	1728.28	C=O Stretch
2000-2170	2168.06	C=C Stretch
2300-2500	2445.11	C-H Stretch
3130-3450	3433.41	O-H Stretch

FTIR studies of Mixture



No.	Peak	Intensity
1	447.50	97.675
2	578.66	98.701
3	671.25	98.406
4	763.84	99.246
5	840.99	99.334
6	949.01	98.892
7	1026.16	98.639
8	1103.32	94.049
9	1249.91	96.470
10	1288.49	95.732
11	1350.22	98.359
12	1519.96	96.545
13	1597.11	97.710
14	1643.41	97.887
15	1728.28	98.615
16	1820.86	98.642
17	2052.33	97.680
18	2337.80	98.571

Figure 7. FTIR Spectra of Mixture

Table 13. FTIR Peaks of Mixture

Reference Peak Wavenumber (cm-1)	Observed Peak Wavenumber (cm-1)	Functional Group
1705-1740	1728.28	C=O Stretch
2000-2170	2052.33	C=C Stretch
2300-2500	2052.33	C-H Stretch
3130-3450	3140.22	O-H Stretch

XRD Studies of Pure Drug and Solid Dispersion

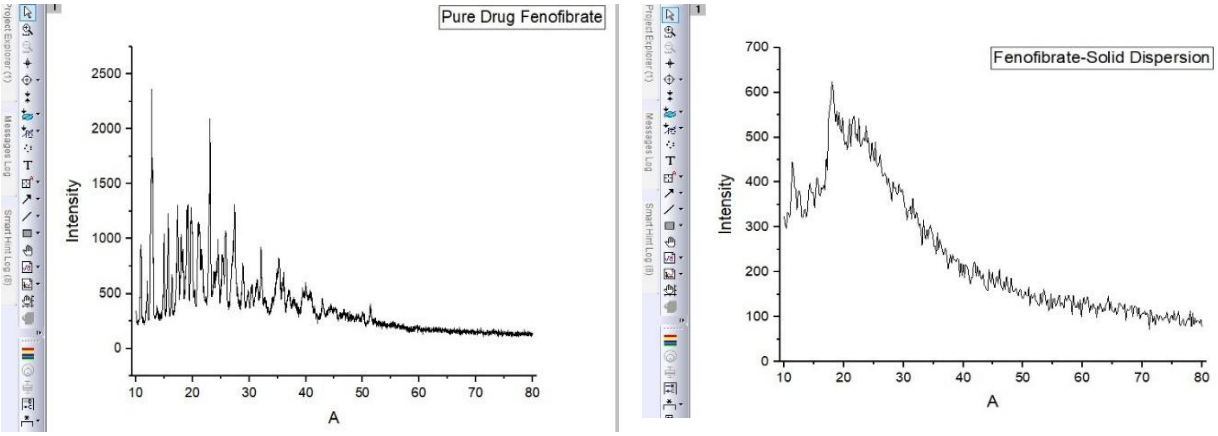


Figure 8. XRD studies

Evaluation of Precompression Properties

For each designed formulation, blend of drug and excipients was prepared and evaluated for precompression properties shown in table 10. Bulk density was found to be between 0.44±0.04 to 0.49±0.01gm/cm3 and tapped density between 0.51±0.01 to 0.570±0.03 gm/cm3 for all formulations. From density data % compressibility was calculated and was found to be between 12.80±0.03% to 16.69±0.04 percent. Angle of repose was found to be in the range of 25.82±0.03 to 32.22±0.02. Hausner ratio was found below 1.22±0.02. All the formulation shows the fair to good flow properties for direct compression and hence tablets were prepared by using direct compression technology.

Table 14. Precompression Properties

Formulation	Angle of repose(o)*	Bulk density (gm/cm3)*	Tapped density (gm/cm3)*	Carr's index (%)*	Hausner's ratio (HR)*
F1	28.41±0.01	0.45±0.03	0.5161±0.02	12.80±0.01	1.146±0.02
F 2	29.34±0.02	0.470±0.02	0.5517±0.01	14.71±0.01	0.8528±0.02
F3	32±0.01	0.444±0.02	0.5330±0.01	16.69±0.02	1.200±0.03
F4	32.47±0.03	0.4637±0.03	05423±0.03	14.49±0.03	0.855±0.01

F5	27.75±0.02	0.4637±0.01	0.5423±0.03	14.44±0.03	1.161±0.03
F6	30.9±0.02	0.4848±0.02	0.5517±0.03	12.12±0.03	1.1379±0.03
F7	30.52±0.01	0.45±0.02	0.5161±0.02	12.80±0.02	1.146±0.01
F8	25.82±0.03	0.477±0.01	0.5517±0.01	13.5±0.03	1.156±0.01
F9	32.22±0.01	0.444±0.03	0.5333±0.01	16.69±0.02	1.200±0.02

*All values are expressed as mean ± SD, n=3

Evaluation of Post Compression Properties of tablets

Tablets were prepared using direct compression technique. Since the powder material was free flowing, Tablets were obtained of uniform weight due to uniform die fill, tablets were obtained of uniform weight variations as per Pharmacopoeial specifications. All the tablets were exhibit in white color, odorless, convex in shape with smooth surface with zero defects. The drug content was found in the range of 97.56 – 101.32% (acceptable limit) and the hardness of the tablets between 3.5 – 4.2 kg/cm2 . Friability of the tablets was found below 1 % indicating a good mechanical resistance of tablets. Thickness of the formulations were varied from 1.9±0.02 to 2.3±0.02 mm, diameter of the formulations were varied from 9.7±0.01 to 10.1±0.01 mm. All the parameters were found well within the specified limit.

Table 15. Post Compression Paramters

Form-ulation	**Diameter (mm)**	**Thickness (mm)**	**Hardness (kg/cm2)**	**Friability (%)**	**Drug content (%)**	**Weigh variati (mg)**
F1	9.7± 0.12	2.1±0.3	3.5± 0.10	0.30± 0.05	96.35±1.3	403± 0.31
F2	9.84± 0.22	2.2±0.1	4.0± 0.31	0.45± 0.05	97.61±2.34	398± 1.6
F3	9.99± 0.43	2.1±0.42	3.9± 0.42	0.56± 0.12	97.53±1.13	397± 2.04
F4	10.1±	2.4±0.03	3.8±	0.61±	96.22±1.12	404±

	0.52		0.52	0.11		2.23
F5	9.75± 0.31	2.0±0.71	4.1± 0.21	0.55± 0.53	97.75±2.1	397± 2.21
F6	9.83± 032	2.0±0.53	4.2± 0.53	0.45± 0.05	98.75±1.23	400± 0.65
F7	9.97± 0.33	2.4±0.12	3.9± 0.31	0.45± 0.14	98.50±1.2	402± 1.63
F8	9.79± 0.34	2.3±0.42	4.0± 0.63	0.69± 0.03	99.41±1.12	402± 0.82
F9	9.87± 0.12	2.0±0.31	3.9± 0.14	0.61± 0.05	99.51±2.32	405± 0.31

In vitro % Drug Release of Drug from Tablet

All the nine formulations were subjected for the in vitro dissolution studies using tablet dissolution apparatus (USP). Phosphate Buffer pH 6.8 was used as dissolution medium. The sample were withdrawn at different time intervals, filter and analyzed at 280nm. Cumulative % drug release was calculated on the basis of mean amount of Fenofibrate present in respective table.

Table 16. Dissolution Profile of Tablets

Time (min.)	**0**	**2**	**4**	**6**	**8**	**10**
F1	0	34.606±0.13	43.906±01.39	61.244±0.62	84.138±1.37	93.513±0.62
F2	0	32.136±0.07	43.904±1.83	61.244±1.73	84.775±1.94	92.896±1.63
F3	0	33.371±0.12	44.436±0.92	57.941±1.94	75.723±0.78	95.924±1.82
F4	0	29.667±0.73	42.666±0.62	60.002±1.7	83.532±0.53	90.147±1.85
F5	0	28.432±1.73	39.571±0.71	55.675±0.82	78.878±1.98	89.78±0.64
F6	0	30.284±1.73	43.284±1.85	58.157±0.7	76.74±0.82	92.879±0.55
F7	0	31.519±1.94	43.903±1.83	62.476±0.64	85.391±0.62	97.217±0.73
F8	0	32.754±1.92	44.522±1.64	64.333±0.98	86.63±1.73	97.458±1.23
F9	0	33.371±0.92	45.758±0.73	65.567±1.63	87.868±1.53	99.08±0.79

*Results are mean of 3 determinations

The rapid dissolution was observed in formulation F9 releases 99.08±0.79 at the end of 10minutes. Rapid dissolution might be due to fast breakdown of particles and rapid absorption of drug.

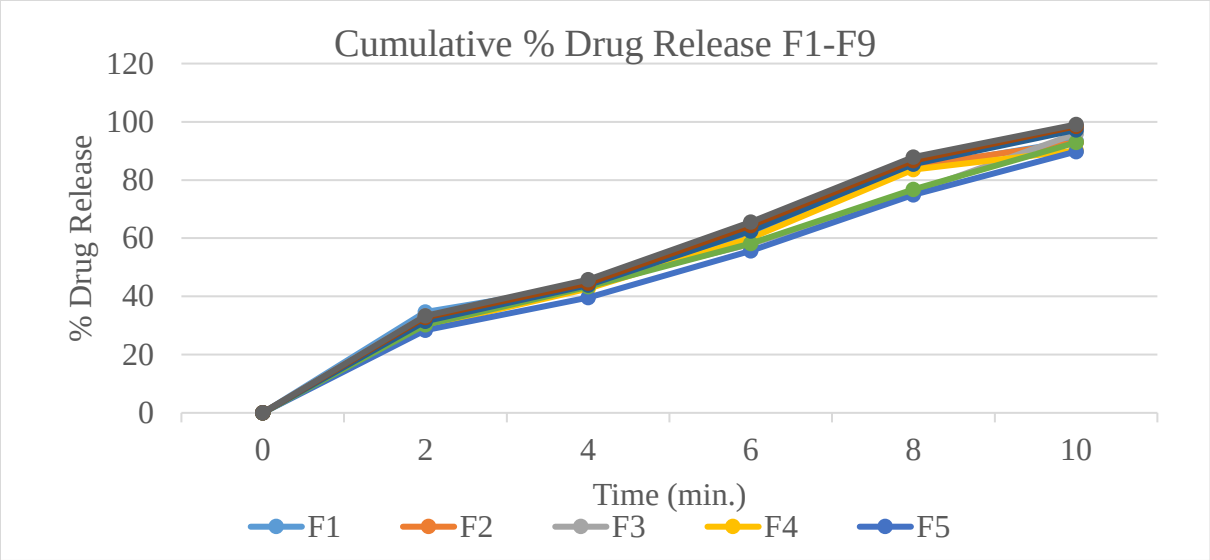


Figure 9. Dissolution Profile of Tablets

In comparative study F9 formulation gives higher percentage drug release compare to other remaining eight formulations at the end of 10minutes and graphical representation is shown in figure 9. Therefore it was concluded that the best batch was found to be F9 because of lesser disintegration time and highest % drug release at the end of 10 min.among all the formulations. Because it contain Crosspovidon superdisintegrant with fast wetting time and highest swelling property.

Stability Study

The mouth dissolving tablets of solid dispersion of Fenofibrate, F9 batch were subjected to stability study at temperature 40ºC± and relative humidity 75% ± for three months. After each month tablets were analyzed for hardness, friability, disintegration time, dissolution time and drug content. The results are as follows.

Table 17. Stability study of Mouth Dissolving Tablets of optimized batch

F9 at 40ºC ± 2ºC/75 % RH ± 5%

Parameters	Initial	After 1month	After 2months	After 3months
Hardness (kg/cm^3)	3.9±0.04	3.9±0.89	3.5±0.84	3.3±0.53
Friability (%)	0.61±0.01	0.66±0.76	0.71±0.7	0.751±0.97
Dintegration time (min.)	1.10±0.01	1.10±0.67	1.17±0.56	1.20±0.01

Content Uniformity (%)	99.51±1.32	99.21±0.12	98.73±1.32	97.32±0.32
Cumulative % Drug release	99.08±0.79	99.08±0.86	98.78±0.46	97.08±0.65

*Results are mean of 3 determinations

From the above table it is concluded that, the Mouth Dissolving Tablets of solid dispersion of Fenofibrate from tablet F9 batch are physically stable and retained their original properties when stored at 40ºc ± 2ºc/75 % RH ± 5% and after three months was no significant difference in disintegration time, cumulative % drug release, hardness, friability and drug content.

CONCLUSION

All the formulations of solid dispersions were successfully prepared and Fenofibrate tablets are prepared and evaluated for solubility and dissolution rate. The saturation solubility of drug was found to be more in the solid dispersions as compared to the phase solubility achieved in the presence of hydrophilic carriers in the dissolution media. This may be due to drug carrier interaction or change in property of drug in the solid dispersion formulations. Highest solubilizing power of Povidone towards Fenofibrate was shown by dissolution studies. From FTIR spectroscopy studies, it was concluded that there was no defined chemical interactions between Fenofibrate and Povidone. It can provide a promising way to enhance its solubility and dissolution rate.

In the present investigation we developed mouth dissolving tablets of fenofibrate by Solid Dispersion technique.The wetting time or simulated saliva penetration was observed to be very fast with batch F9 tablets. The total drug from the optimized batch was found to be released within the first ten minutes of dissolution study. These tablets rapidly dissolved (within 60-70 sec) in saliva. The prepared tablet gives benefit in terms of patient compliance, low dosing, rapid onset of action, increased bio-availability, low side effect and good stability which make these tablets popular as a dosage form for the treatment of hyperlipidemia.

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Formulation and Evaluation of Bilayer tablet of Sustained release Glibenclamide and Immediate release Enalapril Maleate

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ABSTRACT:

The purpose of this study was to formulate and evaluate bilayer tablets of immediate release Enalapril maleate for hypertension and sustained release of Glibenclamide for diabetes. Direct compression method was used to formulate bilayer tablets, which contained hydrophilic HPMC K- 100 and hydrophobic Ethyl cellulose as a sustained release polymer and super disintegrating agent such as Croscarmellose sodium in different proportion from batch F1-F6. Formulation of Bilayer tablets was prepared by the FEM1-FEM6 and FG1-FG6 powder blend for different parameters of formulated bilayer tablets were hardness, friability, thickness, drug content, weight variation, and the in-vitro drug release rate pattern results indicated that the formulation F3 was high as compared to other formulations. In formulation F3, percentage drug release of Glibenclamide sustained release layer was **99.98%** at 12 hrs. and **98.91%** at 90 min for Enalapril Maleate Immediate release layer.

Keywords:
Glibenclamide, Enalapril Maleate, Bilayer Tablets, Sustained Release, Immediate Release, Co-morbidity.

1. INTRODUCTION

The drug Glibenclamide is antidiabetic agent which comes under BCS class II sulfonylureas oral hypoglycemic is used to treat type -2 diabetes and Enalapril maleate is antihypertensive agent which comes under BCS class III used to treat high blood pressure (Hypertension). Glibenclamide (GLB) has elimination half-life is approximately 2-5 h after oral administration, and it is 84±9% absorbed from GIT, but its bioavailability is low due to its poor solubility and extensive first-pass

metabolism in liver.[1] It is used to treat people with Non-Insulin Dependent Diabetes Mellitus (NIDDM). It has a strong yet slow-acting initial insulinemic effect. [1]

Diabetes mellitus is a condition of insulin-controlled metabolic balance that causes abnormalities in lipid and carbohydrate metabolism. There are two fundamental abnormalities that define type 2 diabetes mellitus. Type 2 diabetes occurs when your body becomes resistant to insulin, and sugar builds up in your blood. Insulin deficiency is a symptom of type 2 diabetes, which is characterized by pancreatic-cell failure and insulin resistance in target organs. When insulin secretion is compromised, type 2 diabetes mellitus results because the body is unable to meet the increasing demands placed on it by the insulin-resistant condition. Therefore, a lack of insulin is the only known cause of diabetes mellitus in Type 2 diabetes mellitus has a relative deficiency while type 1 diabetic mellitus has an absolute deficiency.[2]

Enalapril Maleate is the maleate salt of enalapril, a derivative of two amino acid , L-alanine and L-proline. Enalapril maleate is angiotensin converting enzyme (ACE) inhibitor. It lower blood pressure by reducing peripheral vascular resistance without relatively increasing cardiac output, rate or contractility. Hypertension is when blood pressure is too high. Narrow blood vessels, also known as arteries, create more resistance for blood flow. The narrower your arteries are, the more resistance there is, and the higher your blood pressure will be. Over the long term, the increased pressure can cause health issues, including heart disease.[3]

Studies have shown that the use of angiotensin converting enzyme (ACE)inhibitors can prevent the progression of renal damage and delay progression to end stage renal disease in addition to lowering blood pressure.[4] These are the novel drug delivery systems where combination of two drugs in a single unit having different release profiles Immediate release Enalapril Maleate and Sustained release Glibenclamide. Bilayer tablets are the medicines which consist of two same or different drugs combined in a single dose for effective treatment of the disease.[4]

The need for bilayer tablet is to treat Co-morbidity condition in same patients with same pill or tablet at a same time. It reduces the dose frequency and pill burden. An of reducing the lag time and providing faster onset of action to reduce the blood pressure immediately.[5] Bilayer tablet has patient compliance and is beneficial for sequential release of two drugs in combination. Bilayer tablet is an advanced technology that helps in overcoming the limitations of a single-layered tablet. In order to successfully treat a condition, bilayer tablets are prescription drugs that contain two of the same or distinct medications in a single dose. Bilayer tablets, which are excellent for the sequential delivery of numerous drugs, have high patient compliance. These are innovative drug delivery systems that combine two medicines with various release profiles into a single unit. [5]

2. MATERIALS AND METHOD

2.1. MATERIALS –

Glibenclamide an Antidiabetic agent was provided by Arti Pharmaceuticals and Enalapril Maleate an Antihypertensive agent was provided by Yarrow Chem Pharmaceuticals(India). HPMC and Ethyl Cellulose polymer as a free sample. Research Fine Chem Ltd, (India) supplied Croscarmellose sodium, Microcrystalline cellulose (Avicel pH 102), Lactose, Magnesium stearate, Talc. All of the reagents used in this experiment was analytical quality grade.

2.2 METHODS OF FORMUALTION OF BIALYER TABLETS OF ENALAPRIL MALEATE AND GLIBENCLAMIDE

The Direct Compression method is used for formulation of bilayer tablet of Enalapril Maleate and Glibenclamide in which tablets are formulated by direct compressed method from a powder blend of suitable excipients and API. Pre-treatment of blended of powder by dry or wet granulation procedure is not necessary. It provides merits mostly in terms of speedy production, as it's requires less machinery, reduced number of personnel, fewer unit operations and significantly less processing time along with improved product stability.

2.2.1Preparation of Immediate Release powder

Mixing all the powdered polymer ingredients with Enalapril Maleate passing through sieve no.40 using lactose as lubricant and magnesium stearate as binder to get fine smooth powder.

2.2.2 Preparation of Sustain Release powder

The drug Glibenclamide along with polymers like HPMC and Ethyl Cellulose and other exicipients mixing or blending them properly and passing through sieve by using binder to get fine smooth powder.

3. Formulation Table:

Formulation table of Bilayer Tablet of IR Enalapril Maleate and SR Glibenclamide. The tablet blends for different batch formulation (FEM1-FEM6) and (FG1-FG6) are prepared and further studied for Pre-compression properties and subjected for tablet punching by direct compression.

Table.1 Formulation of Immediate Release layer of Enalapril Maleate

Formulations	**FEM1**	**FEM2**	**FEM3**	**FEM4**	**FEM5**	**FEM6**
Ingredients		**Unit formula (mg per tablet)**				

Enalapril Maleate	**5**	**5**	**5**	**5**	**5**	**5**
Croscarmellose	**2**	**4**	**6**	**8**	**10**	**12**
Lactose	**92**	**90**	**88**	**86**	**84**	**82**
Magnesium Stearate	**1**	**1**	**1**	**1**	**1**	**1**
Total	**100**	**100**	**100**	**100**	**100**	**100**

Table.2 Formulation of Sustain Release layer of Glibenclamide

Formulations	**FG1**	**FG2**	**FG3**	**FG4**	**FG5**	**FG6**
Ingredients		**Unit formula (mg per tablet)**				
Glibenclamide	**10**	**10**	**10**	**10**	**10**	**10**
HPMC K-100	**40**	**60**	**80**	**-**	**-**	**-**
Ethyl Cellulose	**-**	**-**	**-**	**40**	**60**	**80**
Magnesium Stearate	**8**	**8**	**8**	**8**	**8**	**8**
Talc	**8**	**8**	**8**	**8**	**8**	**8**
Microcrystalline Cellulose	**134**	**114**	**94**	**134**	**114**	**94**
Total	**200**	**200**	**200**	**200**	**200**	**200**

5. EVALUATION OF TABLETS

A)Pre-compression study:

1) Angle of Repose:-

It is determined by funnel method. The funnel is fixed at a particular height (2.5 cm) on a burette stand. The sample powder was allowed to pass through the funnel allowing it to form a pile. This area is encircled to measure radius. This similar procedure repeated 3 times and the average value all 3 observation is taken. The angle of repose can be calculated by using equation.

$$\textbf{Angle of Repose } (\theta) = \textbf{Tan}^{-1}\ (h/r)$$

Where, h=height of pile,

θ = angle of repose,

r= radius of the base of the powder pile.

2) Bulk Density:- Accurately weighed quantity of the powder(W) is taken in measuring cylinder and volume (V_0) is measured, Bulk density is calculated using the formula.

Bulk density = Weight of the powder / Volume of powder

3)Tapped density determination:-

Accurately weighed quantity of the powder (W) is taken in a measuring cylinder and the volume occupied by powder is measured. The cylinder is fixed in Tapped Densitometer and is tapped for 500, 750 and 150 times till the difference in the volume after successive tapping was less than 2%. The final reading was denotes by (V_f).

Tapped density = W/V_f g/ml

4) Hausner's Ratio

Hausner found that the ratio was related to inter particle friction and as such, could be used to predict powder flow properties.

Hausner's factor = Tapped bulk density/Loose bulk density

5)Carr's Index:

Carr's Index is fast and popular method of predicting powder flow characteristics

Carr's index was calculated using the formula:

Carr's index=(Tapped Density- Bulk density)×100/Tapped Density

B) Post compression studies-

1) Appearance-

All tablets were inspected visually and found white colored round shaped and biconvex.

2) Thickness and Diameter-

Thickness and diameter of tablets was determined using Vernier caliper. Five tablets from each batch were used, and average values were calculated.

3) Weight variation Test-

To study weight variation, 20 tablets of each formulation were weighed using an electronic balance and the test was performed according to the official method.

4) Hardness-

For each type of formulation, the hardness values for 3 tablets were determined using Monsanto hardness tester.

5) Friability-

For each type of formulation, the friability was determined as follows

Twenty tablets were weighed accurately and placed in Roche friabilator. The speed rotation of Roche friabilator was kept 25 rpm for 4 min. The tablets were removed and weighed. The percentage friability was determined using following formula

% Friability = [Initial weight - Final weight] X 100/Initial weight)

Evaluation of prepared tablet blends for pre-compression study: The mass-volume relationship characteristics of a mixed blend were determined by characterization. Angle of repose, bulk density, and tapped density were all examined, with Hauser's ratio and compressibility index.

5) Disintegration time:

The measured disintegration time of tablets of each batch ranged between 5 Min to 8 Min.

6)In- Vitro Dissolution Study:

Speed of Paddle: 50 rpm.

Temperature of Dissolution Medium: 37°C ± 0.5°C.

All of the formulated tablets were disintegrated in vitro using the USP II Paddle technique at 50 rpm in 0.1 N HCL for the first 2 hours and 6.8 pH buffer solution for the remaining 10 hours. The temperature of the dissolving media was kept constant at 37.50°C. After 1, 2, 4, 6, 8, and 12 hours, 1 ml of the sample was extracted. To keep the volume consistent throughout the experiment, 1 ml of 0.1 N HCL and 6.8 pH buffer solution was employed. The samples were appropriately diluted, and the percentage of drug release from each formulation was determined using a UV-Spectrophotometer at 210 nm and 230 nm.

7) In vitro Release Kinetics Studies:

The analysis of drug release mechanism from a pharmaceutical dosage form is important but complicated process and is practically evident in the case of matrix systems. The order of drug release from SR was described by using zero order kinetics or first order kinetics. The mechanism of drug release from SR was studied by using Higuchi equation and the Peppa's-Korsemeyer equation.

1. Zero Order Release Kinetics:

It defines a linear relationship between the fractions of drug released versus time.

$$Q=k_0t.$$

Where, Q is the fraction of drug released at time t and k, is the zero-order release rate constant. A plot of the fraction of drug released against time will be linear if the release obeys zero order release kinetics.

2. First Order Release Kinetics:

Wagner assuming that the exposed surface area of a tablet decreased exponentially with time during as tablets could be described dissolution process suggested that the drug release from most of the slow adequately by the first-order kinetics. The equation that describes first order kinetics is

$\text{Log } C = \text{Log } C_0 - kt/2.303$

Where C is the amount of drug dissolved at time t,

Co is the amount of drug dissolved at t=0 and

k is the first order rate constant.

A graph of log cumulative of log % drug remaining Vs time yields a straight line. Will be linear if the release obeys the first order release kinetics.

3. Higuchi equation: It defines a linear dependence of the active fraction released per unit of surface (Q) and the square root of time.

$Q=K_2t^{1/2}$

Where K_2 is release rate constant. A plot of the fraction of drug released against square root of time will be linear if the release obeys Higuchi equation. This equation describes drug release as a diffusion process based on the Fick's law, square root time dependent.

4. Peppa's-Korseymere equation (Power Law):

In order to define a model, which would represent a better fit for the formulation, dissolution data was further analyzed by Peppa's-Korseymere equation (Power Law).

$$Mt/M_2=K.t^n$$

Where, Mt is the amount of drug released at time t

Mα is the amount released at time a.

$M_t/M\alpha$ is the fraction of drug released at time t,

K is the kinetic constant and n is the diffusion exponent.

To characterize the mechanism for both solvent penetration and drug release n can be used as abstracted. A plot between log drug release upto 60% against log of time will be linear if the release obeys Peppa's-Korsemeye equation and the slope of this plot represents "n" value the kinetic data of the formulations were included. Nature of release of the drug from the designed tablets was

inferred based on the correlation coefficient obtained from the plots of the kinetic models. The data were processed for regression analysis using PCP Disso1.

Table 3: Drug Release kinetics mechanism

Diffusion Exponent	Mechanism
0.45	Fickian diffusion
0.45<n<0.89	Anomalous(Non- Fickian) Diffusion
0.89	Case II transport
n>0.89	Super Case II transport

6.RESULT AND DISCUSSION

1. Pre-compression Studies:

The values of angle of repose (<30) indicates good flow properties of the powder. The bulk density and tapped density were found to range from 0.30±0.03 to 0.33±0.5 and 0.33±0.2 to 0.36±0.08 respectively. These results were satisfactory and may further influences the properties of the tablets. The hausner's ratio and carr's index results were also satisfactory.

Table.4 Evaluation of Powder Blend IR Enalapril Maleate

Batch	Angle of Repose(θ°)	Bulk Density(gm/ml)	Tapped Density(gm/ml)	Hausner's ratio	Carr's Index(%)
FEM1	27.88±0.42	0.31±0.02	0.33±0.02	1.06±0.031	6.42±1.12
FEM2	28.30±1.66	0.33±0.05	0.36±0.08	1.09±0.082	8.33±1.78
FEM3	27.10±1.03	0.31±0.04	0.35±0.02	1.12±0.071	11.42±1.32
FEM4	28.12±0.38	0.30±0.03	0.33±0.03	1.11±0.041	9.09±1.79
FEM5	26.34±0.45	0.31±0.02	0.34±0.04	1.09±0.012	8.82±1.61
FEM6	28.60±0.88	0.30±0.08	0.35±0.07	1.16±0.032	14.28±1.87

Table.5 Evaluation of Powder Blend SR Glibenclamide

Batch	Angle of Repose(θ°)	Bulk Density(gm/ml)	Tapped Density(gm/ml)	Hausner's ratio	Carr's Index
FG1	30.26±0.73	0.28±0.02	0.32±0.04	1.14±0.03	12.5±1.12
FG2	29.93±0.83	0.29±0.03	0.32±0.05	1.10±0.05	9.37±1.34
FG3	28.40±0.67	0.30±0.02	0.34±0.02	1.13±0.06	13.3±1.30
FG4	29.76±0.62	0.29±0.01	0.32±0.02	1.10±0.07	11.76±1.54
FG5	30.72±0.17	0.28±0.03	0.30±0.03	1.07±0.05	6.66±1.33
FG6	28.98±0.81	0.32±0.02	0.36±0.05	1.12±0.04	11.23±1.32

2. Post – Compression Studies

The average percentage deviation for all tablet formulation was found to be with in specified limit and all the formulation complied the weight variation test. All tablets showed hardness and thickness values were found the range between 6.0±0.43 to 7.99±0.77 and 4.13±0.003 to 4.23±0.09 respectively. The friability of all tablet formulations was found to be <1%, indicating that the friability is within the prescribed limits.

Table.6 Post-compression studies of Bilayer Tablet

Batch	Weight Variation	Thickness	Hardness	Friability
F1	296.24 ± 1.12	4.13±0.003	6.0±00.4	0.50±0.02
F2	298.39 ± 1.18	4.34±0.006	7.5±0.28	0.57±0.03
F3	297.50 ± 1.14	4.23±0.009	6.8±0.20	0.34±0.019
F4	299.36 ± 1.04	4.15±0.0012	6.8±0.56	0.45±0.45
F5	296.66 ±1.15	4.23±0.0012	6.0±0.40	0.50±0.20
F6	298.11±1.02	4.28±0.003	7.99±0.77	0.13±0.11

These table show the results of batches of F1-F6 IR Enalapril Maleate and SR Glibenclamide

Table.7 Disintegration Time of Enalapril Maleate Immediate Release Layer

Formulations	Disintegration Time
F1	**70 sec**
F2	**72 sec**
F3	**79 sec**
F4	**85 sec**
F5	**80 sec**
F6	**90 sec**

Dissolution Study: All of the formulated tablets were subjected to In vitro dissolution using the USP II Paddle technique at 50 rpm in 0.1 N HCl for the first 2 hours and 6.8 pH buffer solution for the remaining 10 hours. The temperature of the dissolving media was kept constant at 37°C. For Immediate release layer sample extracted after 5,10,15,30,45,60,90 minutes and for sustained release layer After 1, 2, 4, 6, 8, and 12 hours, 1 ml of the sample was extracted. To keep the volume

consistent throughout the experiment, 1 ml of 0.1 N HCl and 6.8 pH buffer solution was employed. The samples were appropriately diluted, and the percentage of drug release from each formulation was determined using a UV-Spectrophotometer at 210 nm and 230 nm.

Table.8 Dissolution study of Immediate Release Layer of Enalapril Maleate

Time (Min)	F1 %	F2%	F3%	F4%	F5%	F6%
0	0	0	0	0	0	0
5	10.21 ± 0.12	12.15 ± 0.25	14.04 ± 0.32	16.78 ± 0.51	19.02±0.87	22.08±0.67
10	16.11 ± 0.31	18.02 ± 0.65	27.65 ± 0.78	30.72 ± 0.36	34.62±0.13	35.66±0.21
15	29.99 ± 0.63	34.11 ± 0.52	41.77 ± 0.55	49.82 ± 0.62	54.3±0.23	59.41±0.12
30	40.25 ± 0.52	45.91 ± 0.33	56.21± 0.44	65.22 ± 0.47	69.29±0.89	88.41±0.16
45	55.77 ± 0.11	60.21 ± 0.98	71.01± 0.21	89.36 ± 0.34	91.44±0.22	99.43±0.76
60	68.17 ±0.28	72.01 ± 0.17	90.22 ± 0.16	99.4±0.11	99.44± 0.41	-
90	79.59± 0.10	83.35± 0.91	98.91± 0.32	-	-	-

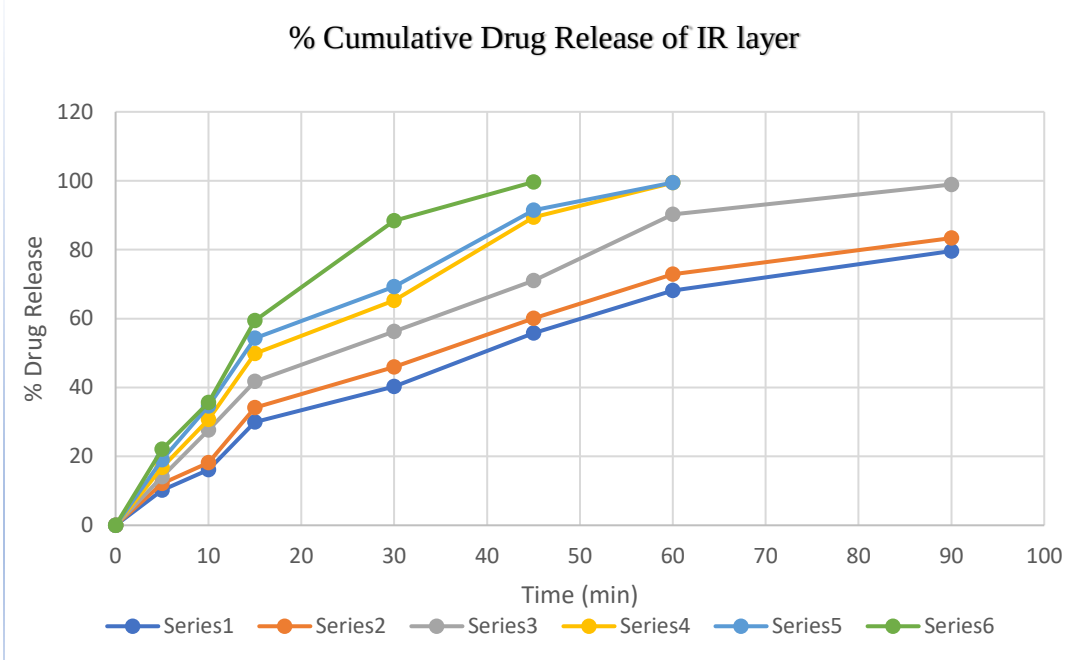


Fig.11 Cumulative Dissolution study of Batch FEM1 to FEM6

Table.9 Dissolution Study of Sustained Release Layer of Glibenclamide

Time (Hour)	FG1	FG2	FG3	FG4	FG5	FG6

0	0	0	0	0	0	0
1	15.44±0.88	13.73±0.83	16.76±0.11	16.76±0.98	17.71±023	20.33±0.22
2	36.32±0.26	32.73±0.89	38.99±0.32	34.07±0.76	35.15±0.76	40.11±0.10
4	69.22±0.89	62.89±0.32	49.99±0.21	63.61±0.85	64.87±0.44	59.13±0.12
6	88.38±0.28	85.99±0.54	61.78±0.65	82.31±0.31	85.45±0.32	70.22±0.33
8	99.43±0.89	90.21±0.43	78.88±0.43	99.12±0.39	90.06±0.98	80.54±0.54
10	-	99.90±0.98	89.65±0.11	-	99.98±0.31	91.11±0.88
12	-	-	99.98±0.34	-	-	97.22±0.23

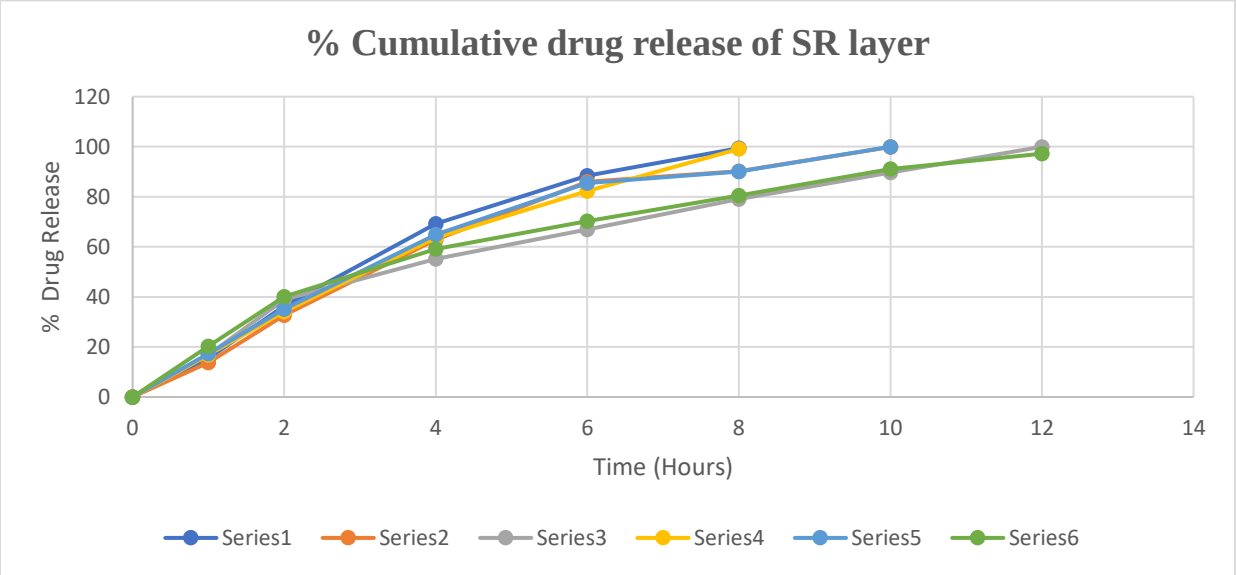
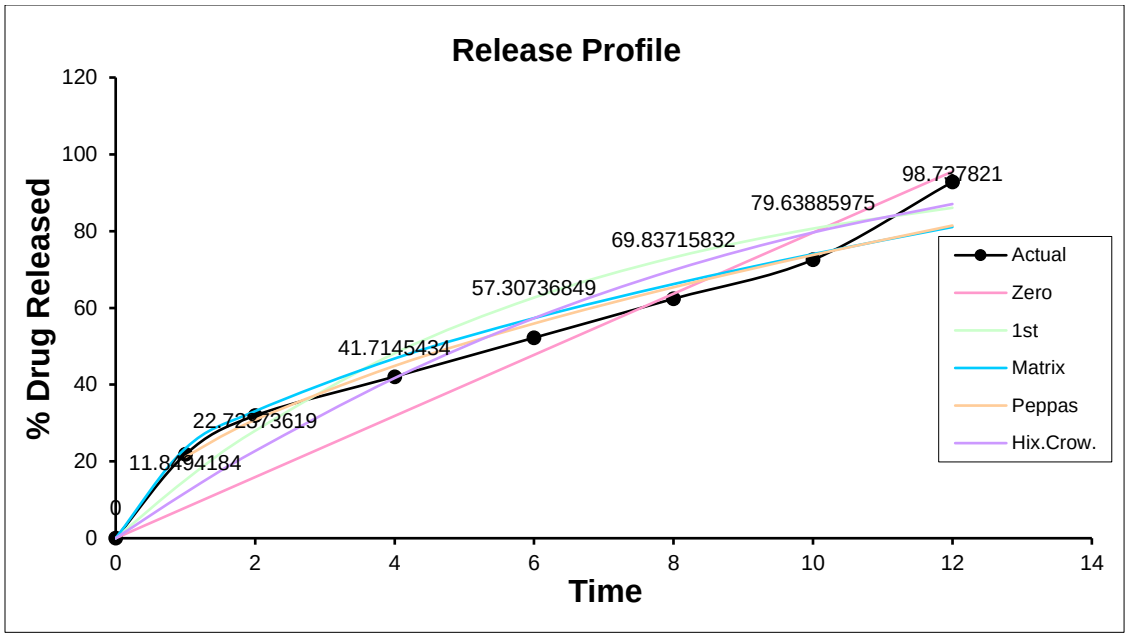


Fig.11 Cumulative Dissolution study of Batch FG1 to FG6

The formulation F3 as an optimized formulation because of these batch showed satisfactory result of the tablets parameter. Result of Glibenclamide and Enalapril Maleate in vitro 99.98% and 98.91 % drug release profile an indicated that formulation (F3) was the most promising formulations as the drug release from this formulation was high as compared to other formulations.

4.4. In-Vitro Release Kinetics Studies:

• The In -vitro dissolution data of Glibenclamide SR formulations was subjected to goodness of fit test by linear regression analysis according to zero order and first order kinetic equations, Higuchi's and Korsmeyer- Peppas models to assess the mechanism of drug release. It was observed from the above, that dissolution of all the tablets followed zero order kinetics with co-efficient of determination (R^2) values above 0.9834. Kinetic data also treated for Peppas equation, the absorbed slope (n) value is that shows 0.9874 • Non-Fickian diffusion mechanism. The kinetic result reveals that, the best fit model for F3 formulation is Korsmeyer- Peppas models with highest correlation coefficient (r2) value i.e. 0.9525.



Model Fitting (Average)-

	R	k
Zero order	0.9834	7.4632
T-test	13.275	(Passes)
1st order	0.9072	-0.1502
T-test	5.282	(Passes)
Matrix	0.9525	21.5373
T-test	7.658	(Passes)
Peppas	0.9874	14.3904
T-test	15.278	(Passes)
Hix.Crow.	0.9535	-0.0381
T-test	7.747	(Passes)

5.5 Stability Study:

Formulation Batch F3 for Enalapril Maleate is 98.91% and for Glibencamide is 99.98 % Stability study: Stability study for the developed formulation F3 were carried out as per ICH guideline by storing at 40°C/75% RH up to three months. The formulation F3 was selected on the basis of their high cumulative percentage drug release.

Table 10. Stability study of Bilayer tablet

Parameters	Initial Release	Final Release
Hardness	5-6	5-6
% of Drug Release (Batch F3)	**99.98 SR** **98.91 IR**	**97.98 SR** **96.01 IR**

The stability study showed that the formulation F3 was physically stable when stored at 40±20°C and 75±5% RH for three months and there was no significant difference in dissolution parameters of optimized formulation.

Conclusion:

In the present study, Glibenclamide is Sulphonylureas agent, an antidiabetic agent Enalapril Maleate is Angiotensin Converting enzyme inhibitor agent was successfully prepared in the form of Bilayer. Glibenclamide and Enalapril maleate was prepared by using HPMC, Ethyl cellulose sustain release polymer and Croscarmellose as Super disintegrating polymer by using Direct Compression method, The Results of the Formulation F3 were was also satisfying was stable in compared to Formulation kept in Room Temperature as no significant growth in particle size was found in the Optimized batch. Comparison of in-vitro % Drug Release of pure drug and optimized Batch Bilayer of Glibenclamide and Enalapril Maleate was also carried out. Optimized Batch F3 Enalapril Maleate showed 99.89% at 90 mins and Glibenclamide showed 98.22 % Drug Release in 12 hr.

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Formulation and Evaluation of Sustained Release Matrix Tablet of Lamivudine

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ABSTRACT-

The main objective of present work was to formulate and evaluate sustained release matrix tablet of lamivudine using different polymers viz. Xanthan Gum, Ethyl Cellulose. Lamivudine an Antiretroviral agent which comes under BCS class III which has high solubility and low permeability, was chosen for the study. Formulation of matrix tablets was prepared by using powder blend of different ratios of polymer to get desirable drug release profile. Direct compression method was used to formulate tablets. The evaluation of physical properties of tablet were done, the in-vitro drug release study was performed in 0.1 N HCL for 2 hours and in phosphate buffer PH 6.8 up to 10 hours. Evaluation parameters of formulated matrix tablets were hardness, friability, thickness, drug content, weight variation, and the in-vitro drug release rate pattern results indicated that the formulation F3 was the most promising formulation as the drug release from this formulation was high as compared to other formulations. In formulation F3, percentage drug release of lamivudine sustained release was 98.81±0.63.

KEYWORDS: Matrix Tablets, Sustained Release, Lamivudine, Xanthan Gum, Direct Compression.

1. INTRODUCTION

A sustained-release dosage form is defined as "any drug or dosage form modification that prolongs the therapeutic activity of the drug". Development of sustained release tablets of highly water-soluble drugs has always been a challenge and therefore, most of these drugs if not formulated properly, may be released at a faster rate resulting in exceeding the maximum therapeutic levels, hence will lead to toxic side effects. Sustained delivery of such drugs ensures improved drug delivery and patient compliance, greater safety and efficacy, desired release kinetics and helps in maintaining the plasma drug concentration within the therapeutic window for extended period of time[1]. In long-term therapeutic concern for the treatment of chronic

disease conditions, conventional formulations are required to be administered in multiple doses and therefore have several disadvantages. However, when administered orally, many therapeutic agents are subjected to extensive presystemic elimination by gastrointestinal degradation or first pass hepatic metabolism which leads to low systemic bioavailability and formation of inactive or toxic metabolites. Controlled release (CR) tablet formulations are preferred for such therapy because they offer better patient compliance, maintain uniform drug levels, reduce dose and side effects, and increase the safety margin for high-potency drugs[2].

Oral controlled drug delivery system represents one of the frontier areas of drug delivery system in order to fulfill the need for a long-term treatment with anti-HIV agents. Among the different controlled drug delivery (CDD) systems, matrix based controlled release tablet formulations are the most popularly preferred for its convenience to formulate a cost effective manufacturing technology in commercial scale. Development of oral controlled release matrix tablets containing water-soluble drug has always been a challenging because of dose dumping due to improper formulation resulting in plasma and fluctuation of accumulation toxic concentration of drug. The use of polymers in controlling the release of drugs has become an formulation important tool in the of pharmaceutical dosage forms. Over many years, numerous studies have been reported in the literature on the application of hydrophilic polymers in the development of controlled release matrix systems for various drug[3].

The acquired immunodeficiency syndrome (AIDS), a disorder in which the immune system begins to fail and life-threatening opportunistic infections develop, can be brought on by the human immune deficiency virus (HIV), a retrovirus. Both HIV-1 and HIV-2 are contagious and AIDS-causing agents. There are two types of HIV. Lack of host immune system control over HIV replication leads to disease development. A person develops aids once HIV infection weakens the immune system to the point where the body can no longer defend itself against other infections and malignancies (fewer than 200 CD4+ cells per microliter of blood). Despite the lack of a medicine to treat this illness, there are ways to limit its progression[4]. A synthetic nucleoside analogue called lamivudine is being used more frequently as the main component of an antiretroviral regimen to treat HIV infection. By competitively inhibiting viral reverse transcriptase and stopping proviral DNA chain extension, nucleoside analogues are phosphorylated intracellularly by endogenous kinases to putatively active 5'-triphosphate (3TC-TP) derivatives that stop HIV replication in vivo. Lamivudine belongs to BCS class III with high solubility and low permeability. Lamivudine is rapidly absorbed after oral administration with an absolute bioavailability of 86% 16%, a peak serum concentration of lamivudine (Cmax) of 1.5 0.5 mcg/mL, and a mean elimination half-life (t) of 5-7 hours, lamivudine is rapidly absorbed after oral administration, necessitating frequent administration to maintain constant therapeutic drug levels[5].Therefore, the objective of present work is to provide a prolong action of pharmaceutical composition containing lamivudine in a sustained release matrix formulation, to maintain constant drug level into blood for prolong period of time.

2. MATERIALS AND METHODS

2.1 MATERIALS:

Lamivudine was obtained from Yarrow chem. Pharmaceuticals, Mumbai, India. Ethyl cellulose, Xanthan gum, Microcrystalline-cellulose, Magnesium stearate and talc were obtained from Research fine lab, Mumbai, India.

2.2 METHOD OF PREPARATION OF MATRIX TABLET OF LAMIVUDINE:

Tablets were prepared by direct compression method. For the preparation of powder blend all ingredients were weighted accurately. Lamivudine, Polymers, MCC were weighed properly and triturate thoroughly. The above blend was lubricated with talc and magnesium stearate and passed through sieve #40 to break any lumps. The powder blends were compressed into tablets by direct compression technique on rotary tabletting machine. Before compression the surface of die and punch were lubricated with talc and powder blend compressed into tablets. These Tablets of each formulation were further evaluated for various properties.

Table1. Formulation of Lamivudine Sustained Release Matrix Tablet

Ingredients (mg)	F1	F2	F3	F4	F5	F6
Lamivudine	150	150	150	150	150	150
Xanthan Gum	40	80	120	-	-	-
Ethyl Cellulose	-	-	-	40	80	120
MCC	97	57	17	97	57	17
Magnesium Stearate	8	8	8	8	8	8
Talc	5	5	5	5	5	5
Total	300	300	300	300	300	300

3. EVALUATION OF PREPARED SUSTAINED MATRIX TABLETS

A) Pre-compression studies: [6,7]

1) Angle of Repose-

Angle of repose was determined by funnel method. The accurately weighted quantity of granules was taken in funnel. The height of the funnel was adjusted in such a way that the tip of the funnel just touched the apex of the heap of the granules.

The granule was allowed to flow through the funnel freely on to the surface. The diameter of the powder cone was measured and angle of repose was calculated using the following equation

$\theta = \tan^{-1} h/r$

Where, h and r are the height and radius of the powder cone.

Table 2. Flow Properties and Corresponding Angle of Repose

Flow Property	Angle of Repose (degrees)
Excellent	25-30
Good	31-35
Fair	36-40
Passable	41-55
Poor	46-55
Very poor	56-65
Very very poor	<65

2) Bulk Density-

Both loose bulk density (LBD) and tapped bulk density (TBD) were determined. A quantity of granules lightly shaken to break any agglomerates formed was introduced into a 10 ml measuring cylinder. After the initial volume observed, the cylinder was allowed to fall under its own height onto hard surface from the height of 2.5 cm at 2 seconds interval. The tapping was continued until no further change in the volume was noted. (LBD) and (TBD) were calculated by using the following formulas

LBD = Weight of the powder / volume of the packing

TBD = Weight of the powder /tapped volume of the packing

3) The compressibility index

The compressibility index of the granules was determined by Carr's compressibility index Carr's index (%) = [(TBD – LBD) x 100]/TBD

4) Hausner's Ratio

The Hausner's ratio was related to inter particle friction and it could be used to predict powder flow properties.

Hauser's factor = Tapped bulk density/Loose bulk density

B) Post compression studies-[8,9]

1) Appearance-

All tablets were inspected visually and found white coloured round shaped and biconvex.

2) Thickness-

Thickness and diameter of tablets was determined using Vernier calliper. Five tablets from each batch were used, and average values were calculated.

3) Weight variation Test-

To study weight variation, 20 tablets of each formulation were weighed using an electronic balance and the test was performed according to the official method.

4) Hardness-

For each type of formulation, the hardness values for 3 tablets were determined using Monsanto hardness tester.

5) Friability-

For each type of formulation, the friability was determined as follows

Twenty tablets were weighed accurately and placed in Roche friabilator. The speed rotation of Roche friabilator was kept 25 rpm for 4 min. The tablets were removed and weighed. The percentage friability was determined using following formula

% Friability = [Initial weight - Final weight] X 100/Initial weight)

6) In-Vitro Dissolution study-

The study was carried out using dissolution apparatus USP Type-II (paddle)

Speed of Paddle: 50 rpm.

Temperature of Dissolution Medium: 37°C ± 0.5°C.

In vitro Dissolution Study 900 ml of 0.1N HC1 was placed in the vessel and the USP-II apparatus (Paddle method) was assembled. The medium was allowed to equilibrate to temperature of 37°C+0.5°C. A tablet was placed in the vessel and was covered; the apparatus was operated up to 2 hours at 50 rpm. After completion of 2 hours remove the 0.1N HCL and add 6.8 phosphate buffer then continue the apparatus up to 10 hours. At definite time intervals, 5 ml of dissolution medium was withdrawn; filtered and again replaced with 5 ml of fresh medium to maintain sink conditions. Suitable dilutions were done with dissolution medium and were analysed spectrophotometrically at λ_{max}=270 nm using a UV-spectrophotometer.

Table 3. Dissolution Parameters

Parameters	**Detail's**
Dissolution apparatus	USP-Type II (Paddle)
Medium	0.1 N HCL and 6.8 Phosphate buffer
Volume	900 ml
Speed	50 rpm
Temperature	37°C ± 0.5°C.
Sample Volume Withdrawn	1 ml
Time Points	1,2,4,6,8,10 and 12 hours
Analytical Method	Ultraviolet Visible Spectroscopy
λ_{max}	270 nm

7) In-Vitro Release Kinetics Studies:

Different kinetics models (Zero-order, First-order, Korsmeyer's and Hixon Crowell) were applied to interpret the release profile from matrix system. The analysis of drug release mechanism from a pharmaceutical dosage form is important but complicated process and is practically evident in the case of matrix systems. The order of drug release from ER was described by using zero order kinetics or first order kinetics. The mechanism of drug release from ER was studied by using Higuchi equation and the Peppa's-Korsemeyer equation.

1. Zero Order Release Kinetics:

It defines a linear relationship between the fractions of drug released versus time.

$Q= k_o t$.

Where, Q is the fraction of drug released at time t and k, is the zero order release rate constant. A plot of the fraction of drug released against time will be linear if the release obeys zero order release kinetics.

2. First Order Release Kinetics:

Wagner assuming that the exposed surface area of a tablet decreased exponentially with time during dissolution process suggested that the drug release from most of the slow release tablets could be described adequately by the first-order kinetics. The equation that describes first order kinetics is

Log C= Log C_o -kt/2.303

Where C is the amount of drug dissolved at time t,

C_o is the amount of drug dissolved at t=0 and

k is the first order rate constant.

A graph of log cumulative of log % drug remaining Vs time yields a straight line. Will be linear if the release obeys the first order release kinetics.

3. Higuchi equation:

It defines linear dependence of the active fraction released per unit of surface (Q) and the square root of time.

$Q=K_2t^1/2$

Where K_2 is release rate constant. A plot of the fraction of drug released against square root of time will be linear if the release obeys Higuchi equation. This equation describes drug release as a diffusion process based on the Fick's law, square root time dependent.

4. Peppa's-Korsemeyer equation (Power Law):

In order to define a model, which would represent a better fit for the formulation, dissolution data was further analysed by Peppa's-Korsemeyer equation (Power Law).

$Mt/ M_\infty=K.t^n$

Where, Mt is the amount of drug released at time t

M_α, is the amount released at time α

M_t/M_α, is the fraction of drug released at time t,

K is the kinetic constant and n is the diffusion exponent.

To characterize the mechanism for both solvent penetration and drug release n can be used as abstracted. A plot between log drug release upto 60% against log of time will be linear if the release obeys Peppa's-Korsemeyer equation and the slope of this plot represents "n" value the kinetic data of the formulations were included. Nature of release of the drug from the designed tablets was inferred based on the correlation coefficients obtained from the plots of the kinetic models. The data were processed for regression analysis using PCPDisso1.

4. RESULTS AND DISCUSSIONS

4.1. Pre-compression Studies:

The values for bulk density and tapped density were found to range from **0.49±0.06** to **0.62±0.02** and **0.38±0.03** to **0.44±0.03** respectively. These results were satisfactory and may further influences the properties of the tablets. The values of angle of repose (<30) indicates good flow properties of the powder. The Hausner’s ratio and Carr’s index results were in the limits.

Table.4 Pre-compression studies of Lamivudine SR tablet

Formulation Code	Angle of Repose (θ)	Bulk Density (g/ml)	Tapped Density (g/ml)	Hauser's ratio	Carr's Index (%)
F1	32.26±0.4	0.61±0.06	0.44±0.03	1.38±0.01	16.88±0.05
F2	31.13±0.7	0.58±0.01	0.41±0.05	1.41±0.04	15.87±0.07
F3	29.20±0.3	0.49±0.06	0.38±0.03	1.28±0.02	14.23±0.04
F4	33.12±0.5	0.62±0.02	0.43±0.06	1.44±0.06	17.32±0.01
F5	32.23±0.4	0.61±0.04	0.42±0.02	1.45±0.03	16.46±0.02
F6	30.46±0.6	0.50±0.05	0.39±0.04	1.28±0.04	15.39±0.03

4.2. Post-compression Studies

The average percentage deviation for all tablet formulation was found to be with in specified limit and all the formulation complied the weight variation test. All tablets showed hardness and thickness values were found the range between 5.8±0.46 to 6.3±0.27 and 4.10±0.13 to 4.21±0.09 respectively. The friability of all tablet formulations were found to be <1%, indicating that the friability is within the prescribed limits.

Table.5 Post- compression studies of Lamivudine SR tablet

Formulation code	Weight variation	Hardness	Friability	Thickness
F1	296.89±0.23	5.9±0.43	0.59±0.58	4.14±0.15
F2	294.88±0.45	5.8±0.46	0.67±0.33	4.10±0.13
F3	297.80±0.33	6.1±0.27	0.59±0.28	4.21±0.09
F4	299.88±0.61	5.9±0.49	0.61±0.36	4.16±0.03
F5	298.96±0.61	6.1±0.39	0.58±0.24	4.18±0.02
F6	298.74±0.56	6.2±0.34	0.59±0.36	4.13±0.07

4.3. In-vitro dissolution Parameters of Lamivudine SR tablets:

The in-vitro drug release study for all the batches of Lamivudine was carried out using paddle method (USP apparatus). Among all the formulations (F1 to F6), the rate and extend of drug release was decreased with increasing polymer concentration. Data for in-vitro drug release study is presented in the following table 6 and graphical representation of graphical drug release vs. time graph is shown in the figure 1.

Table.6 Percentage Drug Release of Each Batch of Lamivudine

Time (Hours)	F1	F2	F3	F4	F5	F6
0	0	0	0	0	0	0
1	23.31±0.47	17.88±0.23	22.51±0.42	35.86±0.55	19.36±0.41	21.26±0.36
2	41.25±0.24	32.55±0.29	31.09±0.54	59.36±0.61	29.65±0.36	30.56±0.37
4	61.63±0.31	46.22±0.44	42.86±0.56	84.25±0.45	42.25±0.21	42.59±0.51
6	83.22±0.40	65.84±0.35	55.36±0.35	99.96±0.32	56.24±0.25	52.68±0.46
8	99.86±0.28	88.23±0.65	68.25±0.84		74.96±0.34	66.89±0.71
10		98.47±0.54	83.76±0.25		97.78±0.43	84.58±0.44

12			98.81±0.63			92.78±0.53

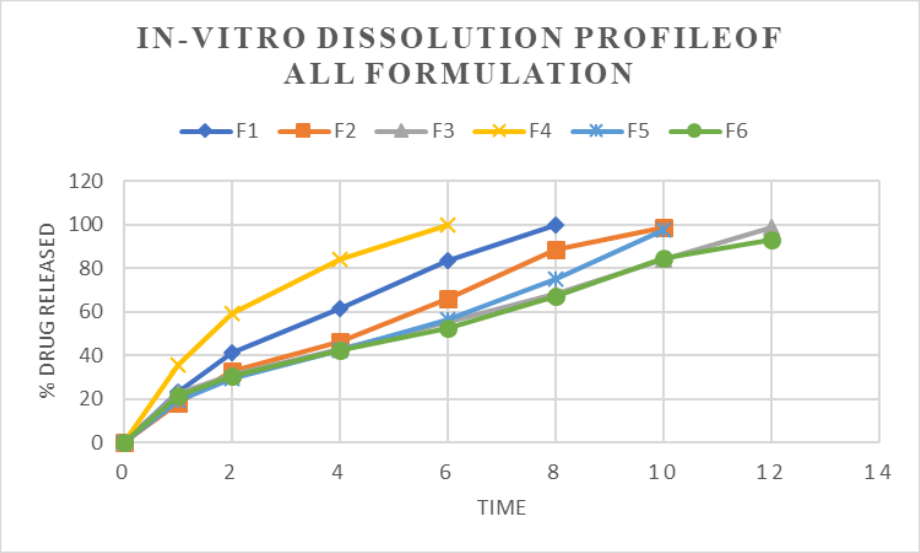

Figure.1 In-vitro dissolution profile of all formulations

4.4. In-Vitro Release Kinetics Studies:

The In -vitro dissolution data of lamivudine SR formulations was subjected to goodness of fit test by linear regression analysis according to zero order and first order kinetic equations, Higuchi's and Korsmeyer-Peppas models to assess the mechanism of drug release. The results of linear regression including regression coefficients are summarized in Table 7 and plots shown in Fig.2. It was observed from the above, that dissolution of all the tablets followed zero order kinetics with co-efficient of determination (R^2) values above 0.984. Kinetic data also treated for Peppas equation, the absorbed slope (n) value is 0.9935 that shows Non-Fickian diffusion mechanism. The kinetic result reveals that, the best fit model for F3 formulation is Korsmeyer-Peppas models with highest correlation coefficient (r2) value i.e. 0.9935.

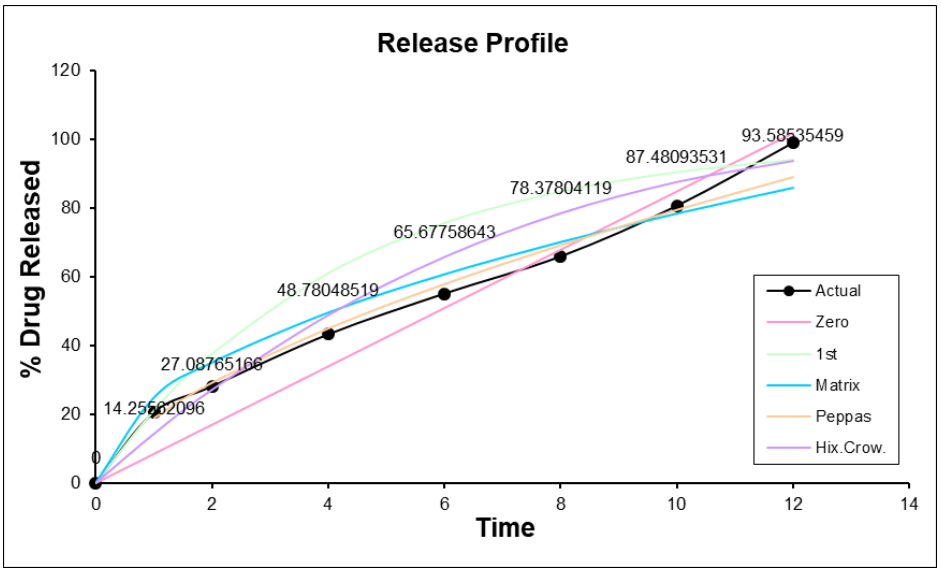

Figure.2 In-vitro Kinetics profile of F3 formulation

Table 7. Kinetics Study Table

	R	K
Zero Order	0.9927	8-4847
First Order	0.8868	-0.2358
Matrix	0.9777	24-7541
Peppas	0.9935	19-0911
Hix-Crow.	0.9374	-0-0500

4.5. Stability Study:

Stability study for the developed formulation F3 were carried out as per ICH guideline by storing at 40°C/75% RH up to three months. The formulation F3 was selected on the basis of their high cumulative percentage drug release.

Table 8. Stability study

Parameters	Initial	Final
Hardness	5-6	5-6
% of Drug Release (Batch F3)	98.81	97.65

The stability study showed that the formulation F3 was physically stable when stored at 40±20°C and 75±5% RH for three months and there was no significant difference in dissolution parameters of optimized formulation.

CONCLUSION

The present work was to formulate and evaluate sustain release matrix tablets of Lamivudine by using natural and semi-synthetic polymer to sustain the drug release from matrix tablet. The sustained release drug delivery was a promising approach to achieve a prolonged therapeutic action of drug. The matrix forming polymers, Xanthan gum, Ethyl Cellulose were studied. The amount of drug release for optimized formulation F3 was found to be 98.81±0.63. The cumulative percentage drug was decreased by increase in polymer concentration.

Physiochemical characteristics were used to assess the prepared tablet. The physiochemical analysis of the tablet reveals a white colour, a round form, and a smooth look. The formulation F3 as an optimized formulation because of this batch showed satisfactory result of the tablets parameter. Result of in vitro % drug release profile an indicated that formulation (F3) was the most promising formulations as the drug release from this formulation was high as compared to other formulations. So, F3 was found to be optimized formulation and was selected for further stability study. Also, Stability study of optimized batch is showed satisfactory result.

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FORMULATION AND EVALUATION OF ANTIDIABETIC BILAYER TABLET OF GLIMEPIRIDE AND PIOGLITAZONE HCL

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Abstract: *The aim of present study was to develop and submit antidiabetic bilayer tablet of sustain release Glimepiride and Immediate release Pioglitazone HCl. The drug procurement was completed from the gift sample from various companies. Sustained release of Glimepiride developed by using polymer like HPMC K4M and HPMC K100M. Super disintegrants such as Crospovidone and Kyron T-314 are used to prepare the immediate release layer. The drug excipient compatibility study was done with help of FTIR and there was no chemical interaction found in the study. The powder blend was evaluated for the various aspects such as the angle of repose, bulk density, tapped density, Hausner's ratio, Carr's index. The results were satisfied and showed the good result hence the conclusion that we can go for the tablet manufacturing. The tablets were manufactured with help of the direct compression method. The prepared tablets were evaluated for different tests and found in limit of uniformity of weight, hardness, thickness, diameter, and friability. Tablets from each batch studied for the drug content and was found within range of 96-99%, disintegration test was performed, the time was found 62-85sec. The In vitro dissolution was performed by using USP Type II apparatus. The release data further indicated that HPMC K100M can give the sustained release with maximum drug release up to 12 hrs. Which shows minimize the burden of dosing. Immediate Release layer IR6 containing Kyron T314 shows maximum drug release about 98.10 % up to 40 min. than other formulations. HPMC K100M polymer controlled the release of Glimepiride up to 12 hr. intended for once daily administration. The release data of In vitro study indicates that formulation follows zero order, Higuchi equation and diffusion takes place via non-fickian transport. The optimized tablets were studied for the stability study for period of 3 months. Formulation F3 found to be stable at accelerated stability as per the ICH guidelines for a period of 3 months.*

Keywords: *Type 2 diabetes, Bilayer Tablet, Glimepiride, Pioglitazone HCl, HPMC K100 M, HPMC K4M, Kyron T 314, Crospovidone.*

INTRODUCTION

Oral route is one of the most popular routes of drug delivery due to its ease of dministration, patient compliance, least sterility constraints and flexible design of dosage form. The oral route of administration is the most preferred route due to its many advantages like ease of administration, accurate dosage, self-medication, pain avoidance, versatility and patient compliance. Tablets and capsules are

the most popular dosage forms. Ideally a drug to provide desired therapeutic action should arrive rapidly at the site of action in optimum concentration, remain there for the desire time, be excluded from other site[1,2]. The fact that absorption rate of drug into the body can be decreased by reduction of the rate of release of the drug from the dosage form is one of the most recent and interesting result of pharmaceutical research, but one important condition including stroke, Parkinson's disease, neurological disorders, AIDS etc. Dual release Tablets is a unit compressed Tablets dosage for intended for oral Application. It contains two layers in which one layer having conventional or immediate release part of single or multiple actives; another layer is sustained or controlled release part of single or multiple actives. They are also called as Bilayer Tablets, multi-layer Matrix Tablets. A bilayer Tablets is a type of multiple compressed Tablets. Tablets are composed of two layers of granulation compressed together. Monograms and other distinctive marking may be compressed in the surface of the bilayer Tablets. Coloring the separate layer provide many possibilities for unique Tablets identity. There are some applications like Bilayer Tablets are mainly used in the combination therapy. Bilayer Tablets are used to deliver the loading dose and sustained dose of the same or different drugs. Bilayer Tablets are used to deliver the two different drugs having different release. They are used as an extension of a conventional technology Potential use of single entity feed granules. Patient compliance is enhanced leading to improved drug regimen efficacy. Patient convenience is improved because fewer daily doses are required compared to traditional drug delivery system. Maintain physical and chemical stability. Retain potency and ensure dose accuracy [3-4].

Pioglitazone HCl is Thiazolidinedione (TZD) class of drug with hypoglycemic, antihyperglycemic and antidiabetic action. Chemically Pioglitazone is (RS)-5-(4-[2-(5-ethylpyridin-2-yl) ethoxy] benzyl) thiazolidine-2, 4-dione. Pioglitazone is used for the treatment of diabetes mellitus type 2 (previously known as non-insulin-dependent diabetes mellitus, NIDDM) in monotherapy and in combination with a sulfonylurea, Metformin. Pioglitazone has also been used to treat non-alcoholic fatty liver. Pioglitazone has also been found to reduce the risk of conversion from prediabetes to diabetes mellitus type 2 by 72%. It has short biological half-life of 3-5 hrs. [5,6]. Glimepiride acts at ATPase-dependent potassium channels in β cells of the pancreas to stimulate insulin release. using glycemic and hyperglycemic clamp studies it has been shown to improve both first- and second-phase insulin secretion.15 Glimepiride binds to 65-kD proteins on β cells. In healthy volunteers, a linear relationship was shown between serum Glimepiride concentrations and insulin release during Euglycaemia and a nearly linear relationship under Hyperglycemic conditions [7,8].

MATERIAL AND METHOD

The API's and excipients were obtained as gift sample from various companies. The Pioglitazone obtained from Brundavan Chemicals, Hydrabad. Glimepiride obtain from the Surya chemicals, Mumbai. The Signet Excipients Pvt. Ltd. Mumbai provided various excipients of pharmacopeial grades such as Lactose, Magnesium Sterate, Talc. Nb Entrepreneurs, Nagapur Provide Avicel Ph 101 Ip. Superdisintegrant Such As Kyron T-314 Supplied By Corel Pharma Chem, Ahmedabad. Crospovidone Given By Prachin Chemical, Ahmedabad.

All ingredients were collected and weighed accurately. Sifted API's and polymers through sieve no. 60# and then mixed with remaining excipients. Sifted talc and magnesium stearate separately, through sieve no. 60#. Pre-blending of all ingredients (except lubricant magnesium stearate) in blended for 15 minutes. Blend then again blended for 5-6 min then added magnesium stearate blended 5 min. Lubricated powder was compressed by rotary machine. Compressed tablets were examined as per official standards and unofficial tests. Prior to the compression the drug and polymers were evaluated for several tests.

EXPERIMENTAL WORK

Determination of Absorption Maxima of drugs

Confirmation of Pioglitazone HCl through UV spectral analysis:

The stock solution of, prepared by about 10 mg of Pioglitazone HCl was accurately weighed and dissolved in 100 ml of methanol to obtain a concentration 100 µg/ml. From stock solution different aliquots were taken in series of 0.2, 0.4, 0.6, 0.8, 10 ml in 10 ml volumetric flask and diluted with methanol to obtain a series of concentration. The solutions were scanned in spectrophotometer in UV range 200 - 400 nm. The absorption maxima of Pioglitazone were found to be 268 nm. The standard curve was plotted and values of slope, intercept and coefficient of correlation were calculated. The standard curve was plotted and values of slope, intercept and coefficient of correlation were calculated.[9]

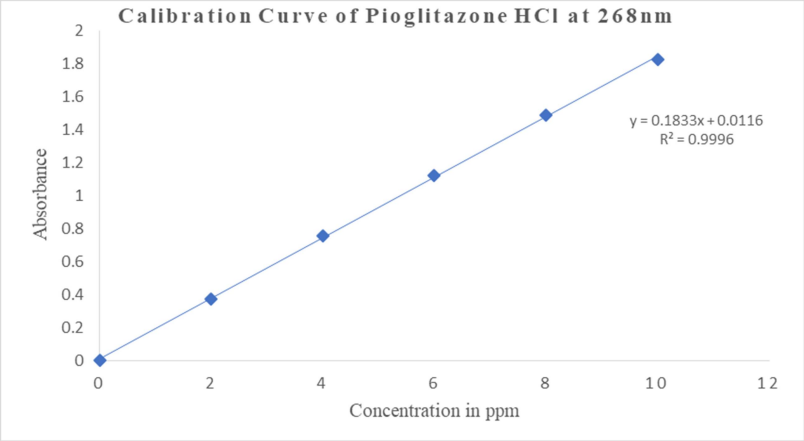

Fig 1: Calibration curve of Pioglitazone HCl in methanol.

Estimation of Glimepiride

Preparation of calibration curve of Glimepiride in Methanol: -

The stock solution of, prepared by about 10 mg of Glimepiride was accurately weighed and dissolved in 100 ml of methanol to obtain a concentration 100 µg/ml. From stock solution different aliquots were taken in series of 0.2, 0.4, 0.6, 0.8, 10 ml in 10 ml volumetric flask and diluted with methanol to obtain a series of concentration. The solutions were scanned in spectrophotometer in UV range 200 - 400 nm. The absorption maxima of Glimepiride were found to be 228 nm. The standard curve was plotted and values of slope, intercept and coefficient of correlation were calculated.[10]

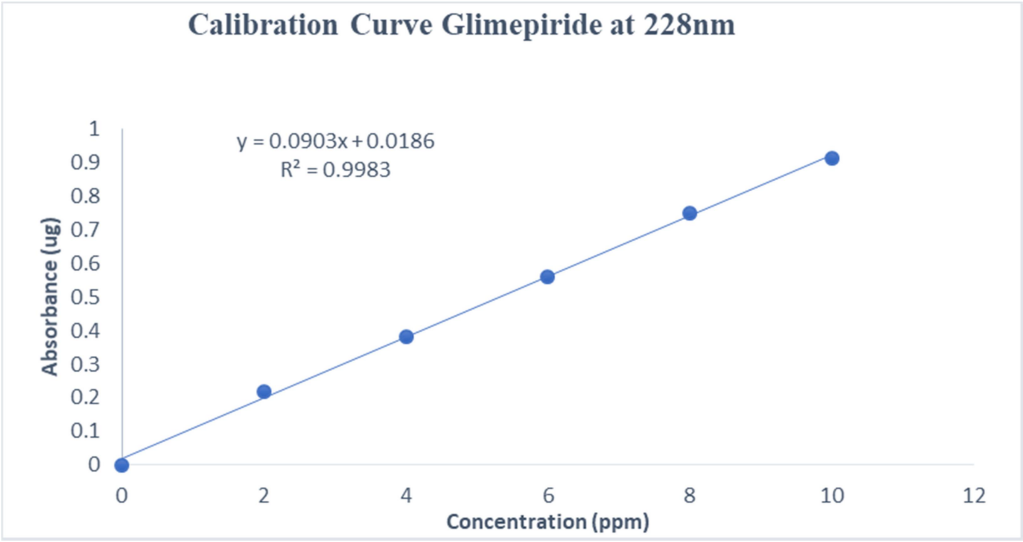

Fig 2: Calibration curve of Glimepiride in Methanol

Ftir Spectroscopy

The active pharmaceutical ingredient was identified by FTIR analysis of the sample obtained from sources. The sampling technique was mixing the API with the KBr and forming of the pellet which was then analyzed in 400-4000 wave number range by the FTIR Spectrophotometer.

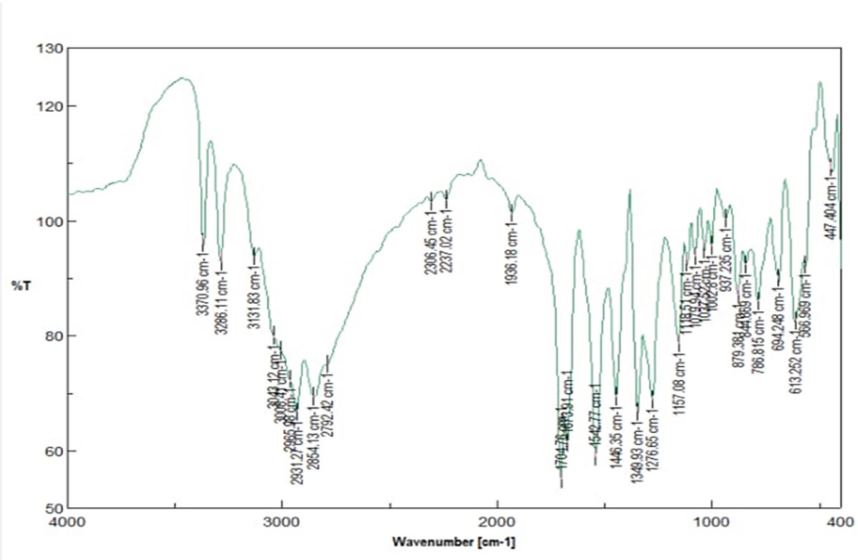

Fig 3: FT-IR of pure Glimepiride

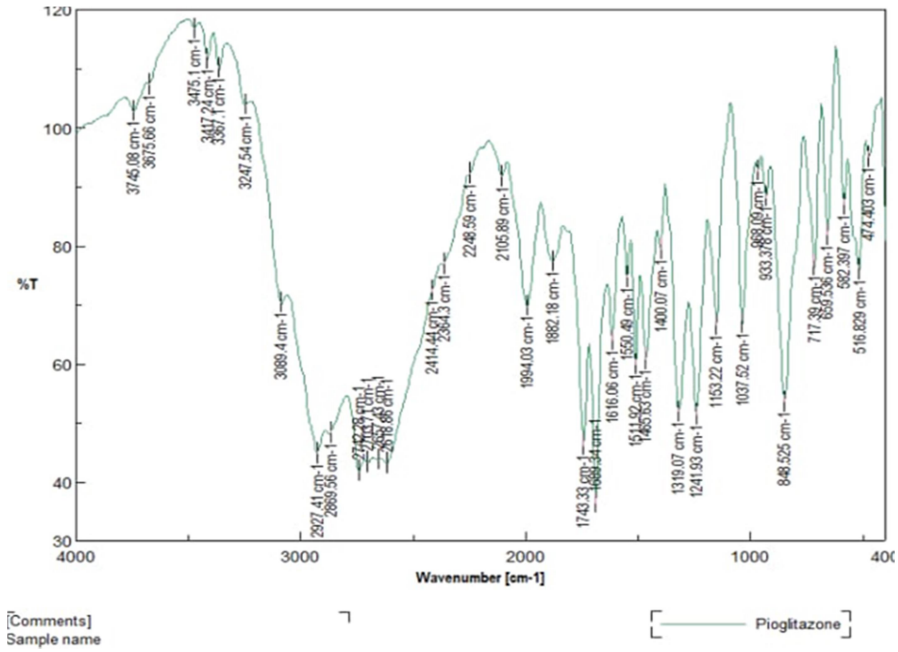

Fig 4: FT-IR of pure Pioglitazone HCl

Drug excipient Compatibility study

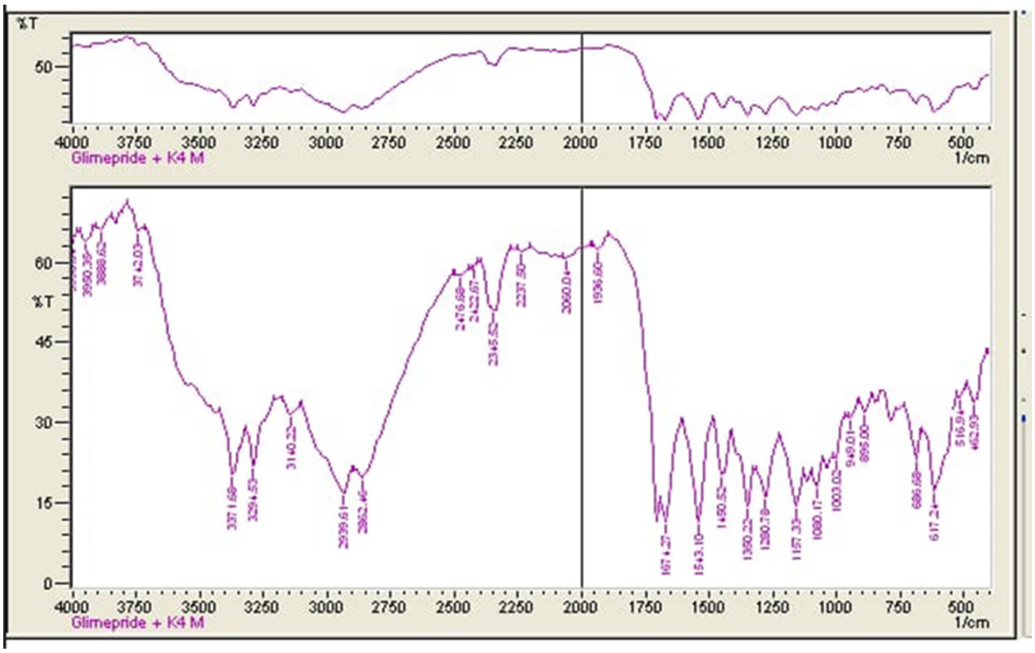

Fig 5: FTIR of Physical Mixture of Glimepiride + HPMC K4M

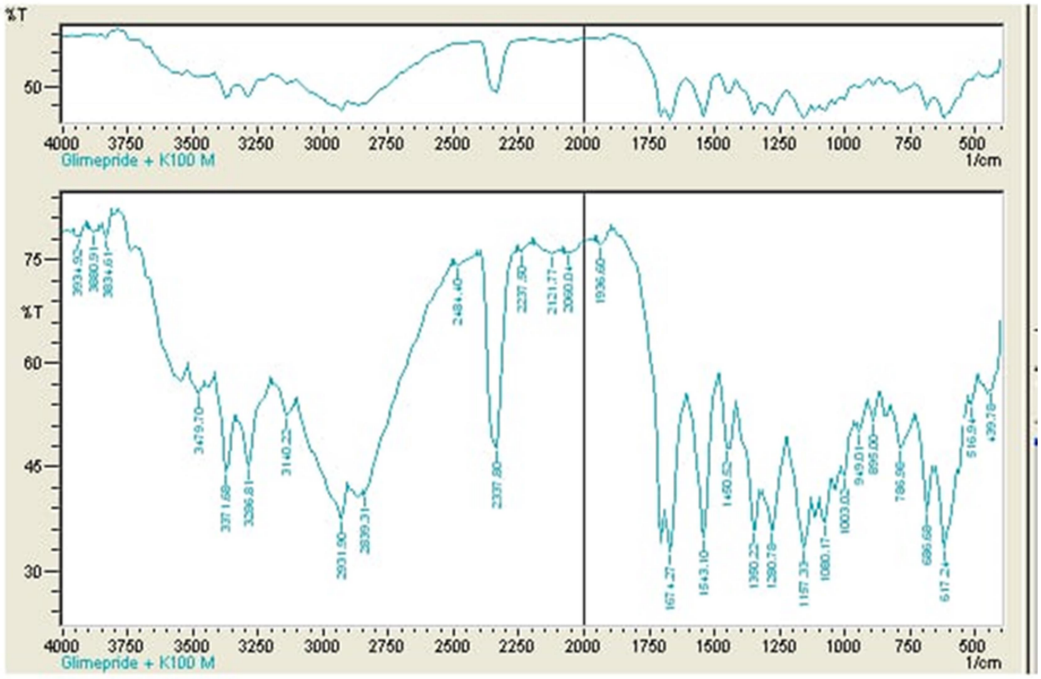

Fig 6: FT-IR of Physical Mixture of Glimepiride + HPMC K100M

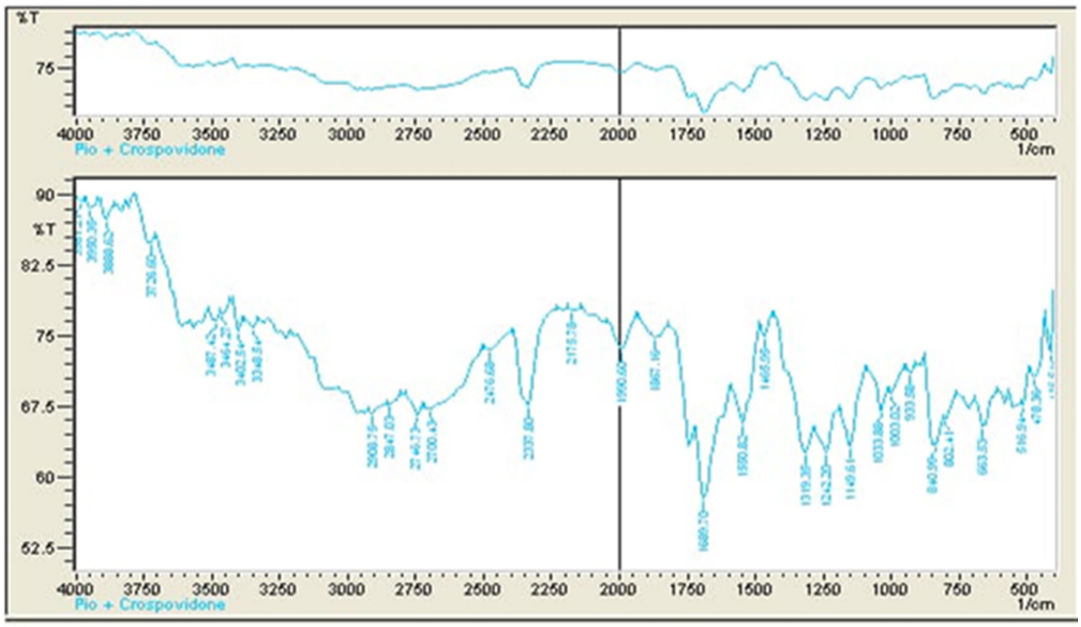

Fig 7: FT-IR of Physical Mixture of Pioglitazone HCl + Crospovidone

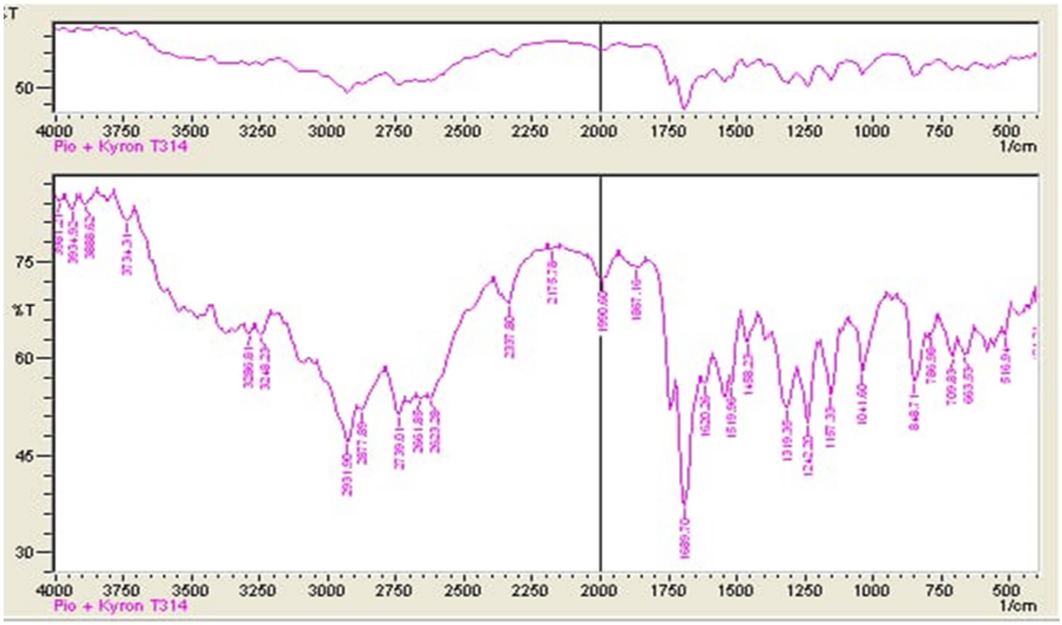

Fig 8: FT-IR of Physical Mixture of Pioglitazone HCl + Kyron T314

Preparation of tablet

Immediate Release Layer

The immediate layer was prepared by mixing the ingredients in the proper proportion. The following steps were followed during the preparation of the direct compression layer. Sifting of API (Pioglitazone HCI) and Excipients is the first step in the formulation. All ingredients were weighed accurately and sieved. Dry mix: Pioglitazone, microcrystalline cellulose, KYRON T-314 and lactose were mixed together. The mixing was done till the through mixing was confirmed. The lubricant (Mag Stearate) was

shifted and transferred to the mixture to aid the flow property. Compression: the mixture was compressed into the tablet using the low force compression

Preparation of Sustained Layer

All the ingredients including active drug (Glimepiride) were weighed properly, sieved and mixed thoroughly. Individually every tablet was prepared by direct compression method with a pressure of 5 ton by hand compression machine**.**

Table 1: bilayer tablet formulation table

Formulation	F1	F2	F3	F4	F5	F6
Sustained release layer (mg)	**SR1**	**SR2**	**SR3**	**SR4**	**SR5**	SR6
Glimepiride	8	8	8	8	8	8
HPMC K4M	38	45	52	-	-	-
HPMC K100M	-	-	-	38	45	52
MCC (Avicel 101)	100	93	86	100	93	86
Magnesium stearate	2	2	2	2	2	2
Talc	2	2	2	2	2	2
Total	**150**	**150**	**150**	**150**	**150**	150
Immediate release layer (mg)	**IR1**	**IR2**	**IR3**	**IR4**	**IR5**	IR6
Pioglitazone	30	30	30	30	30	30
Crospovidone	4	6	8	-	-	-
Kyron T-314	-	-	-	1	2	3
Avicel 101	62	60	58	65	64	65
Magnesium stearate	2	2	2	2	2	2
Talc	2	2	2	2	2	2
Total	**100**	**100**	**100**	**100**	**100**	100
Overall Total	250	250	250	250	250	250

Evaluation [11,12,13]

The prepared bilayer tablets from each optimized formulation batches were tested against the official standard evaluation parameters to ensure the proper manufacturing and release rate of the dosages of the drug. Following evaluation parameters were performed:

Size, shape and thickness

The size and shape of the tablets can be dimensionally described, monitored and controlled. The thickness of the tablets is the only dimensional variable related to the process of tableting. At a constant compressive load, tablet thickness varies with the change in the die fill, with particle size distribution and packing of the particle mix being compressed, and with the tablets weight, while with the constant die fill, thickness varies with the variations in compressive load. The tablet thickness should be maintained well within a ± 5% variation of the standard value.

Weight variation

Weigh individually 20 tablets selected at random and calculate the average weight. Not more than two of the individual weights deviate from the average weight by more than the percentage shown in the table and none deviates by more than twice that percentage

Friability

This test is applicable to compressed tablets and is intended to determine the physical strength of tablets and is measured by Roche Friabilator. For tablets with an average weight of 0.65 g or less take a sample of whole tablets corresponding to about 6.5 g and for tablets with an average weight of more than 0.65 g

take a sample of 10 whole tablets. The tablets were de-dust carefully and weighed accurately. The tablets were place in the drum and the drum was rotated 100 times. The tablets were removed after 100 revolutions, any loose dust was removed from them and weighed accurately again. The test is run only once unless the results are difficult to interpret or if the weight loss is greater than the targeted value, in which case, the test is repeated twice and the mean of the three tests is determined. A maximum loss of weight (from a single test or from the mean of the three tests) not greater than 1.0 % is acceptable for most tablets. If obviously cracked, chipped or broken tablets are present in the sample after tumbling, the sample fails the test15.

Hardness

Hardness is the measure of the strength of the tablet to withstand the mechanical shock of manufacturing, packaging and transportation. Hardness is sometimes also referred to as tablet crushing strength. The hardness of the tablets is estimated by Pfizer hardness tester or Erweka tester.

Drug content

10 tablets are taken randomly and weighed. The average weight is calculated and the tablets are then crushed in the mortar. The weight equivalent to the label claim is weighted accurately and is dissolved in 100 ml of the solvent being used for the dissolution study. The solution thus prepared is analyzed spectro-photometrically and the concentration is determined.

In vitro drug release

In vitro dissolution studies of bilayer tablets were studied using USP dissolution test apparatus-II employing a paddle stirrer. 900 ml of 0.1N HCl (pH 1.2) was used as a dissolution medium for first two hours and then was replaced with Phosphate Buffer solution (pH 6.8) for specified time of 10hrs. The temperature of the dissolution medium is maintained to 37 ± 0.5ºC. One tablet from each batch was used in each test. 5 ml of the sample of dissolution medium was withdrawn by means of pipette at known intervals of time and the sample was filtered using the Whatman filter paper. The volume withdrawn at each interval was replaced with same quantity of fresh dissolution medium. The sample is analyzed, for drug release and release kinetics, spectrophotometrically using UV-visible spectrophotometer (Shimadzu-1800) after suitable dilutions. The for immediate release layer its analyses for 228nm λ_{max} range and for sustain its aliases for 268nm λ_{max}. [14]

Stability testing of formulated bilayer tablet of optimized batch

The formulated bilayer tablets were kept at different storage conditions. The test samples were kept at was kept at 25°C±2°C, 60% RH and at 40°C±2°C, 75% RH according to ICH guidelines. The Hardness, friability, drug content of the tablets was determined initially and then at the interval of 15 days and one month. The hardness, friability and drug content of the optimized formulation after 30 days were reported in table

RESULT AND DISCUSSION

Compatibility study

Spectra of the pure drug, excipient and physical mixture of drug and excipient were recorded in between 400-4000 wavenumber (cm^{-1}). The FTIR spectral analysis showed that there is no appearance or disappearance of any characteristic peaks of pure drug Glimepiride and Pioglitazone HCl and in the physical mixture which confirms the absence of chemical interaction between drug and polymers.

Evaluation of the powder bled

The angle of repose was found to be ranging from 21°30'+0.04 to 26° 16'40.02 for the granules of all the formulations. Compressibility index was found to be ranging from 11.92 0.07 to 14.77±0.04 % for the granules of all the formulations. The results of Hausner's ratio were found to be lesser than 1.25 which

indicates better flow properties. The results of angle of repose (<30) indicates good flow properties of the powder. This was further supported by lower compressibility index values. Generally, compressibility values up to 15% results in good to excellent flow properties.

Table 2: Evaluation of prepared tablet blends for pre compression study of Immediate release

Formulations	Angle of Repose(θ˚)	Bulk Density (gm/ml)	Tapped Density (gm/ml)	Hauser's Ratio (HR)	Carr's Compressibility Index (%)
F1	29.03±0.14	0.285±0.06	0.324±0.04	1.13±023	12±0.14
F2	27.74±0.35	0.292±0.02	0.33±0.04	1.14±0.31	12.31±0.25
F3	27.11±0.17	0.30±0.06	0.375±0.05	1.17±0.45	14.77±0.17
F4	28.36±0.28	0.290 ±0.06	0.315±0.01	1.07±0.41	7.3±0.85
F5	27.74±0.84	0.307±0.9	0.333±0.09	1.08±0.32	7.8±0.35
F6	28.81±0.24	0.307±0.01	0.342±0.09	1.11±0.12	10.23±0.36

Layer

Formulations	Angle of Repose(θ˚)	Bulk Density (gm/ml)	Tapped Density (gm/ml)	Hauser's Ratio (HR)	Carr's Compressibility Index (%)
F1	27.69±0.35	0.457±0.05	0.5±0.02	1.09±0.5	8±0.19
F2	27.74±0.54	0.465±0.01	0.509±0.04	1.096±0.87	8.64±0.84
F3	28.19±0.45	0.473±0.05	0.529±0.06	1.12±0.54	10.59±0.33
F4	27.744±0.65	0.465±0.02	0.509±0.04	1.09±0.51	8.64±0.14
F5	27.203±0.78	0.473±0.09	0.519±0.08	1.09±032	8.88±0.48
F6	27.699±0.25	0.482±0.08	0.540±0.04	1.12±0.47	10.74±0.37

Table 3: Evaluation of prepared tablet blends for pre compression study of sustaine release Layer

Post Compression Study Of Bilayer Tablet

Table 4: Evaluation of prepared tablet blends for post compression study of Bilayer tablet

Formulations	Weight variation (mg)	Thickness (mm)	Hardness (Kg/cm^2)	Friability (%)	Dug content (%)		Disintegration time(sec)
					IR	**SR**	
F1	249.3±1.25	3.6±0.14	3.1±	0.47±0.02	98.40±1.3	97.53±0.30	84±5
F2	251±0.94	3.4±0.21	3.6±0.14	0.439±0.03	96.36±0.27	97.6±1.8	75±1
F3	248.9±0.59	3.4±0.36	3.5±0.24	0.396±0.01	97.86±1.39	97.73±0.30	71±3
F4	249.7±0.95	3.3±0.29	3.4±0.16	0.83±0.09	97.54±0.61	97.70±0.63	72±4
F5	251.2±0.74	3.4±0.18	3.7±0.36	0.51±0.08	96.79±0.14	97.5±1.3	69±4
F6	250.8±0.65	3.5±0.41	3.5±0.25	0.516±0.04	98.51±0.09	99.26±0.19	62±2

Dissolution Study

Table 5: In Vitro Drug Release Profile of Formulations of Sustained Released Tablet

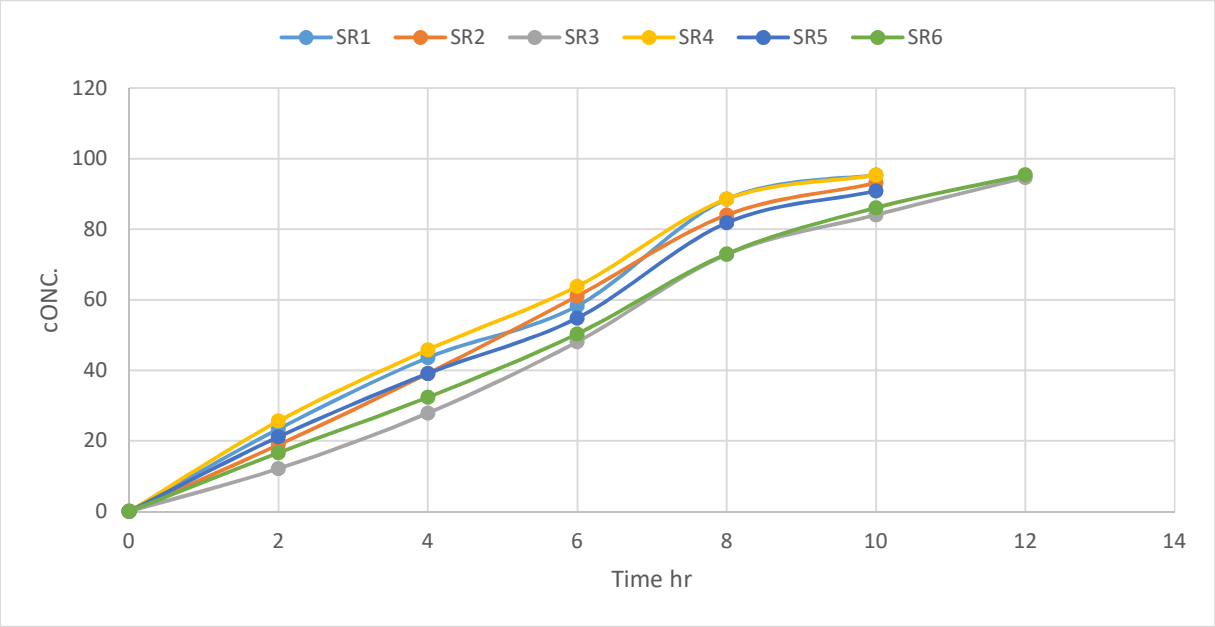

Fig 9: cumulative drug release in sustained release Glimepiride

The effect of polymer concentration on drug release could be clearly seen from the variation of dissolution profiles. It was found that drug release from SR3 composed of HPMC K4M in high concentration was 12 hrs. And also shows significantly higher drug release rate than other formulations. Formulation SR6 containing 52 mg of HPMC K100M of Cumulative drug release which comparatively greater than other formulation batches so SR6 was selected for further formulation of bilayer tablet of Glimepiride

In Vitro Drug Release Study for Immediate release Tablet of Pioglitazone HCl

Table 6: Immediate release layer dissolution profile

Time in hour	% Cumulative drug release					
	IR1	**IR2**	**IR3**	**IR4**	**IR5**	**IR6**
0	0.000	0.000	0.000	0.000	0.000	0.000
5	10.378±0.14	12.178±0.75	14.141±0.37	12.669±0.62	15.941±0.49	20.195±0.75
10	15.952±0.25	21.353±0.49	24.628±0.62	23.399±0.51	31.420±0.43	34.778±0.86
15	33.394±0.25	37.656±0.51	41.589±0.62	35.696±0.38	39.717±0.86	42.343±0.86
20	40.303±0.25	44.733±0.65	49.815±0.62	46.370±0.62	53.913±0.38	59.896±0.38
25	54.827±0.25	58.608±0.75	63.205±0.49	57.874±0.99	62.071±0.51	70.352±0.25
30	68.059±0.14	71.762±0.79	75.218±0.28	71.436±0.62	74.411±0.86	80.574±0.29
35	85.231±0.25	89.102±0.43	86.836±0.86	81.250±43	85.046±0.71	88.516±0.14
40	89.416±0.25	92.146±0.43	96.013±71	91.484±0.62	94.139±0.99	99.249±0.62

Time in hour	% Cumulative drug release					
	SR1	**SR2**	**SR3**	**SR4**	**SR5**	**SR6**
0	0.000	0.000	0.000	0.000	0.000	0.000
2	23.36±0.35	21.50±0.65	12.90±0.65	23.36±2.24	18.88±0.58	16.64±2.24
4	43.57±0.47	39.45±0.65	29.36±0.65	43.67±2.25	39.16±0.54	32.42±2.24
6	63.79±0.65	55.26±0.78	50.24±1.8	64.09±2.27	66.28±0.58	50.54±2.27
8	88.52±0.56	82.15±1.14	74.28±0.59	89.85±2.24	84.58±0.55	73.23±2.26
10	97.58±0.97	94.57±0.78	84.45±0.65	96.32±0.98	94.01±0.64	87.09±0.47
12	---	--	97.99±1.71	-------	-----	96.53±2.68

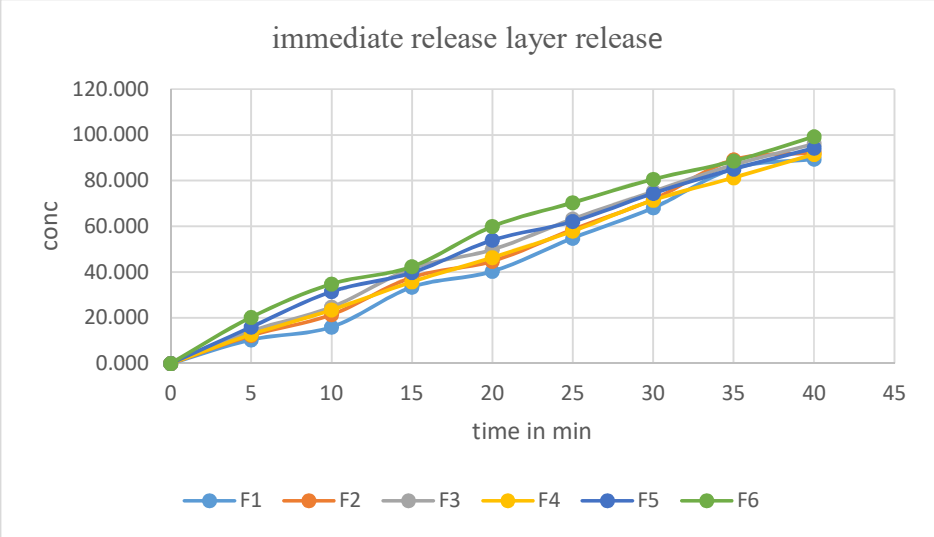

Fig 10: cumulative drug release immediate Pioglitazone HCl

The effect of Super-disintegrants concentration on drug release could be clearly seen from the variation of dissolution profiles. It was found that drug release from IR6 composed of Kyon T314 in high concentration was 98.10% drug release after 40 min. and also shows significantly higher drug release rate than other formulations. Formulation IR6 containing 3 mg of Kyron T314 of Cumulative drug release which comparatively greater than other formulation batches so IR6 was selected for further formulation of bilayer tablet of Pioglitazone HCl.

Stability Studies of Bilayer Tablet

Stability study for the developed formulation F3 were carried out as per ICH guideline by storing at 40°C/75% RH for the two months. The formulation F3 was selected on the basis of their cumulative percentage drug release in the dissolution test. In the comparative study for immediate release F6 was the optimized match and F6 in sustain release containing HPMC K 100M optimized**.**

Table 10: Comparative % drug release of immediate release layer F6 Batch

Time in Minutes	% Cumulative drug release	
	IR6 Initial	IR6 after three Months
0	0.000	0.000
5	20.35±0.54	16.92±0.52
10	35.84±0.45	28.14±0.34
15	42.26±0.54	40.61±0.57
20	58.50±0.45	53.33±0.36
25	70.10±0.81	65.26±0.46
30	80.98±0.46	73.52±082
35	89.17±0.48	83.08±0.55
40	98.10±0.74	96.59±0.74

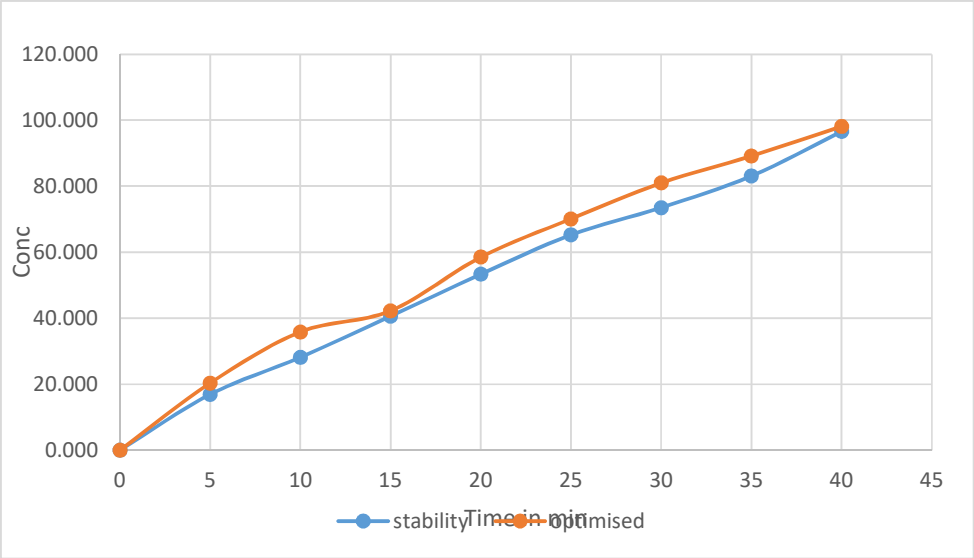

Fig 10: Comparative drug % release of SR6 batch

Table 11: % cumulative drug release of stability study of SR6

Time Hr.	% Cumulative drug release	
	SR1	**After 3 months**
0	0	0
2	12.16±0.19	10.29±0.34
4	27.86±0.16	25.84±0.22
6	48.07±0.2	44.18±0.56
8	72.78±0.16	70.83±0.34
10	84.07±0.28	82.64±0.72
12	97.61±0.37	95.81±0.45

The stability study graphical representation

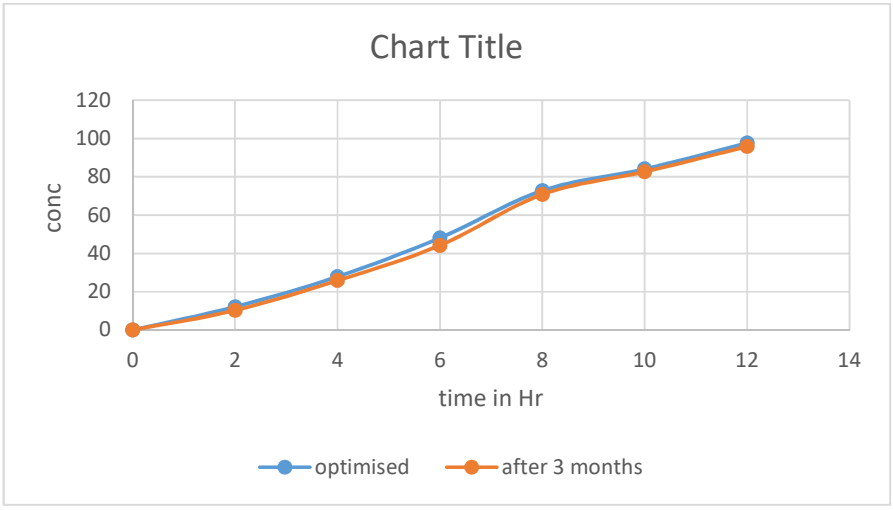

Fig 11: Comparative drug release of sustained release IR 6 batch

CONCLUSION

Sustained release of Glimepiride developed by using polymer like HPMC K4M and HPMC K100M and incorporation of superdisintegrants like Crospovidone and Kyron T314 in immediate release layer. The release data further indicated that HPMC K100M can give the sustained release with maximum drug release up to 12 hrs. Which shows minimize the burden of dosing. Immediate Release layer IR6 containing Kyron T314 shows maximum drug release about 98.10 % up to 40 min. than other formulations. HPMC K100M polymer controlled the release of Glimepiride up to 12 hrs intended for once daily administration. The release data of in vitro study indicates that formulation follows zero order, Higuchi equation and diffusion takes place via non-fickian transport. Formulation F3 found to be stable at accelerated stability as per the ICH guidelines for a period of 3 months

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FORMULATION AND EVALUATION OF BILAYER TABLET OF METFORMIN HCL AND ENALAPRIL MALEATE

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Abstract: *The present research work was to establish the bilayer tablet of Metformin HCl and Enalapril Maleate for patients having Diabetes Mellitus and Hypertension. The bilayer tablet of Metformin HCl and Enalapril Maleate was developed by direct compression method. The bilayer tablet contained the Sustained release layer of Metformin HCl and Immediate release layer of Enalapril Maleate. The SR layer of Metformin HCl developed by using the HPMC K4M and HPMC K100M for controlled release of drug. While, the IR layer of Enalapril Maleate was developed by using Croscarmellose Sodium (CCS) and Sodium Starch Glycolate (SSG). The pre-compression study was performed and the results were reported. In pre-compression study the angle of repose, bulk density, tapped density, Hausner's ratio and Carr's index was calculated and results shown the good compliance as per IP standards. The tablets parameters were evaluated by testing thickness, hardness, weight variation, friability test etc. The % drug content was found to be in the range of 97.7±0.33 to 99.12±0.56 for SR layer of Metformin HCl and for IR layer Enalapril Maleate was found to be 97.03±0.62 to 99.53±0.35. The disintegration time for IR layer was found to be 45.31±0.39 to 78.27±0.55 sec. The formulation F3 shown the best results of dissolution study containing IR3 and SR3 layer of Croscarmellose and HPMC K4M respectively. The IR3 layer of F3 formulation shown 98.93±0.37 % drug release at the end of 40 min. and which was the best drug release than other formulations. The SR3 layer of F3 formulation shown the controlled drug release for 12 hrs. with maximum drug release than other formulations and was found to be 97.99±0.46 % drug release. Further, the stability study of the bilayer tablet was checked as per ICH guidelines and the results were complies to the IP standards. From the drug release study it is possible to reduce the frequency of dose.*

Keywords: *Diabetes Mellitus, Hypertension, Metformin HCl, Enalapril Maleate, CCS, SSG, HPMC K4M, HPMC K100M*

INTRODUCTION

Diabetes mellitus (DM) is a clinical syndrome characterized by varying degrees of insulin hyposecretion and/or insulin insensitivity leading to hyperglycaemia. Lack of insulin affects the metabolism of carbohydrates, protein and fat, which lead to metabolic alterations, and it causes a significant disturbance of water and electrolyte homeostasis [1]. According to the International Diabetes Federation, in 2019, approximately 463 million adults (20-79 years) were living with diabetes worldwide, and this number is projected to increase to 700 million by 2045 [2]. Diabetes is classified into several types, including type 1

diabetes [Insulin-dependent DM (IDDM)], type 2 diabetes [Noninsulin-dependent DM (NIDDM)], gestational diabetes, and other specific types [3].

Type 1 diabetes (T1D)

Type 1 diabetes, also known as insulin-dependent diabetes. T1D is less common in India than type 2 diabetes and is characterized by an absolute deficiency of insulin due to the destruction of pancreatic beta cells. The prevalence of T1D in India is estimated to be around 0.5% of all diabetes cases [4].

Type 2 diabetes (T2D)

Type 2 diabetes, on the other hand, is characterized by insulin resistance, which leads to a relative deficiency of insulin. T2D is the most common form of diabetes in India, accounting for around 90% of all cases. Risk factors for T2D in India include age, obesity, physical inactivity, and a family history of diabetes. According to the International Diabetes Federation (IDF), the global prevalence of diabetes in adults was estimated to be 9.3% in 2019, with type 2 diabetes accounting for the majority of cases [5].

Approximately, 30-60% of diabetics have systemic arterial hypertension (SAH), which shows the close relationship between such diseases [6]. SAH, in turn substantially contributes to morbidity in patients with diabetes [7], with oxidative stress (OS) configuring an important mechanism in the pathophysiology of DM and SAH [8-10]. Increased oxygen free radical activity, coupled with reduced protection against OS, could play a role in the etiology of neurovascular abnormalities in experimental DM [11]. Production of reactive oxygen species (ROS) is increased in diabetic patients, especially in those with poor glycaemic control. Diabetes and hypertension (high blood pressure) are two common chronic conditions that often coexist and can have a significant impact on a person's health. The exact mechanisms underlying the relationship between diabetes and hypertension are complex and not fully understood. However, several physiological processes contribute to the interaction between the two conditions. These include insulin resistance, endothelial dysfunction, sympathetic nervous system overactivity, and abnormal renal sodium handling [12].

Anti-hyperglycaemic medication metformin HCL helps type II diabetics' ability to tolerate glucose. According to reports, 50–60% of metformin HCL administered orally has a 100% bioavailability. The proximal small intestine is the primary location of metformin HCL's absorption, and its biological half-life is 1.5–1.6 hours [13]. It belongs to the class of drugs known as biguanides and is considered a first-line treatment for diabetes. Metformin HCl works by reducing glucose production in the liver and increasing the body's sensitivity to insulin. It does not stimulate the pancreas to produce insulin [14].

Enalapril maleate is a medication commonly prescribed for the treatment of hypertension (high blood pressure) and heart failure. It belongs to the class of drugs known as angiotensin-converting enzyme (ACE) inhibitors. Enalapril maleate works by inhibiting the action of the ACE enzyme in the body. ACE is responsible for the conversion of angiotensin I to angiotensin II, a potent vasoconstrictor. By blocking ACE, enalapril reduces the production of angiotensin II, leading to vasodilation (widening of blood vessels) and a decrease in blood pressure [15,16].

Metformin HCl and Enalapril Maleate, these two medications are used to treat type 2 diabetes and high blood pressure, respectively. Combination medication treatment minimizes dosage burden because 40–80% of persons with diabetes also have hypertension. When anti-diabetic and anti-hypertensive medications are taken, they lead to synergistic pharmacodynamic effectiveness. While Metformin HCl and Enalapril Maleate have different mechanisms of action and primary indications, they may have some potential pharmacodynamic synergistic effects when used together. This means that their combined action may provide additional benefits beyond what each medication can achieve on its own.

Both Metformin HCl and Enalapril Maleate have been shown to have positive effects on cardiovascular health. Metformin has been associated with reduced cardiovascular events and improved outcomes in patients with diabetes, while enalapril has been shown to reduce blood pressure and improve heart function. When used together, these medications may have a complementary effect in managing conditions such as diabetes and hypertension.

Additionally, the combination of enalapril maleate and metformin may also have potential benefits for patients with kidney disease. Both medications have been shown to have Reno protective effects, meaning they can help protect the kidneys from damage and slow down the progression of kidney disease. Studies have indicated that the combined use of enalapril maleate and metformin in patients with diabetic nephropathy (kidney disease caused by diabetes) may have synergistic effects on renal function and reduce proteinuria (excessive protein in the urine).
Bilayer tablet of Immediate released Enalapril Maleate and Sustained released Metformin HCl may help to reduce the dosage burden of patients having Diabetes and Hypertension due to which the patient compliance will be improved. The sequential release of Metformin HCl and Enalapril Maleate shows the synergistic effect by reducing the Oxidative Stress. Bilayer tablet is an advanced technology that helps in overcoming the limitations of a single-layered tablet.

MATERIALS AND METHOD

Materials

Metformin HCl by Aarti Chemicals and Distributors Mumbai, Enalapril Maleate from Yarrow Chemicals Mumbai. HPMC K4M, HPMC K100M, Croscarmellose, Sodium Starch Glycolate (SSG) are received from Research lab Fine Chem. Industries, Mumbai. Microcrystalline Cellulose from Prachin Chemical, Ahmedabad and Magnesium stearate and Talc is received from Signet Excipients Pvt. Ltd. Mumbai.

Method

Identification of pure drug [17]

Identification of pure drug was carried out by UV Visible spectroscopy and Fourier Transform Infra-Red Spectrophotometry scanned in the range of 200-400nm and shown in Fig.7 to Fig.14.

Drug-excipient compatibility study [18]

Studies of drug-excipient compatibility are important to ascertain drug and excipients are compatible with each other. Fourier Transform Infra-Red Spectrophotometry (FTIR) are used to study drug excipient compatibility.

Calibration curve of Metformin HCl Enalapril Maleate in 0.1N HCl and Metformin HCl and Enalapril Maleate in pH 6.8 Phosphate buffer [19]

100 mg of pure drug was dissolved in 100 mL of 0.1N HCl / pH 6.8 Phosphate buffer (stock solution-I; 1000 µg/mL) and then placed in a Sonicator for 10 min, from this 10 mL of solution was taken and the volume was adjusted to 100 mL with 0.1N HCl / pH 6.8 Phosphate buffer (stock solution-II; 100 µg/mL). The stock solution-II; was suitably diluted with 0.1N HCl/ pH 6.8 Phosphate buffer to obtain the series of working dilutions: 2, 4, 6, 8, 10 µg/mL of drug solution. The median concentration was scanned for λ_{max} and at the respective λ_{max} working dilutions were analyzed by using a double beam UV-Vis. spectrophotometer (Shimadzu, Japan UV-1800 double beam Spectrophotometer). The λ_{max} of pure drug in 0.1N HCl and 6.8 phosphate buffer shown in Fig.1,3,5. The standard calibration curve was plotted by taking concentration on X-axis and absorbance on Y-axis was shown in Fig.2,4,6.

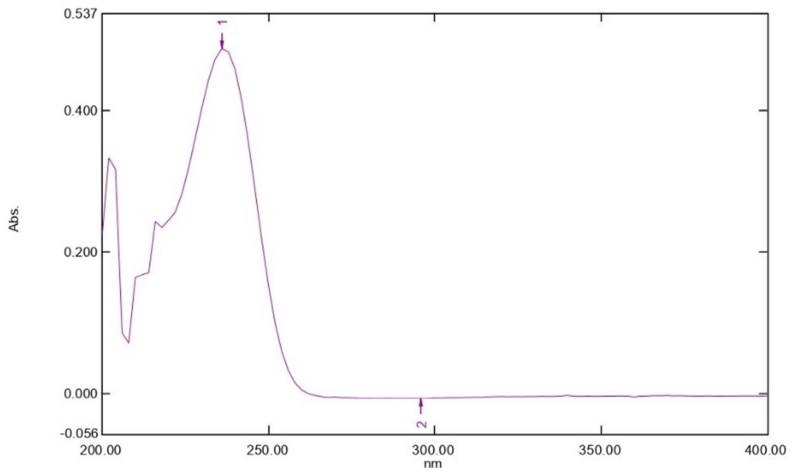


Fig 1: UV Spectrum of Metformin HCl in 0.1N HCl

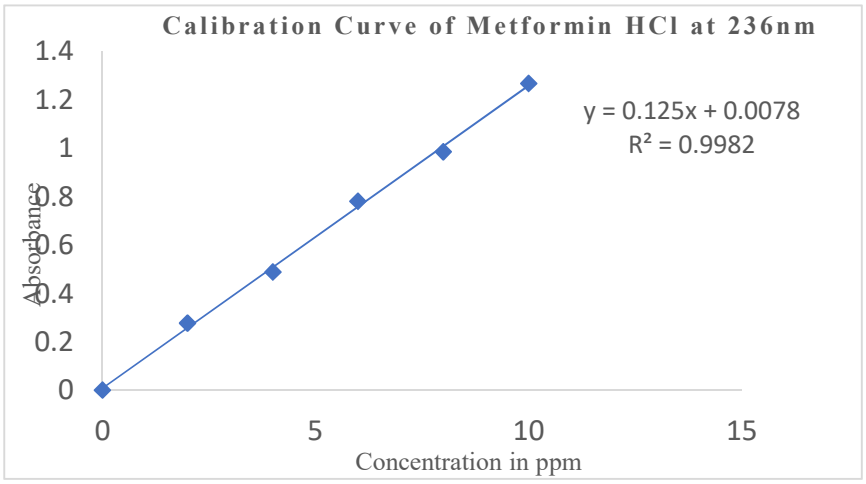


Fig 2: Calibration curve of Metformin HCl in 0.1 N HCl

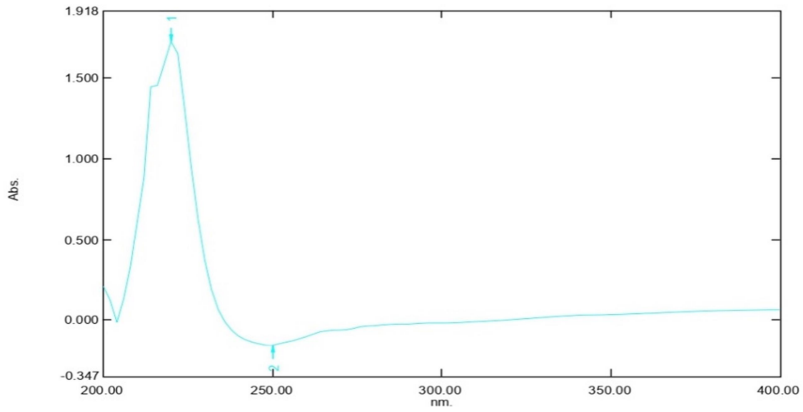


Fig 3: UV Spectrum of Metformin HCl in 6.8 Phosphate Buffer

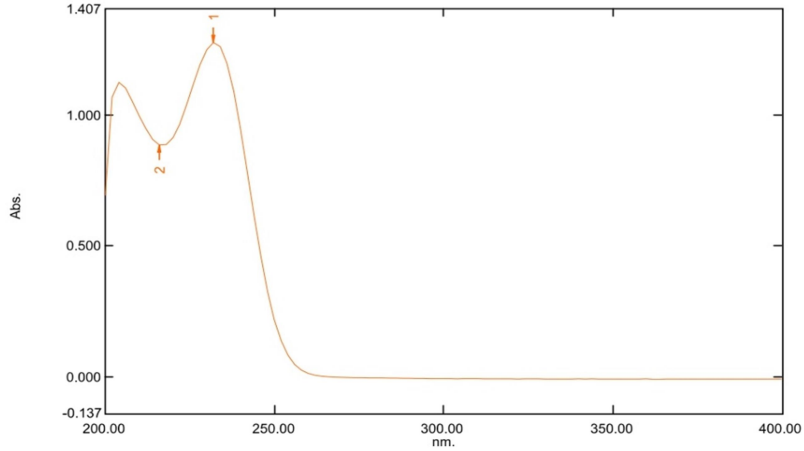


Fig 4: Calibration Curve of Metformin HCl in 6.8 pH Phosphate Buffer

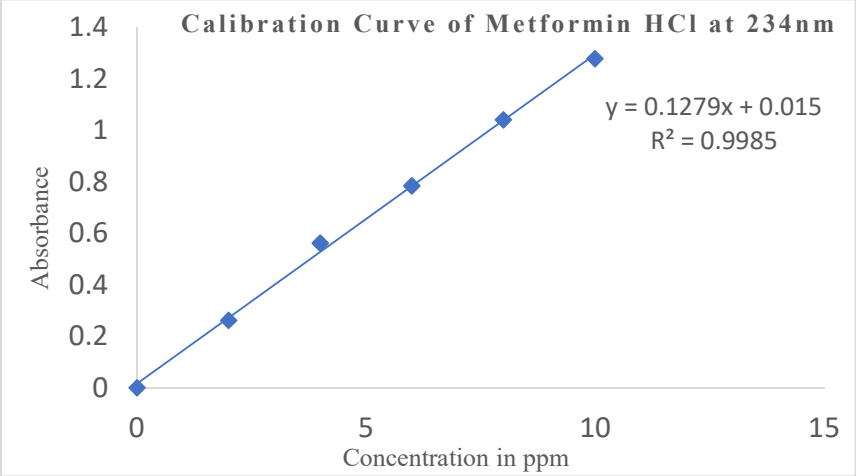
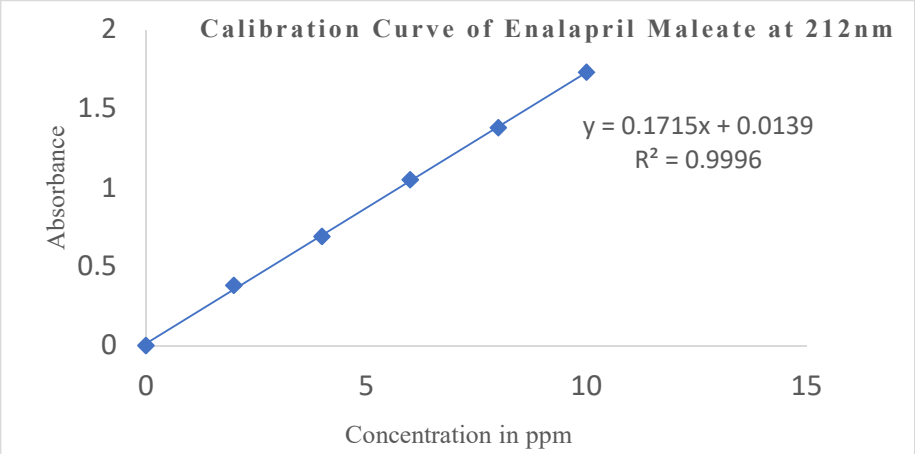


Fig 5: UV Spectrum of Enalapril Maleate in 0.1N HCl

Fig 6:



Calibration Curve of Enalapril Maleate in 0.1 N HCl

Drug – Excipient Compatibility Study

Fourier Transform Infrared Spectroscopy (FTIR) [20]

FT-IR spectrum of Metformin HCl and Enalapril Maleate was recorded to confirm its purity on FTIR spectrophotometer (FTIR 8400S, Shimadzu) by using KBr powder press technique. The base line correction was done using dried potassium bromide. The instrument was operated under dry air purge with resolution of cm^{-1} over the region 4000-400 cm-1. The scans were evaluated for presence of principle peaks of drug. The identified peaks were compared with peaks of reported IR spectrum.

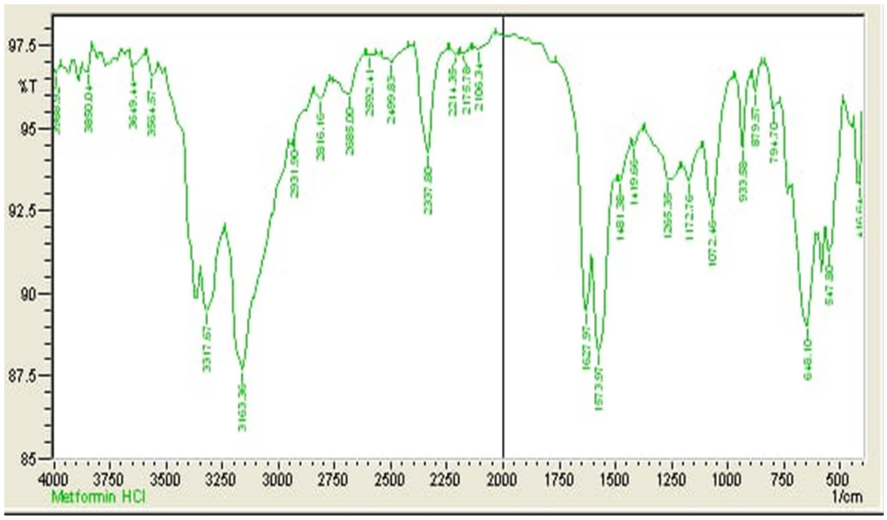


Fig 7: FT-IR Spectra of Metformin HCl

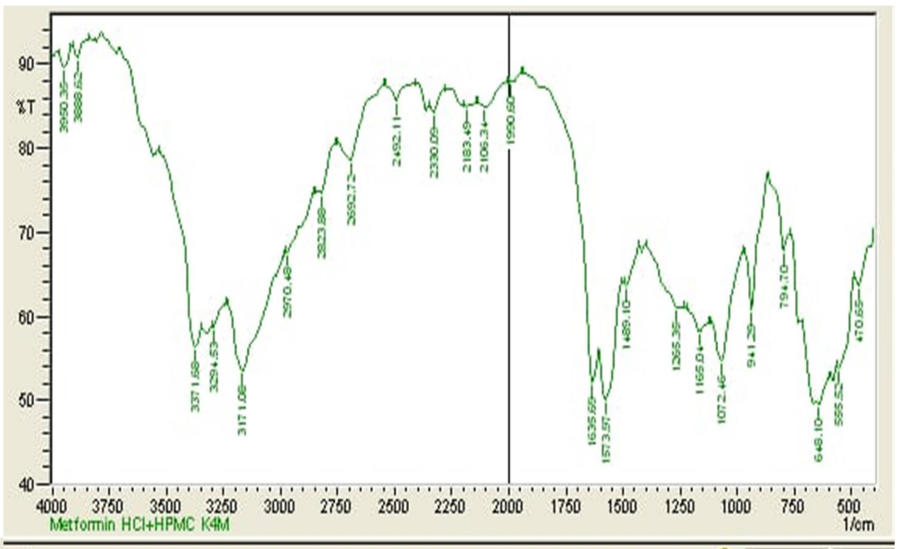


Fig 8: FT-IR Spectrum of Physical Mixture of Metformin HCl +HPMC K4M

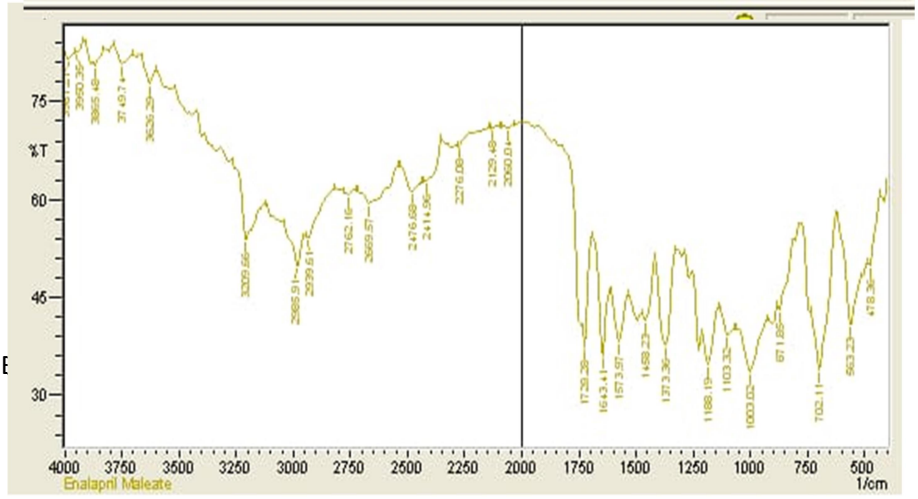


Fig 9: FT-IR Spectrum of Physical mixture of Metformin HCl + HPMC K100M

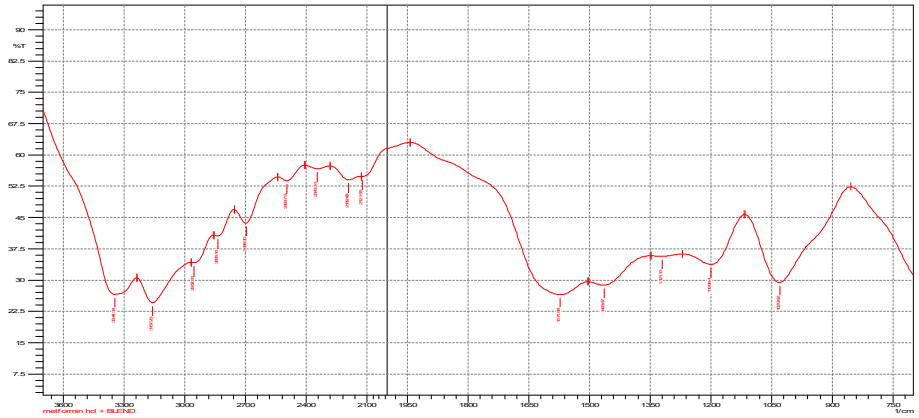


Fig 10: FT-IR Spectrum of Physical mixture of Metformin HCl + other Excipients

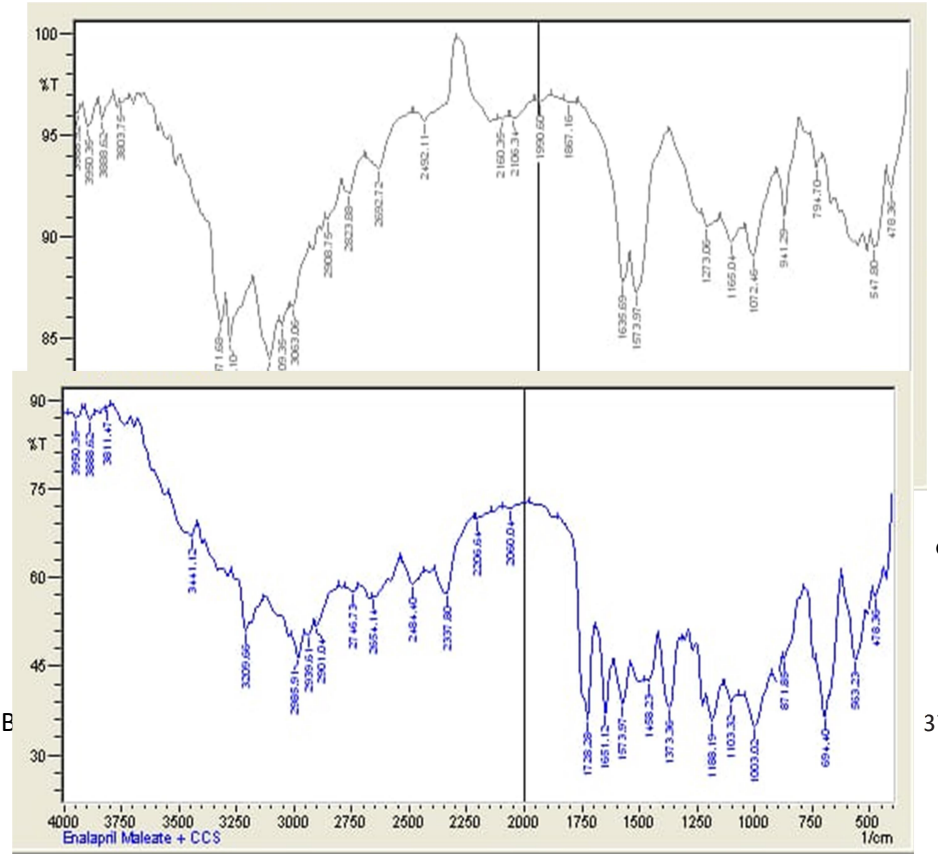


Fig 11: FT-IR Spectrum of Enalapril Maleate

Fig 12: FT-IR Spectrum of Physical mixture of Enalapril Maleate + Croscarmellose

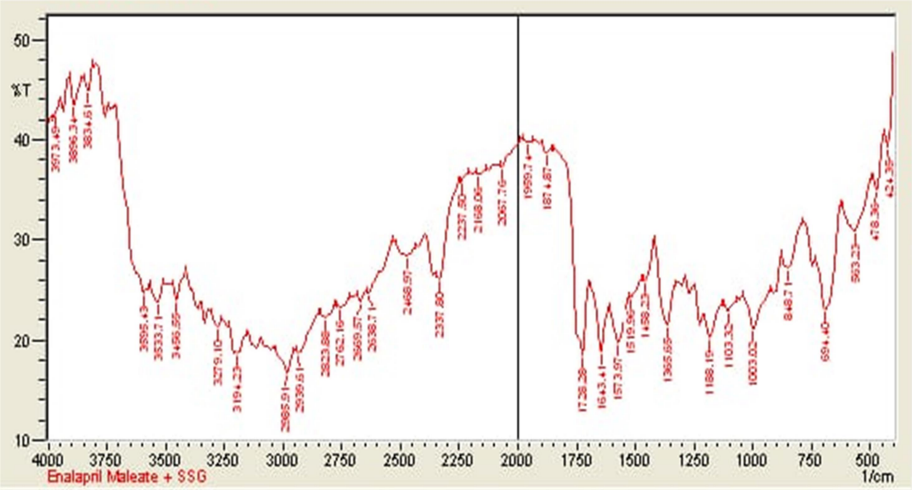


Fig 13: FT-IR Spectrum of Physical mixture of Enalapril Maleate + Sodium Starch Glycolate

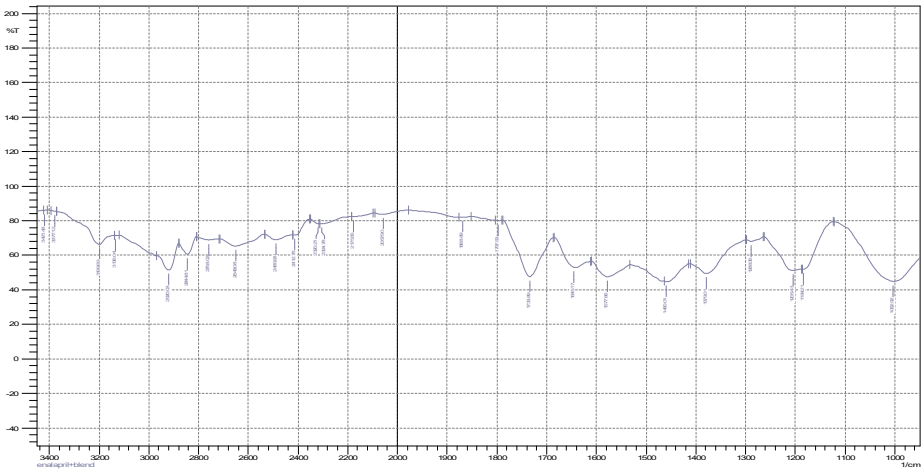


Fig 14: FT-IR Spectrum of Physical mixture of Enalapril Maleate + other Excipients

Preparation Of Bilayer Tablet

Preparation of Immediate Release Layer

The Immediate release layer were prepared by Direct Compression technique by blending Enalapril Maleate uniformly with Sodium Starch Glycolate and Croscarmellose sodium as a superdisintegrants, Microcrystalline cellulose as a direct compressible agent, Magnesium Stearate as a diluent as per the formula. The blend obtained was passed through a 40# sieve. The powder blend was mixed with talc.

Preparation of Sustained Release Layer

The sustained release layer was prepared by direct compression method by blending Metformin hydrochloride uniformly with HPMC K4M and HPMC K100M as a sustained release polymer, Microcrystalline Cellulose as a compressible agent. The blend powder was mixed with talc and magnesium stearate.

Table 1: Formulation of Sustained Release Metformin HCl Layer

Immediate Release Layer	IR1	IR2	IR3	IR4	IR5	IR6
Enalapril Maleate	5	5	5	5	5	5
Croscarmellose Sodium	5	10	15	---	---	---
Sodium starch Glycolate	---	---	---	5	10	15
Microcrystalline cellulose	88	83	78	88	83	78
Magnesium stearate	1	1	1	1	1	1
Talc	1	1	1	1	1	1
Ferric oxide	Q. S	Q. S	Q. S	Q. S	Q. S	Q. S
Total(mg)	100	100	100	100	100	100

Table 2: Formulation of Immediate Released Enalapril Maleate Layer

Formulation in mg	F1	F2	F3	F4	F5	F6
Sustained Release Layer	**SR1**	**SR2**	**SR3**	**SR4**	**SR5**	SR6
Metformin HCl	500	500	500	500	500	500
HPMC K100M	150	170	190	---	---	---
HPMC K4M	---	---	---	150	170	190
Microcrystalline Cellulose	84	64	44	84	64	44
Magnesium Stearate	8	8	8	8	8	8
Talc	8	8	8	8	8	8
Total (mg)	750	750	750	750	750	750

Pre-Compression Studies [21]

Directly compressible tablet blends of Enalapril Maleate-IR layer and Metformin HCl-SR layer were evaluated for angle of repose (θ), bulk density (BD), tapped density (TD), Carr's Index (CI) & Hausner's Ratio (HR). The obtained results of pre-compression studies of IR and SR layers were reported in **Table 3** and **Table 4.**

Angle of Repose [22]

This is the maximum angle possible between the height of pile of blend powder and horizontal plane. The frictional forces in the lose powder can be measured by angle of repose. The tangent of angle of repose is equal to the coefficient friction (θ) between the particles. Hence the rougher and more irregular the surface of particles the greater will beangle of repose.

$\theta = \tan^{-1} (h/r)$

Where, h = height of the pile r = radius of the pile

Bulk Density [23]

Bulk density is the ratio of the mass by the volume of an untapped powder sample. The bulk density is measured in g/ml. The bulk density depends on both the density of the powder particles and the arrangement of the powder particles. The bulk density influences preparation, storage of the sample. The mathematical re-presentation is given below.
Bulk density = Weight of the drug / Bulk volume

Tapped Density [24]
In tapped density, the bulk powder is mechanically tapped in a graduated cylinder until the volume is observed. Here the tapped density is calculated as mass divided by the final volume of the powder
Tapped density = Weight of the granules / tapped volume

Carr's Index [25]
It is one of the most important parameters to characterize the nature of granules.
Carr's index (%) = (Tapped density – Bulk density/ Tapped density) × 100

Hausner's ratio [25]
It is an important character to determine the flow property of granules in the presence of different compositions of polymers. The following formula can calculate this.
Hausner's ratio = Tapped density / Bulk density
Values less than 1.18 indicate good flow, and greater than 1.18 indicate poor flow.

Post-Compression Studies Of Bi-Layered Tablets [21,22,23,24,25]
Average weight of tablets 20 tablets was randomly selected from each batch and their weight was determined by an electronic balance (Shimadzu, Japan) the results shown in **Table 5.**

Thickness
For the thickness, tablets were randomly selected from each batch and their thickness was measured using a vernier calipers (Mitutoyo Corporation, Japan.).

Hardness
To check the hardness of the tablet, tablets were randomly selected from each batch and their hardness was measured using a Monsanto hardness tester (Pfizer mLabs-SE-276).

Friability
The friability of the 20 tablets from each batch was tested by a Friabilator (Roche-Friabilator, Germany) at a speed of 25 RPM for 4 min. The tablets were then de-dusted, re-weighed, and percentage weight loss was calculated by the equation below,
% Friability = (Initial Wt.− Wt. after friability) / Initial Wt. × 100

Disintegration test
The disintegration time for the tablet was determined using the disintegration apparatus. One tablet was placed in each of six tubes placed in a beaker containing 1000 ml of purified water maintained at 37±50ºC and the apparatus was operated. The time taken for the tablets to disintegrate and pass through the mesh was noted.

Drug Content Uniformity
Twenty tablets were finely powdered and an amount equivalent to 500 mg of Metformin hydrochloride and 5mg of Enalapril Maleate was accurately weighed and transferred to a 100 mL volumetric flask and was shaken for 10 min. with 70 mL of methanol. Finally, the volume was made up to mark with methanol. This was filtered through Whatman filter paper No.41 and suitably diluted. Drug content was determined using U.V spectrophotometer at 232nm and 208nm respectively

In vitro Dissolution study of IR layer

Dissolution test was carried out using dissolution apparatus USP Type-II using 0.1N HCl as the dissolution medium, maintained at a temperature of 37±0.5ºC. Randomly selected three tablets from each batch were taken for the evaluation undergone dissolution in the USP-II (paddle) dissolution apparatus (Electro lab, Mumbai (Model TDT-08L)), each flask was filled with 900 mL of 0.1N HCl; speed of paddle was maintained at 50 rpm, the temperature was kept constant at 37°C ± 0.5°C. At time points 0, 5, 10, 15, 20, 25, 30, 35, 40 min. 5 mL of dissolution media was withdrawn, filtered through 0.45µm membrane filter, suitably diluted and analyzed at respective λ_{max} of Enalapril Maleate using a double beam UV-Vis. spectrophotometer (Shimadzu-1800, Japan). Each sample withdrawn was replaced with an equal amount of fresh 0.1 N HCl, to keep the volume constant. In vitro dissolution profiles of Enalapril Maleate-IR tablets were shown in **Table 6** and **Fig.15**.

In vitro Dissolution study of SR Layer

Dissolution test was carried out using dissolution apparatus USP Type-II using 0.1N HCl as the dissolution medium, maintained at a temperature of 37±0.5ºC. Randomly selected three tablets from each batch were taken for the evaluation undergone dissolution in the USP-II (paddle) dissolution apparatus (Electro lab, Mumbai (Model TDT-08L)), each flask was filled with in 900 mL of 0.1N HCl for first 2 hr. and in 900 mL of pH 6.8 Phosphate buffer up to 12 hr. Speed of paddle was maintained at 50 RPM, the temperature was kept constant at 37°C ± 0.5°C. Samples were collected at time points 1,2,4,6,8,10,12hr, 5 mL of dissolution media was withdrawn, filtered through 0.45µm membrane filter, suitably diluted and analyzed at respective λ_{max} of Metformin HCl other time points using a double beam UV is spectrophotometer (Shimadzu-1800, Japan). Each sample withdrawn was replaced with an equal amount of fresh 0.1N HCl, to keep the volume constant. In vitro dissolution profiles of SR bi-layered tablets were shown in **Table 7** and **Fig.16**.

Stability testing of formulated bilayer tablet of optimized batch

The formulated bilayer tablets were kept at different storage conditions. The test samples were kept at was kept at 25±2^{o} C, 60% RH and at 40±2^{o} C, 75% RH according to ICH guidelines. The hardness, friability and drug content of the optimized formulation after 3 months were reported in **Table 10**.

RESULT AND DISCUSSION

Calibration curve of Glyburide and Metformin HCl

λ_{max} of Metformin HCl in 0.1N HCl; Enalapril Maleate in 0.1N HCl and Metformin HCl in pH 6.8 Phosphate buffer are 236 nm, 212 nm and 232 nm respectively. The standard curves are following linearity with a regression coefficient of (r^2=0.999). They are obeying the Beer's law in the conc. range of 0-10 µg/ml. As the excipients used in the study were not interfering and good % recovery of drug(s) indicates this spectrophotometric method was suitable for the estimation of drug(s) in dissolution studies and % assay of formulations.

Drug-excipient compatibility studies by FT-IR

An interpretation of FT-IR spectrum of Metformin HCl and Enalapril Maleate (pure drugs) reveals that the IR bands of pure drug and drug(s) + excipients show no significant shifts or reduction in intensity of the FT-IR bands. Hence there was no incompatibility problem between the drug and excipients used in the study.

Pre-Compression Studies

Table 3: Evaluation of prepared tablet blends for pre-compression study of Sustained Release Layer

Formulations	Angle of Repose(θ˚)	Bulk Density (gm/ml)	Tapped Density (gm/ml)	Hauser's Ratio (HR)	Carr's Index (%)
F1	30.46±0.73	0.27±0.02	0.32±0.04	1.18±0.02	15.62±0.23
F2	29.05±0.83	0.28±0.06	0.33±0.05	1.17±0.03	15.15±0.26
F3	31.60±0.67	0.27±0.03	0.31±0.06	1.14±0.04	12.9±0.17
F4	29.74±0.69	0.27±0.05	0.31±0.04	1.14±0.06	12.9±0.34
F5	30.83±0.79	0.29±0.03	0.32±0.02	1.10±0.03	9.3±0.28
F6	28.39±0.8	0.28±0.05	0.32±0.03	1.14±0.05	12.5±0.15

Table 4: Evaluation of prepared tablet blends for pre-compression study of Immediate Release Layer

Formulations	Angle of Repose(θ˚)	Bulk Density (gm/ml)	Tapped Density (gm/ml)	Hauser'sRatio (HR)	Carr's Index(%)
F1	27.55±0.61	0.33±0.02	0.37±0.06	1.15±0.0.21	12±0.3
F2	28.50±0.49	0.31±0.05	0.33±0.04	1.06±0.015	6.06±0.024
F3	27.24±0.45	0.31±0.04	0.35±0.02	1.12±0.023	11.4±0.018
F4	28.08±0.63	0.33±0.03	0.35±0.05	1.06±0.012	6.06±0.034
F5	26.56±0.028	0.31±0.02	0.37±0.03	1.19±0.013	16.21±0.027
F6	28.95±0.83	0.3±0.03	0.33±0.01	1.1±0.025	9.09±0.023

Post-Compression Parameters

Table 5: Post-Compression Parameter of Bilayer Tablet

Formulations	Weight variation (mg)	Thickness (mm)	Hardness (Kg/cm^2)	Friability (%)	Drug Content		Disintegration time(sec)
F1	849.7±0.38	5.7±0.034	6.4±0.15	0.87±0.03	97.82±0.73	97.96±0.32	68.45±0.47
F2	848.3±0.55	5.6±0.087	6.5±0.23	0.83±0.06	98.2±0.53	99.53±0.21	54.82±0.33
F3	850.1±0.32	5.5±0.064	6.9±0.37	0.8±0.02	98.6±0.62	98.72±0.46	45.31±0.29
F4	848.5±0.23	5.7±0.043	6.7±0.28	0.84±0.05	98.20±0.33	97.55±0.35	78.27±0.55
F5	852.2±0.62	5.5±0.053	6.9±0.10	0.91±0.02	97.7±0.37	99.01±0.57	67.59±0.25
F6	850.8±0.46	5.6±0.027	6.3±0.2	0.85±0.04	99.12±0.29	97.03±0.60	52.87±0.39

The bilayer tablets of Metformin HCl and Enalapril Maleate were evaluated for post compression parameter like weight variation test, friability, thickness, hardness, and drug content as shown in the **Table 5**. The percent variation was under the pharmacopoeia limit of 5%, thus all the tablets passed the weight variation test. It ranged from 848.5±0.23 to 852.2±0.62 mg. Thickness was found in the range from 5.5±0.053 to 5.7±0.043 mm. Hardness test was performed by Monsanto hardness tester. Hardness was maintained to be within 6.3±0.2 to 6.9±0.10 kg/cm^2. The friability was found well within the approved range of 0.8±0.02 to 0.91±0.02 % i.e., less than1%. The % drug content was found to be in the range of 97.7±0.33 to 99.12±0.56 for SR layer of Metformin HCl and for IR layer Enalapril Maleate was

found to be 97.03±0.62 to 99.53±0.35. The disintegration time for IR layer was found to be 52.87±0.39 to 78.27±0.55 seconds.

Dissolution Study

Table 6: In Vitro Drug Release Profile of Immediate Release Layer

Time in minute	% Cumulative Drug Release					
0	0	0	0	0	0	0
5	9.21±0.47	12.15±0.37	14.04±0.50	7.60±0.38	10.02±0.21	12.08±0.37
10	22.44±0.42	26.18±0.48	27.65±0.42	20.72±0.35	24.61±0.11	25.66±0.21
15	30.99±0.37	37.30±0.42	41.77±0.37	29.80±0.47	33.03±0.28	38.41±0.47
20	47.25±0.63	51.32±0.59	56.51±0.58	45.22±0.38	49.29±0.37	53.98±0.43
25	60.77±0.48	66.03±0.32	71.64±0.32	59.36±0.53	63.40±0.58	67.64±0.21
30	70.17±0.69	74.07±0.37	78.81±0.39	68.45±0.34	71.47±0.11	76.07±0.42
35	82.17±0.43	84.02±0.48	86.28±0.48	79.40±0.38	83.02±0.43	85.10±0.31
40	93.59±0.64	96.35±0.54	98.93±0.37	92.53±0.37	94.41±0.53	96.67±0.57

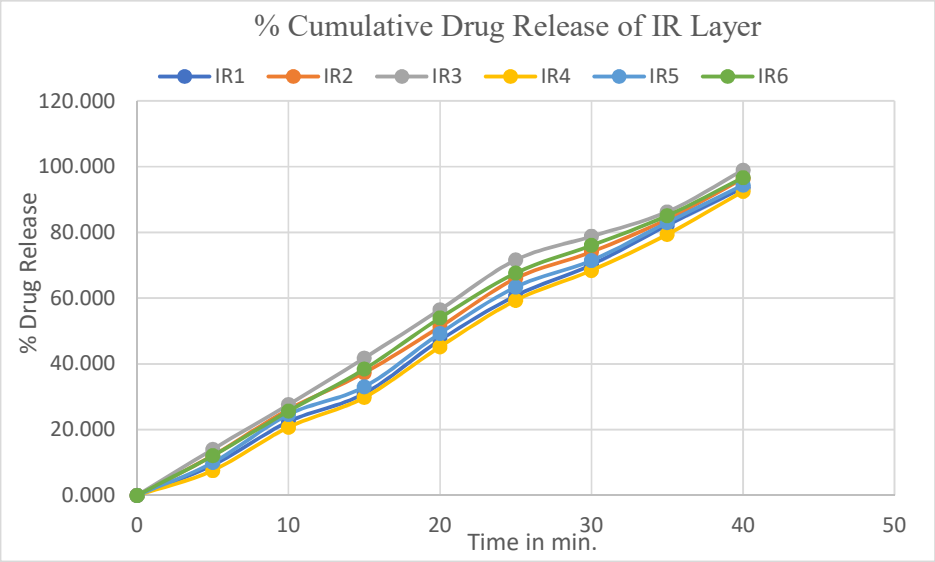


Fig 15: % Cumulative Drug Release of Immediate Release Layer

Immediate released tablets of Enalapril Maleate were prepared by using Croscarmellose and Sodium Starch Glycolate super-disintegrants. The release profiles of Enalapril Maleate Immediate Released tablet were plotted as in **Fig.15**. The effect of Super-disintegrants concentration on drug release could be clearly seen from the variation of dissolution profiles. It was found that drug release from IR3 composed of Croscarmellose in high concentration was 98.93±0.37% drug release up to 40 min. and also shows significantly higher drug release rate than other formulations. Formulation IR3 containing 15 mg of

Croscarmellose Sodium of Cumulative drug release which comparatively greater than other formulation batches so IR3 was selected for further formulation of bilayer tablet of Enalapril Maleate.

Table 7: In Vitro Drug Release Profile of Formulations of Sustained Released Tablet

Time in hour	% Cumulative Drug release					
0	0	0	0	0	0	0
1	12.82±0.44	10.34±0.65	7.64±0.53	10.26±0.53	8.50±0.45	6.10±0.34
2	23.43±0.50	18.63±0.33	17.31±0.51	20.36±0.42	18.05±0.60	16.14±0.36
4	44.20±0.91	40.47±0.50	29.34±0.55	38.55±0.49	34.61±0.46	27.44±0.44
6	64.35±0.71	57.75±0.48	50.77±0.40	57.86±0.58	55.79±0.55	47.06±0.37
8	79.45±0.55	76.69±0.71	69.52±0.47	75.05±0.47	73.81±0.40	66.93±0.4
10	97.44±0.71	95.18±0.73	83.39±0.58	96.24±0.46	93.85±0.55	81.13±0.35
12	-----	-----	97.99±0.46	------	-------	93.10±0.38

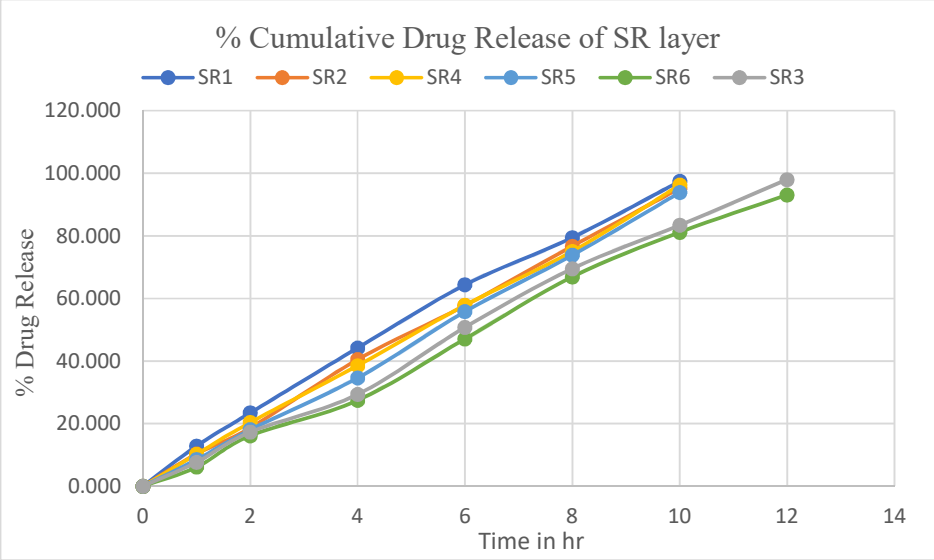


Fig 16: % Cumulative Drug Release of Sustained Release Layer

Sustained release tablets of Metformin HCl were prepared by using HPMC K4M and HPMC K100M polymers. The release profiles of Metformin HCl sustained Released tablet were plotted as in **Fig.16**. The release rate of Metformin HCl mainly controlled by the hydration and swelling properties of polymers. The formulation F1, F2, F4, and F5 shows the drug release up to 10 hrs. which shows the maximum drug release up to 10 hrs. after 10 hrs. the drug release was constant. The effect of polymer concentration on drug release could be clearly seen from the variation of dissolution profiles. It was found that drug release from SR3 composed of HPMC K4M in high concentration was 97.99±0.46 at the end of 12 hrs. and also shows significantly higher drug release rate than other formulations. Formulation SR3 containing 190 mg of HPMC K4M of Cumulative drug release which comparatively greater than other formulation batches so SR3 was selected for further formulation of bilayer tablet of Metformin HCl.

Stability Studies of Bilayer Tablet

Stability study for the developed formulation F3 were carried out as per ICH guideline by storing at

40°C/75% RH up to three months. The formulation F3 was selected on the basis of their cumulative percentage drug release. **Fig.17** and **Fig.18** shows cumulative percent release of F3 Formulation after three months which shows stability of formulation.

Table 8: Comparative drug % of Sustained release layer of SR3 batch

Time in hr.	% Cumulative drug release	
0	0	0
1	7.64±0.53	6.13±0.034
2	17.31±0.51	16.10±0.36
4	29.34±0.55	28.31±0.49
6	50.77±0.40	47.13±0.37
8	69.52±0.47	67.13±0.52
10	83.39±0.58	79.72±0.35
12	97.99±0.46	93.25±0.34

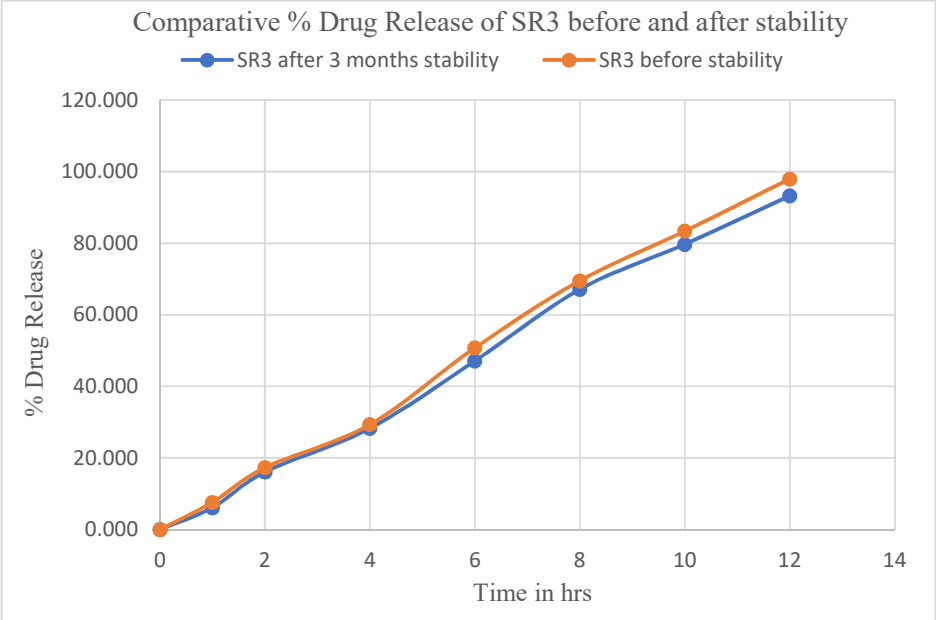


Fig 17: Comparative drug % release of Sustained Release layer of SR3 layer before and after 3 months stability

Table 9: Comparative % drug release of Immediate release layer IR3 Batch

Time in Minutes	% Cumulative drug release	
0	0	0
5	14.04±0.50	13.06±0.42

10	27.65±0.42	25.83±0.58
15	41.77±0.37	39.04±0.23
20	56.51±0.58	51.22±0.42
25	71.64±0.32	65.47±0.43
30	78.81±0.39	73.94±0.69
35	86.28±0.48	84.40±0.65
40	98.93±0.37	95.16±0.80

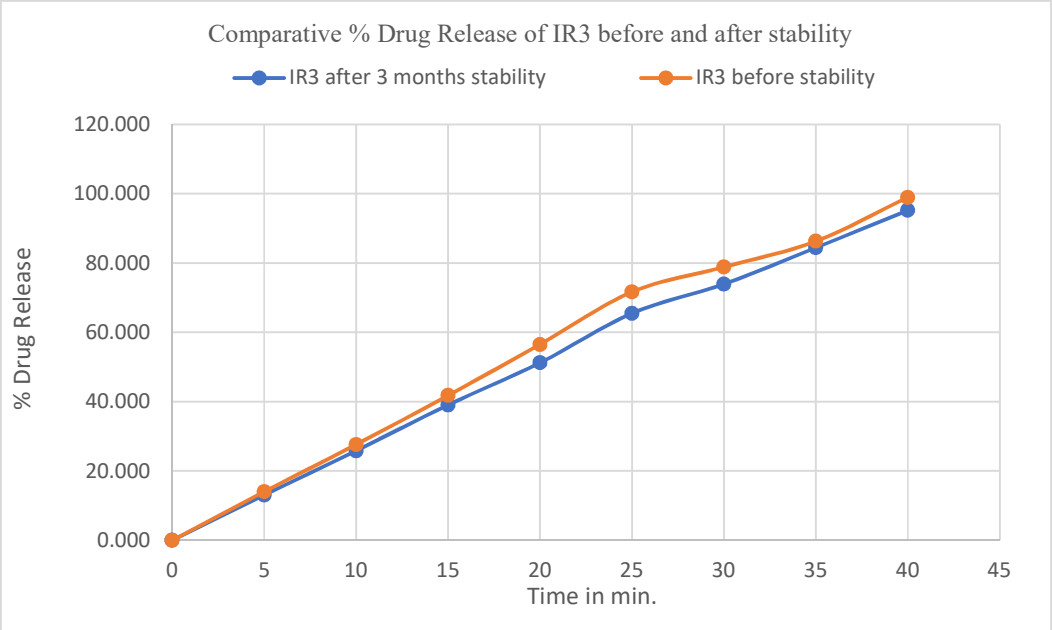


Fig 18: Comparative drug % of release Immediate Release layer of IR3 batch before and after 3 months stability

As there were no significant differences in post compression studies (weight variation, thickness, hardness, friability and in vitro dissolution studies) of initial and accelerated stability samples of optimized formulation IR3 and SR3 of batch F3 in the final up to three months, it passes the test for stability as per ICH guidelines. Comparative FT-IR spectra of optimized F3-Initial and 40°C / 75% RH-2M, reveals there is no significant change in the functional group's peaks of the Enalapril Maleate and Metformin HCl due to interaction with polymers and other excipients in the accelerated stability studies.

Table 10: Comparative stability study of Bilayer Tablet

Sr.No.	Parameter	Initial	After three months
1	Hardness (kg/cm$^{2)}$	6.9±0.37	6.5±0.24
2	Thickness(mm)	5.5±0.064	5.2±0.05
3	Friability (%)	0.8±0.02	0.85±0.06
4	Weight Variation(mg)	850.1±0.32	848±0.96

5	Disintegration Time (Sec)	45.31±0.29	54.63±0.34
6	Drug content (%)	98.6±0.62 (SR3) 98.72±0.46 (IR3)	97.96±0.32 (SR3) 97.42±0.57 (IR3)
7	% Drug Release	97.99±0.46 (SR3) 98.93±0.37 (IR3)	93.25±0.34 (SR3) 95.16±0.80 (IR3)

CONCLUSION

The present research work was carried out to develop antidiabetic bilayer tablet of Metformin HCl and Enalapril Maleate using polymer HPMC K4M and HPMC K100M at high concentration for Sustained Release layer and super disintegrant Croscarmellose Sodium and Sodium Starch Glycolate for Immediate Release layer.

The pre-compression study was performed and the results were reported. In pre-compression study the angle of repose, bulk density, tapped density, Hausner's ratio and Carr's index was calculated and results shown the good compliance as per IP standards. The tablets parameters were evaluated by testing thickness, hardness, weight variation, friability test etc. The % drug content was also calculated and the disintegration time study was performed by using disintegrating apparatus.

The formulation F3 shown the best results of dissolution study containing IR3 and SR3 layer of Croscarmellose Sodium and HPMC K4M respectively. The IR3 layer of F3 formulation shown 98.93% drug release at the end of 40 min. and which was the best drug release than other formulations. The SR3 layer of F3 formulation shown the controlled drug release for 12 hrs. with maximum 97.99 % drug release than other formulations. Further, the stability study of the bilayer tablet was checked as per ICH guidelines and the results were complies to the IP standards. The dissolution study shows the sustain release of the Metformin HCl for 12 hrs. which reduces the dose frequency and increases the patient compliances. The Metformin HCl and Enalapril Maleate show the combining effect that reduces Oxidative Stress.

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Section A-Research paper

DEVELOPMENT OF NOVEL HPTLC METHOD FOR SIMULTANEOUS ESTIMATION OF LISINOPRIL AND AMLODIPINE IN TABLET USING QBD APPROACH

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ABSTRACT

The purpose of this research was to develop a robust, rapid, and novel high-performance thin layer chromatographic (HPTLC) method for quantitation and separation of Lisinopril and Amlodipine in combine tablet dosage form using a quality by design approach. A central composite experimental design with response surface methodology was utilized to study the effects of chromatographic chamber saturation time, band length on R_f value. The R_f value was predicted for Lisinopril and Amlodipine between 0.25 and 0.85 to optimize the chromatographic conditions based on the preliminary trials. The optimized chromatographic conditions were 15 Minute saturation time, 6 mm band length and Methanol: Toluene: Formic acid (8:2:0.2 v/v/v) as a mobile phase. The optimized HPTLC method was validated according to ICH Q2 (R1) guideline. The result of this research clearly shows that QbD approach is successfully applied to optimize HPTLC method through minimum number of experimental runs.

KEYWORDS: Central composite experimental design, optimization, quality by design, validation.

INTRODUCTION

In order to build analytical method that produce quality result with desired conditions, the quality by design (QbD) methodology is used. [1] Quality by design is substantially applicable for finding the effects of independant variable (factors) on responses by carrying out different experimental sets that are obtained from central composite design. This design is also applicable for giving maximum information about methods and factors from the minimum experimental run. At actual QbD is significant model applicable in pharmaceutical industries and is defined as per ICH regulations as "a systematic approach to the development that begins with predefined objectives and emphasizes product and process understanding and process control, based on sound science and quality risk management". [2]

From the literature, it is found that response surface method (RSM) has considerably used to optimize an analytical method, because in a response surface methodology multivariate analysis is possible, that is several factors can be optimized simultaneous [3] and the level of several factors in an experimental design domain is expected to contain optimum. The quality of (HPTLC) method is very important and is best developed by using a QbD approach, as per this approach the HPTLC method is verified at early stage of method development which give assurance about quality of method. From the literature it is clear that there are many reported papers on HPTLC method development but very few are by using a QbD environment, [4, 5] therefore in this research paper we develop HPTLC method systematically from central composite design. Amlodipine (Amlo) is calcium channel blocker, chemically it is 3-O-ethyl-5-O-methyl-2-(2-aminoethoxy methyl)-4-(2-chlorophenyl)-6- methyl-1,4-dihydro pyridine-3,5-dicarboxylate, it and used to treat heart disorders like hypertension and coronary artery diseases. Chemically Lisinopril (Lisino) is (2S)-1-[(2S)-6-amino-2-([(1S)-1-carboxy- 3-phenylpropyl] - amino) hexanoyl] pyrrolidine-2-carboxylic acid. Angiotensin converting enzyme (ACE) inhibitor and is a standard drug used to treat of critical situations of heart failure. Both these drugs are given in combination to show effects on heart disease. [6]

Literature survey revealed that there are several HPLC [7] and HPTLC [8, 9] methods for simultaneous evaluation of Lisinopril and Amlodipine in tablet and various formulations, but not a single method was developed by systematic application of QbD methodology. The rationale of this research work was to

reconnoite the importance of QbD approach in the development of HPTLC method for simultaneous evaluation of Lisinopril and Amlodipine in tablet dosage form.

EXPERIMENTAL

Instrumentation

HPTLC method development applying QbD for simultaneous evaluation of Lisinopril and Amlodipine was utilizes a Camag HPTLC system fitted with Camag thin layer chromatography (TLC) ScannerIII and sample applicator of Linomat V which is semi-automatic. The Hamilton syringe with 100 µL capacity was used to apply sample. The marketed precoated silica gel aluminum plate and flat bottom 10cm by 10 cm twin trough TLC developing chamber were used for Chromatographic separation. For the densitometric analysis TLC scanner III having Camag win CATS software was used and design expert of version 8.0 software was applied for data analysis.

Materials and Reagents

Amlopress L marketed tablet comprising 5 mg dose of Lisinopril and Amlodipine each was purchased. Methanol, Formic acid, Ethyl acetate and Toluene with analytical grade were utilized from D. Y. Patil Research centre.

Standard solution

The standard 500 µg/ml solution of Lisinopril and Amlodipine was prepared by dissolving precisely weighed 5mg of pure drug of Lisinopril and Amlodipine in a 10 ml of methanol using 10ml volumetric flask.

Application of sample

The standard solution with different concentrations and sample solution obtained from formulation were spotted on activated precoated HPTLC plates as narrow bands with 6mm band length separated by 9mm distance and dried with steam of nitrogen gas.

Optimized chromatographic specifications

Mobile phase (Methanol: Toluene: Formic acid; 8:2:0.2 v/v/v) was optimized using Central Composite response surface methodology. The 6mm of band length (optimized with Central Composite design) of sample was applied on activated HPTLC plates and were developed using above mobile phase in a specified TLC developing chamber for saturation time of 15 min at room temperature (optimized with Central Composite design). The spots on HPTLC plates were scanned using Camag TLC scanner III at absorbance mode at 338 nm.

Central composite experimental design

A Central Composite experimental design through three levels, two factors, 13 runs with four center points was selected as response surface design to evaluate quadratic, interactive, and main effects of saturation time and band length on response of R_f value of both the drug. The experimental model was analyzed for Optimization of factor levels on the response of R_f value of Lisinopril and Amlodipine.

Method validation

The optimized method obtained from experimental model was validated as per guidelines given by ICH.[17]

Linearity

The standard concentrations of 500, 1000, 1500, 2000, 2500, 3000 ng/spot of Lisinopril and Amlodipine were prepared, the peak area were determined for these concentrations using optimized method and calibration curve was obtained by plotting a graph of peak area vs.concentrations.

Precision

The peak area was determined for three different standard concentrations of 500 ng/spot, 1000 ng/spot, 1500 ng/spot of Lisinopril and Amlodipine for intraday and interday variation respectively. The method was analyzed for standard deviation, mean and relative standard deviation for obtained value of peak area.

Method Precision

Weigh 20 tablets and calculate average weight of tablet, crushed 20 tablets and weigh accurateky 245.2 mg powder and dissolve it in 10 ml of methanol. Shake the mixture for about 20 min. then filter it through whatmann filter paper, a clear solution was obtained. The 3 µl solution spot was given thrice and percent label claim was calculated from average value of peak area.

Accuracy

To determine accuracy of developed method a known amount of standard solution of 80%, 100%, and 120% were spiked in known concentration of sample solution of Lisinopril and Amlodipine and it was analyzed for three consecutive days to calculate drug recovery.

Limit of Detection (LOD) and Limit of Quantification (LOQ)

The formula used to calculate LOD and LOQ is LOD 3.3*SD/Slope, LOQ 10*SD/Slope. where SD is standard deviation of responses.

RESULTS AND DISCUSSION

Optimization restraint and solution

The restraints in optimization design are based on specifications of final response. The basic objective was to achieve R_f value for Lisinopril and Amlodipine in the 0.2–0.8 range. After applying a design expert software for the data obtained for the responses, the optimum condition for saturation time was 15 min and band width of 6 mm, for the mobile phase of Methanol: Toluene: Formic acid; 8:2:0.2 v/v/v.

From the preliminary data it was shown that R_f value obtained for Lisinopril was below 0.2, for the various mobile phase trials and finally Methanol: Toluene: Formic acid were found as a suitable solvent system for the above combination. As the responses of R_f value of Lisinopril and Amlodipine was found to be vary with critical factor of saturation time and band width. Lisinopril peak was observed at 0.17-0.23 R_f value for higher saturation time. The responses were also varying along with band width and optimum band width was found 6 mm. The Factors, minimum and maximum levels of factors with their units are given in Table 1 and information about responses given in Table 2.

Table 1. Chromatographic factors for central composite experimental design

Factor	Name	Units	Type	Subtype	Minimum	Maximum
A	Saturation time	Min	Numeric	Continuous	10	20
B	Band Length	Mm	Numeric	Continuous	4	8

Table2. Chromatographic response for central composite experimental design

Response	Name	Units	Obs	Analysis
Y1	R_f of Lisinopril	No unit	13	Polynomial
Y2	R_f of Amlodipine	No unit	13	Polynomial

Central composite experimental design

The random orders of experimental runs of design were accomplished to get the accurate data and results obtained are shown in Table 3. A central composite design with quadratic model was applied for finding effects of factors on R_f value of Lisinopril and Amlodipine, respectively, the equations were obtained (1) and (2) as shown below.

$$\text{Rf of Lisinopril (Y)} = +0.22+0.000*A-0.020*B-0.013*A*B-0.059*A2-0.049*B2 \quad (1)$$

$$\text{Rf of Amlodipine (Y)} = +0.82-0.020*A+0.015*B-0.035*A*B-0.11*A2-.552E-003*B2 \quad (2)$$

Where Y is the response, A and B are the factors.

Table 3. Experimental design and its responses

		Factor 1	Factor 2	Response 1	Response 2
Std	**Run**	**A:Saturation time Minute**	**B:Band Length mm**	**R_f of Lisinopril**	**R_f of Amlodipine**
13	1	15	6	0.2	0.82
7	2	15	4	0.23	0.82
3	3	10	8	0.12	0.82
8	4	15	8	0.12	0.77
5	5	10	6	0.17	0.69
12	6	15	6	0.23	0.89
10	7	15	6	0.23	0.8
9	8	15	6	0.21	0.81
1	9	10	4	0.1	0.68
2	10	20	4	0.13	0.69
11	11	15	6	0.23	0.81
4	12	20	8	0.1	0.69
6	13	20	6	0.16	0.69

Table 4 and 5 shows the ANOVA for response of R_f value of Lisinopril and Amlodipine. As per the model

probable value was found 0.0063 and 0.0351 and from this it is confirmed that model was statically significant.

Table 4. Summary of Results of ANOVA

ANOVA for central composite quadratic model for Rf of Lisinopril						
Source	**Sum of squares**	**df**	**Mean square**	**F value**	**p-value Prob > F**	
Model	0.02878	5	0.005756	8.780656	**0.0063**	Significant
A-Saturation time	-3.5E-18	1	-3.5E-18	-5.3E-15	1.0000	
B-Band Length	0.0024	1	0.0024	3.661093	0.0973	
AB	0.000625	1	0.000625	0.95341	0.3614	
A^2	0.009491	1	0.009491	14.47805	0.0067	
B^2	0.006529	1	0.006529	9.959797	0.0160	
Residual	0.004589	7	0.000656			
Lack of Fit	0.003789	3	0.001263	6.314655	0.0536	Not significant
Pure Error	0.0008	4	0.0002			
Cor Total	0.033369	12				

Table5. Summary of Results of Analysis of Variance (ANOVA)

ANOVA for central composite quadratic model for Rf of Amlodipine						
Source	**Sum of squares**	**df**	**Mean square**	**F value**	**p-value Prob > F**	
Model	0.045745	5	0.009149	4.611942	**0.0351**	Significant
A-Saturation time	0.0024	1	0.0024	1.209834	0.3077	
B-Band Length	0.00135	1	0.00135	0.680531	0.4366	
AB	0.0049	1	0.0049	2.470077	0.1600	
A^2	0.031357	1	0.031357	15.8068	0.0054	
B^2	6.65E-06	1	6.65E-06	0.003352	0.9554	
Residual	0.013886	7	0.001984			
Lack of Fit	0.008566	3	0.002855	2.146919	0.2370	Not significant
Pure Error	0.00532	4	0.00133			
Cor Total	0.059631	12				

Values for A^2B, AB^2 are not obtained due to reduced model for selected responses. Bold values given in the tables indicate that there is a significant influence of the selected factors on response. Different response surface plots for various values of factors were studied.

Figure 1 and 2 indicate the effect of factors like band length and saturation time on R_f value of Lisinopril and Amlodipine. Curve-line in plots obtained here which indicate that there is a non-linear effect of parameters of factors on R_f value.

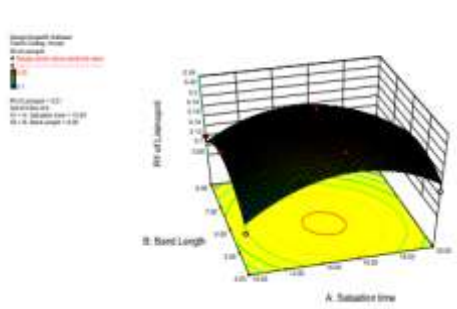

Fig. 1 Response surface plot for Lisinopril

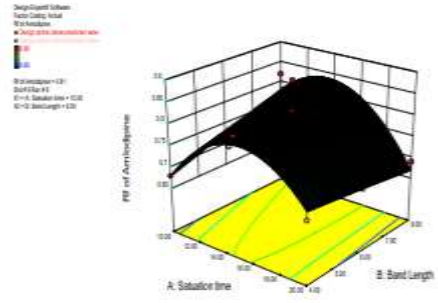

Fig.2. Response surface plot for Amlodipine

By using optimized chromatographic conditions from the central composite design the chromatographic separation of Amlodipine and Lisinopril was achieved and it shown in Figure3.

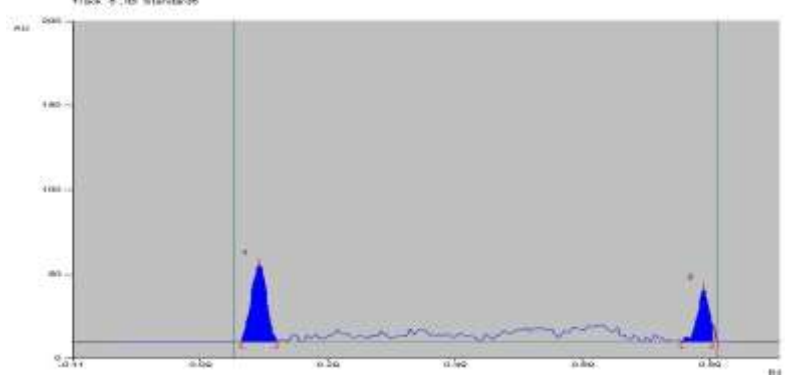

Fig. 3. Seperation of standard Lisinopril (500 ng/spot) and Amlodipine (500 ng/spot) having R_f value of 0.15 and 0.79 respectively.

METHOD VALIDATION

Linearity

The linearity curves obtained from the data was found to be linear when we plot a graph of peak area verses analyte concentration. The accepted value of correlation was obtained which is mentioned in Figure 4 and 5. The figure 6 shows a linearity curve for simultaneous evaluation of Lisinopril and Amlodipine.

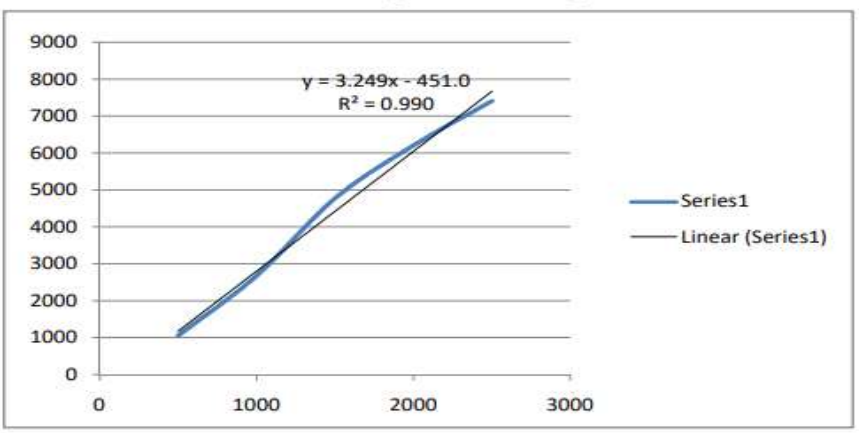

Fig. 4. Linearity curve for Lisinopril

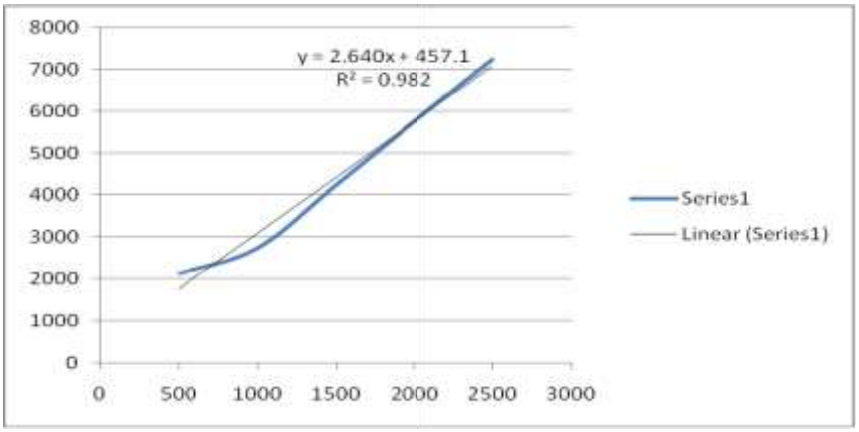

Fig. 5. Linearity curve for Amlodipine

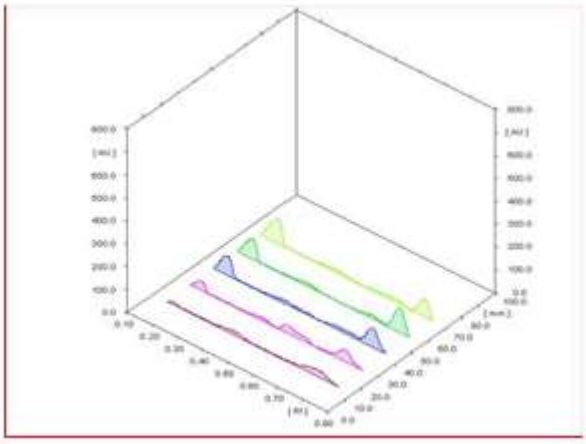

Fig. 6. Linearity for simultaneous estimation of Lisinopril and Amlodipine

Method Precision

Weigh accurately 245.2 mg of crushed powder of 20 tablet and dissolve it in 10 ml of methanol. Shake the mixture for about 20 min. then filter it through whatmann filter paper, a clear solution was obtained. The 3 µl solution spot was given thrice and percent label claim was calculated from average value of peak area. Percent label claim is mentioned in table6.

Table 7. Method Precision data

Sr. no.	**Drug content in powder**		**Percent Label Claim**	
	Amlodipine	Lisinopril	Amlodipine	Lisinopril
1.	5.09mg	5.12 mg	98.20	102.4

Precision

In case of precision we have carried out intra and inter day study, from this study data obtained were used to calculate percent relative standard deviation for the above optimized method and were found less than 2%, this concludes that the given method is precise. The data for precision study is shown in the Table 7.

Table 7. Intraday Precision Study where (n= 3)

SR. NO.	Intra Day Precision			Intra Day Precision		
	PEAK AREA OF LISINO			PEAK AREA OFAMLO		
	MORNING	AFTERNOON	EVENING	MORNING	AFTERNOON	EVENING
1	2822.4	2822.4	2726.5	3487	3261.6	3053
2	2821.8	2820.3	2762.2	3475.7	3208.2	3058
3	2821.8	2830.2	2757.3	3475.7	3250.3	3049.8
AVERAGE	2822	2824.3	2748.66	3479	3240.03	30530.7
SD	0.346	5.21	19.35	6.52	28.14	4.23
%RSD	0.012	0.18	0.7	0.17	0.86	0.138

Table 8. Inter-day Precision Study where (n= 3)

SR. NO.	Inter Day Precision			Inter Day Precision		
	PEAK AREA OF LISINO			PEAK AREA OFAMLO		
	DAY 1	DAY 2	DAY 3	DAY 1	DAY 2	DAY 3
1	3538.5	3320.6	3251	4117.6	3845.9	3470.4
2	3537.5	3340	3241	4105.2	3852.9	3485.2
3	3536.4	3314	3261	4126.3	3830	3452.3
AVERAGE	3537.46	3324.8	3251	4116.37	3842.93	3469.3
SD	1.05	13.51	10	10.6	11.73	16.47
%RSD	0.029	0.4	0.3	0.257	3842.93	0.474

Accuracy

Weigh accurately 5mg amlodipine, 5mg Lisinopril and 242.25mg of tablet powder of 20 tablets; dissolve this in 10 ml of methanol. This is a 100 percent solution, similarly the 80% and 120% solution obtained by taking 4mg, 6 mg of amlodipine and lisinopril each in 242.25 mg of powder respectively. The percent recovery was calculated and from the data given in table it is confirmed that the given method is accurate.

Table 9. Accuracy Results (n=3)

Components	Amount of standard drug added in mg	Amount estimated per tablet in mg	Amount of drug recovered in mg	Percent recovery
Amlodipine	4	9.1	4.1	102.5
	5	9.9	4.9	98
	6	10.85	5.85	97.5
Lisinopril	4	9.02	4.02	100.5
	5	10	5	100
	6	11	6	100

LOD and LOQ

Actually LOD means the lowest conc. of Lisinopril and Amlodipine which can be detected but not quantified by the method and LOQ the lowest conc. of Lisinopril and Amlodipine that can be quantified accurately and precisely by using a given method. The LOD was found 6.58 mg and 22.72 mg for Lisinopril and Amlodipine, LOQ was found 19.94 mg and 69.17 mg for Lisinopril and Amlodipine.

APPLICATION

The 20 tablets of AMLOPRESS L were weighed accurately using an analytical balance and powdered using a mortar pestle. The weight of powder was taken so that it contains 5 mg conc. of Lisinopril and Amlodipine each. By dissolving this in a methanol sample solutions were prepared, the concentration was found using a proposed method. These results shown in figure 7 indicate that method is applicable for determining concentration of drug in a marketed drug formulaton.

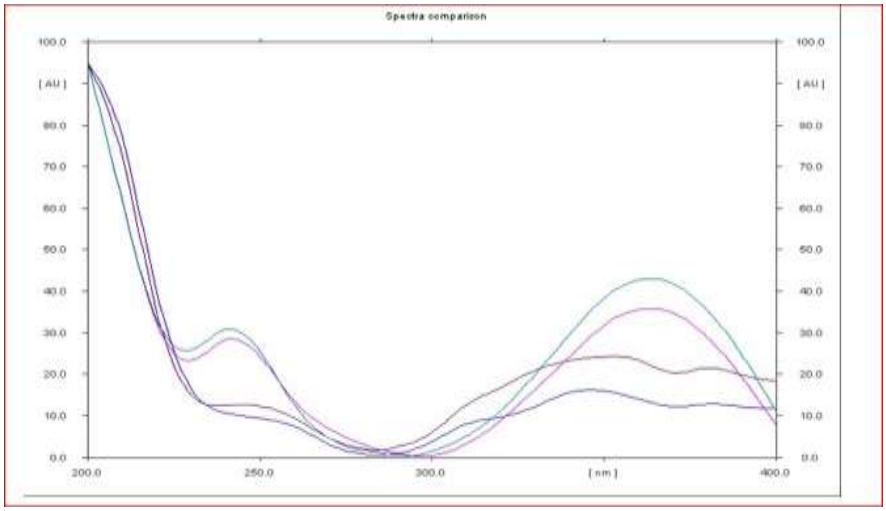

Fig. 7. Graph for standard and marketed formulation

CONCLUSION

Proficient QbD approach was used to develop a validated HPTLC method for finding a concentration of Lisinopril and Amlodipine. This approach gives better understanding of the factors which influence chromatographic separation and assure that method gives an expected result. In this study two factors were analyzed to determine their effect on response with the least number of experiments which will be possible by applying a Central composite design from design expert of version 8.0. The two factors band length and saturation time was considered in this experimental design and HPTLC method was developed. The developed method was validated for specificity, accuracy, linearity so it is concluded here that the QbD approach is successfully used to develop HPTLC method to estimate the concentration of Lisinopril and Amlodipine in a marketed tablet dosage form.

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Formulation Development and Evaluation for Mouth Dissolving Tablet Containing Bromelain

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ABSTRACT:

Bromelain is a proteolytic enzyme that is used as an anti-inflammatory drug. Administration of Bromelain through the oral route is a challenge in children, who have difficulty swallowing tablets. In the present study, six batches of Bromelain Mouth Dissolving tablets (MDT) dosage form at the dose of 100 mg were formulated and evaluated. Results showed that the thickness, weight variation, friability, hardness, and content uniformity of all six formulations were within acceptable limits. But in the in-vitro dissolution study formulation 3 demonstrated better cumulative drug release than other formulations. Hence the study concludes that Bromelain month dissolving tablets formulated using crospovidone (Formulation 3) showed better characteristics of mouth dissolving tablets.

KEYWORDS: Bromelain, Mouth dissolving tablets, Drug-Excipient Compatibility, Crospovidone.

INTRODUCTION:

Many patients, particularly old find it difficult in swallowing tablets, capsules, and fluids and subsequently do not comply with prescriptions, which results in a high frequency of resistance situated research has resulted in bringing out many secure, safe new drug delivery system. Among the several dosage forms developed to improve the difficulty of administration, the Mouth Dissolving Tablet (MDT) is the most favoured commercial product. [1] The oral cavity is an appealing site for the administration of drugs because of the simplicity of administration. Several dosage forms like Tablets, Capsules, and Liquid preparations are administered by oral route. During the most recent decade, Mouth Dissolving Tablet (MDT) advances that make tablets disintegrate in the mouth without chewing and additional water intake have drawn a lot of consideration. The MDTs are also known as fast liquefying, rapid dispersing, rapid dissolve, rapid melt, as well as speedy disintegrating tablets. [2-4] MDTs can be prepared by various conventional methods like direct compression, wet granulation, molding, spray drying, freeze-drying, and sublimation. Firstly, MDTs disintegrate and then dissolve quickly in the saliva without any need for solvents, releasing the drug. A few drugs are absorbed from the mouth, pharynx, and oesophagus as the saliva goes down into the stomach. In many cases, the bioavailability of these drugs is significantly more than those observed from conventional tablet dosage forms. [5,6]

Bromelain is a proteolytic enzyme with anti-inflammatory activity. It is found in pineapple juice and in the pineapple stem. Anti-inflammatory activity by activation of plasmin production from plasminogen and reduction of kinin via inhibition of the conversion of kininogen to kinin. Bromelain has low oral bio-availability because of high first pass metabolism rate. Hence, the formulation in orodispersible form of Bromelain upgrades the bioavailability, decreases side effects, low dosing, patient compliance, and rapid onset of action with great steadiness. In the present work, Orodispersible tablets of Bromelain were prepared by direct compression method using sodium starch glycollate, and crosspovidone as the superdisintegrants. The aim of the study was to evaluate the effect of the superdisintegrants on the wetting time, disintegration time, and drug release profile of the orodispersible tablets. The present investigation deals with the improvement of an effective and stable MDT of Bromelain having a sufficient hardness, low disintegration time, and pleasant taste.

MATERIAL AND METHODS:

Materials:

Bromelain was obtained from Arti Pharma, Mumbai, India. Crospovidone and sodium starch glycolate were obtained from Research Lab Fine Chemical Industries Pvt. Ltd. Mumbai. Microcrystalline cellulose and mannitol were also obtained from Research Lab Fine Chemical Industries Pvt. Ltd. Mumbai. All other chemicals of analytical grade were purchased from commercial sources.

Methods:

Preparation of Mouth Dissolving Tablets by Direct Compression Method:

Mouth dissolving tablets of Bromelain were prepared by direct compression. All the ingredients (except granular directly compressible excipients) were passed through # 60-mesh separately. The ingredients were then weighed and mixed in geometrical order and compressed into tablets of 250 mg using 6 mm round concave punches on an 8-station rotary tablet machine (Table 1).

Table 1: Formulation Table of Bromelain Mouth Dissolving Tablet

Formulations	**F1**	**F2**	**F3**	**F4**	**F5**	**F6**
Ingredient	**Unit Formula (mg per tablet)**					
Bromelain	100	100	100	100	100	100
Crosspovidone	5	10	15	-	-	-
Sodium starch glycolate	-	-	-	5	10	15
Mannitol	50	50	50	50	50	50
Menthol	10	10	10	10	10	10
Avicel PH 102	81	76	71	81	76	71
Sodium stearate	2	2	2	2	2	2
Talc	2	2	2	2	2	2
Total	250	250	250	250	250	250

Evaluation of Bromelain Mouth Dissolving Tablet:

Evaluation of pre-compression parameters of powder:

Preformulation study:

Angle of repose (Ɵ) [7]

The angle of repose was determined by using the funnel method. The accurately weighed blend was taken in a funnel. The height of the funnel was adjusted in such a way that the tip of funnel just touched the apex of the heap and the drug-excipient blend was allowed to flow through the funnel freely to the surface. The diameter of the powder cone was measured and angle of repose calculated using the following equation.

$$\mathbf{Tan\ \theta = h/r}$$

Different ranges of flowability in terms of angle of repose (Table II) are given below (Bikshapathi et al., 2011).

Table 2: Relationship Between Angle of Repose and Flowability

Flow property	Angle of repose
Excellent	25-30
Good	31-35
Fair (aid not needed)	36-40
Passable (may hang up)	41-45
Poor (must agitate, vibrate)	46-55
Very Poor	56-65
Very Very Poor	>66

Bulk Density [8]

Bulk density was determined by pouring presieved drug excipient blend into a 100 ml graduated cylinder. The sample occupied volume and its weight has been recorded It is expressed in g/mL and calculated by using following formula:

$$\mathbf{\rho b = M / Vp}$$

Where,

ρb = Bulk density

M = Weight of sample in grams

Vp = Final volumes of Powder in cm3

Tapped Density [9]

It was carried out by pouring powder blend in 100ml graduated cylinder. The cylinder was tapped mechanically by Tap density apparatus until a constant volume was obtained. Volume occupied by the sample after tapping were recorded and tapped density was calculated by using following formula:

$$\rho t = M / VT$$

Where,

ρt = Tap density

M = Weight of sample in grams

VT = final tap volume of powder in cm3

Hausner's ratio [10]

Hausner's ratio is the ratio of tapped density to bulk density. The lower the value of Hausner's ratio the better the flow property. The ratio is calculated by the following formula

$$\text{Hausner's ratio} = \frac{\text{Tapped density}}{\text{Bulk density}}$$

Lower Hausner's ratios (<1.25) indicate better flow properties than higher ratios (>1.25) (Sayeed et al., 2011).

Carr's Index (Compressibility Index) [10]

It is also one of the simple methods to evaluate flow property of a powder by comparing the bulk density and tapped density. The percentage compressibility of a powder is a direct measure of the potential powder arch or bridge strength and stability. The grading of compressibility of powder according to carr's index is shown in table no.3. It can be calculated by following formula:

$$\text{Carr's Index} = \frac{\text{Tapped density} - \text{Bulk density}}{\text{Tapped density}} \times 100$$

Table 3: Relationship Between Carr's Index and Flowability

Carr's Index	Flow property
5-10	Excellent
12-16	Good
18-21	Fair to possible
23-35	Poor
33-38	Very poor
>40	Extremely poor

Drug-excipient compatibility study

Studies of drug-excipient compatibility are important to as certain drug and excipients are compatible with each other. IR spectra are used to study drug-excipient compatibility.

FTIR study [11]

The study was carried out to determine the molecular structure, serving as an identification test to ascertain the purity of the molecule. IR spectroscopy was obtained by a FTIR spectrophotometer (H400-84100, Shimadzu, Japan) using KBr pellets. The scanning range used was 4400 to 400 cm-1 at a scan period of 1min. Spectra of pure drug and the blend are shown in Figures 1 and 2. There is no change in the shape of the peak or shift of the peak, hence the drug and excipients are compatible (Prameela et al., 2010).

Evaluation of Post-Compression Parameters of Tablets:

Weight variation test [12]

Weight variation was calculated as per method described in Indian pharmacopeia (I.P.2007). Twenty tablets were weighted individually by using Electronic balance (Shimatzu) and the average weight is calculated. The tablets meet the test if no more than 2 tablets are outside the percentage limit and no tablet differs by more than 2 times the percentage limit. The limit of weight variation in tablet are listed in Table 4.

Table 4: Limits for Weight Variation in Tablet as Per I.P. 2007

Average weight of tablet (mg)	Percentage deviation allowed
80 mg or less	± 10
More than 80 mg but less than 250 mg	±7.5
or more	±5

Hardness test [13]

Hardness indicates the ability of a tablet to withstand mechanical shocks while handling. The hardness of the tablets was determined using Digital hardness tester. It is expressed in Kg/cm2. Digital hardness tester was used to measure hardness of the tablet. In which the tablet was placed in the tester and pressure needed to break the tablet was measured.

Thickness [14]

The thickness of the tablets was determined using a Vernier Caliper. Ten tablets from formulation batch were used and average values were calculated.

Friability [15]

Friability is the measure of tablet strength. It was carried out by using Roch friability apparatus, in which the accurately weighed 20 tablets was allowed to rolling and free fall at 25 rpm, after 100 revolutions weight of tablet was again measured and % friability was calculated by following formula

$$\% \text{ Friability} = \frac{\text{Initial weight of tablet} - \text{Final weight of tablet}}{\text{Initial weight of tablet}} \times 100$$

Disintegration time [16]

The disintegration time of tablet was determined by using Disintegration test apparatus. Tablets were placed in disintegration test assembly and disc was placed was placed on tablets in each glass tube of assembly. The assembly was dipped in a vessel containing 900 ml phosphate buffer 6.8 pH. The time for disappearance of tablet residue above mesh was noted as disintegration time.

Wetting time [17]

About 6-8 ml of phosphate buffer 6.8 pH was taken in 10 mL of measuring cylinder. Tablet was placed in the cylinder and complete dispersion of tablet in the cylinder was recorded as the disintegration time. Wetting time in that the tissue paper has been folded twice and placed in petri dish above that tablet is placed. A small quantity of amaranth red color was put on the upper surface of the tablet and 10 ml distilled water was added. The time required to get the tablet completely wet and indicate red color was measured.

Uniformity of drug content [18]

This method is performed as per Indian Pharmacopoeia. Two tablets were crushed and added to 30 ml of 0.1M NaOH in 100 ml volumetric flask sonicated to disintegrate, then diluted by acetonitrile, then this solution was filtered and diluted the filtrate with a mixture of seven volumes acetonitrile and three volumes of 0.1M NaOH. Absorbance was measured by UV spectroscopy at 280 nm and drug content was calculated.

In-vitro Dissolution study [16]

The dissolution study of selected Bromelain formulations was conducted by using USP dissolution apparatus Type – II (Electrolab Mumbai) by taking 900 ml phosphate buffer pH 6.8 as dissolution medium which maintained at 37 ± 0.5°C. At every 5 min interval upto 30 min 1 ml samples was withdrawn and the same volume was replaced to maintain the sink condition. The samples were analyzed using UV spectroscopy at wavelength maxima 280 nm. The % drug release was calculated and is reported in Table 10.11 and drug release profile of selected formulations tablets are depicted in Figure 12.

RESULTS AND DISCUSSION:

Spectroscopic Analysis:

UV spectroscopy:

Determination of λmax of Bromelain in Water:

In UV spectroscopy study, the maximum wavelength (λmax) of Bromelain in water was found to be 279.40 nm. The reported λmax value of Bromelain in water was also 279.40 nm respectively, so the values similar with the reported values indicates that the given sample of Bromelain was in pure form.

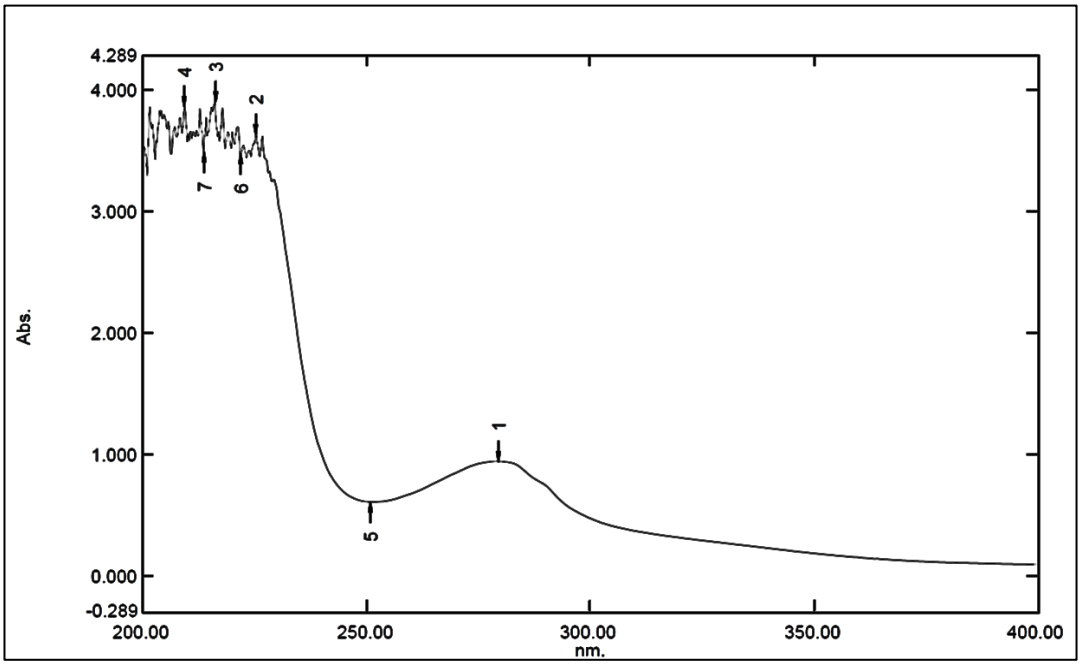

Figure 1: UV spectra of Bromelain in water at 279.40 nm

Calibration Curve of Bromelain in Water:

The linearity of the response of Bromelain was verified at 2–10 µg/ml concentrations. The calibration curve was obtained by plotting the absorbance versus the concentration data and was treated by linear regression analysis. The equation of the linearity curve for Bromelain was y = 0.0127x +0.0033. The linearity curve was found to be linear in the a for mentioned concentrations (the correlation coefficient (r^2) of determination was 0.9922) (Figure 2).

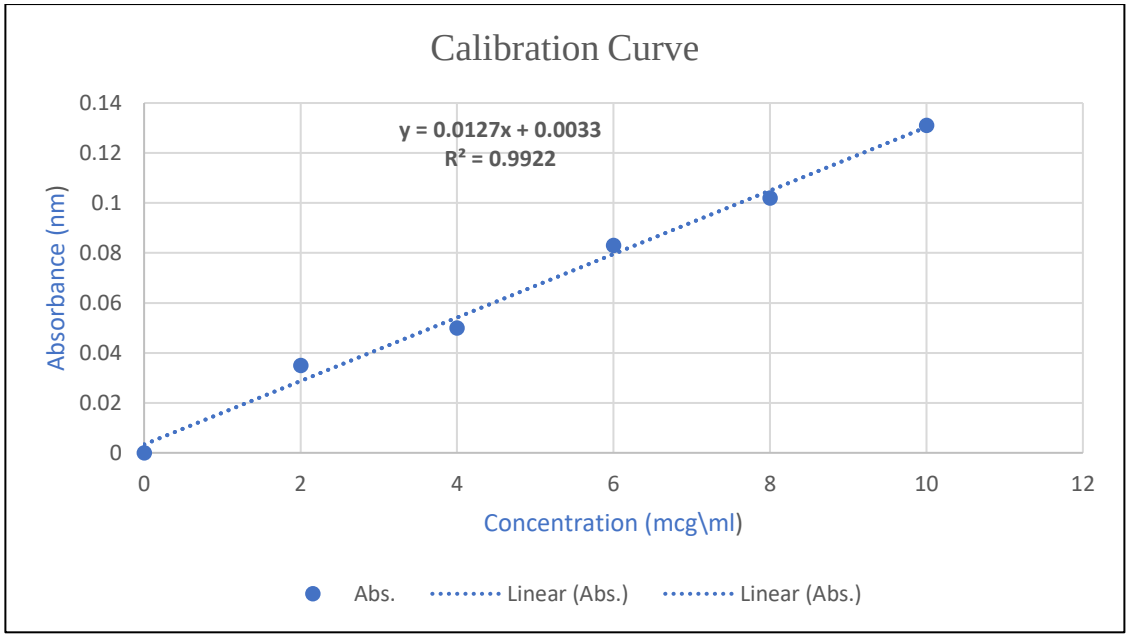

Figure 2: Calibration Curve of Bromelain in Water

FTIR spectroscopy:

The FTIR spectrums of pure Bromelain and physical mixtures of drugs and polymers were studied separately as per the excipients used in the formulation. It was observed that there were no major shifts in the main peaks of either drug. This indicates that there were no compatibility problems with the drug with the polymers and excipients used in the formulation. Bromelain had peaks at 3433 (-OH elongation), 1643 (CO elongation), 3487 (NH stretch), 1384 (C-N stretch), 2862 (C-H).

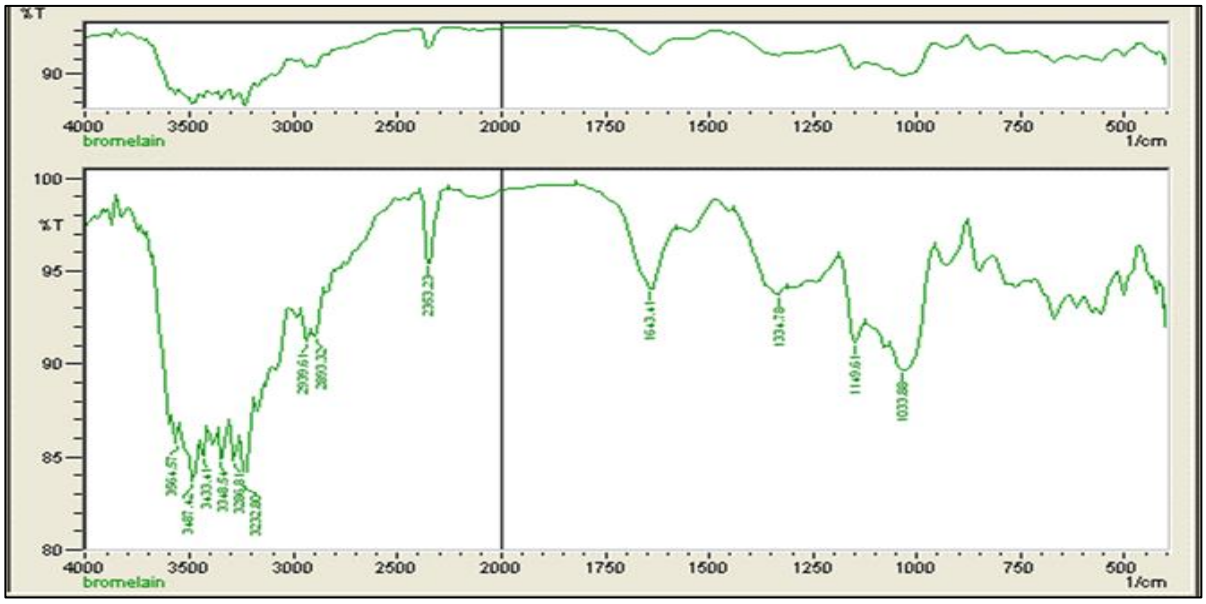

Figure 3: FTIR Studies of Bromelain

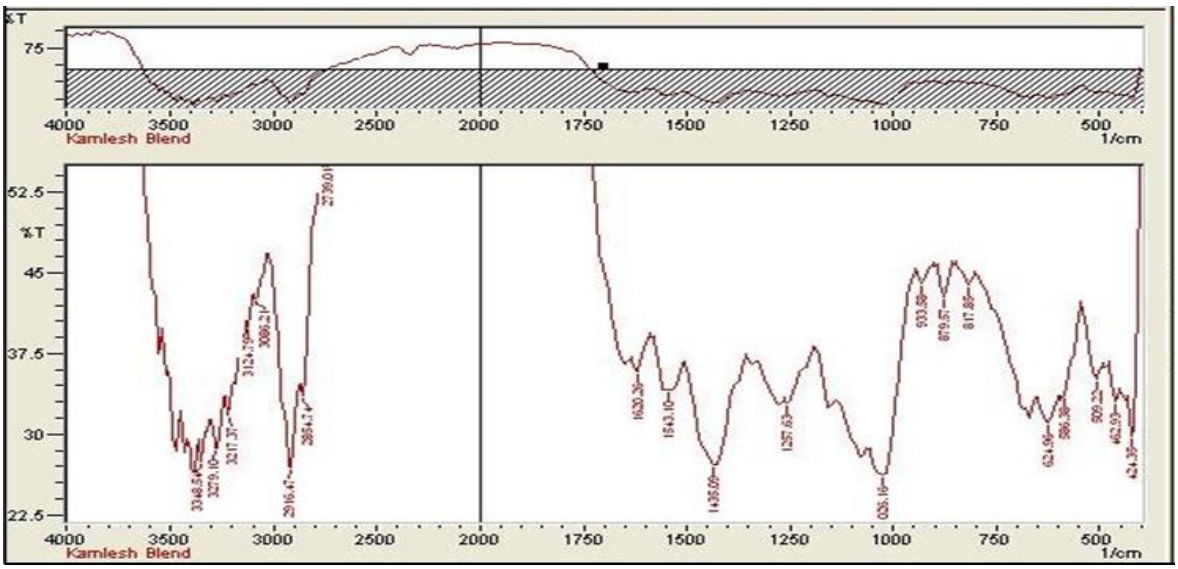

Figure 4: FTIR Studies of Bromelain Tablet Blend

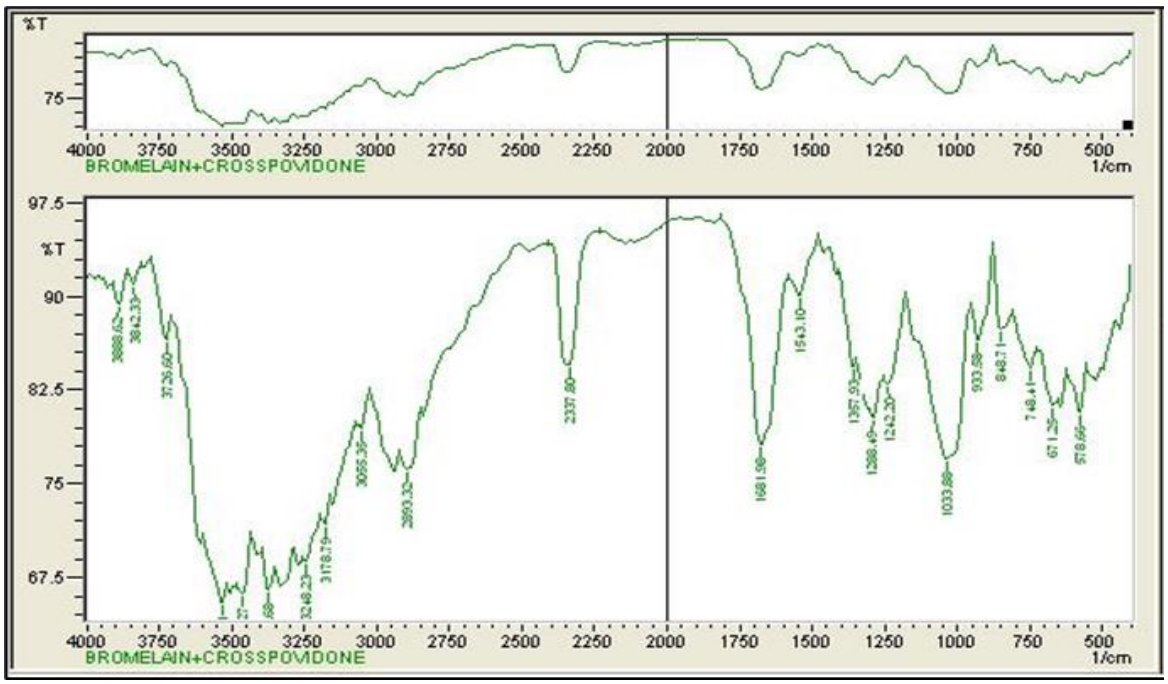

Figure 5: FTIR Studies of Bromelain + Crospovidone

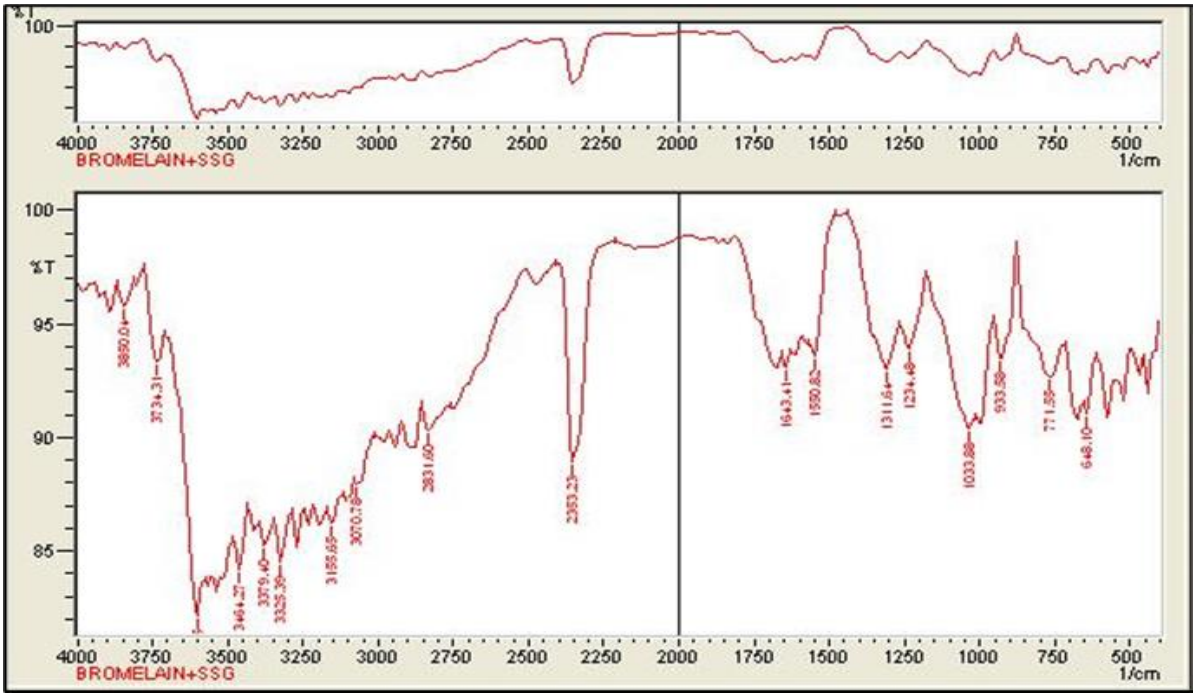

Figure 6: FTIR Studies of Bromelain + SSG

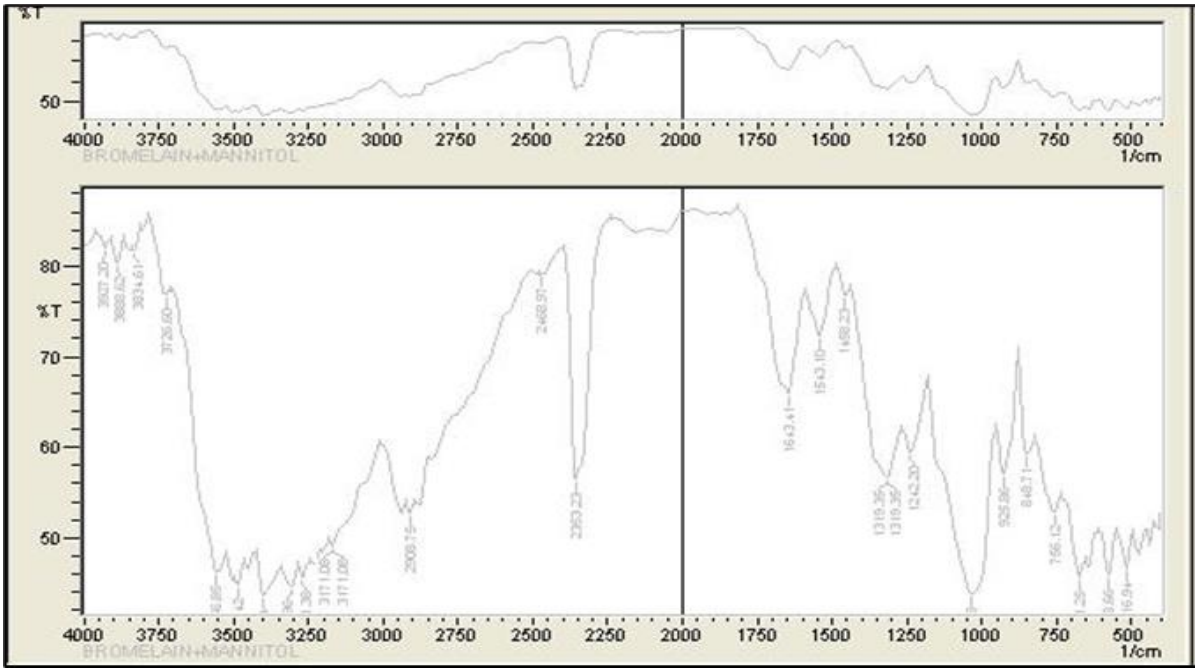

Figure 7: FTIR Studies of Bromelain + Mannitol

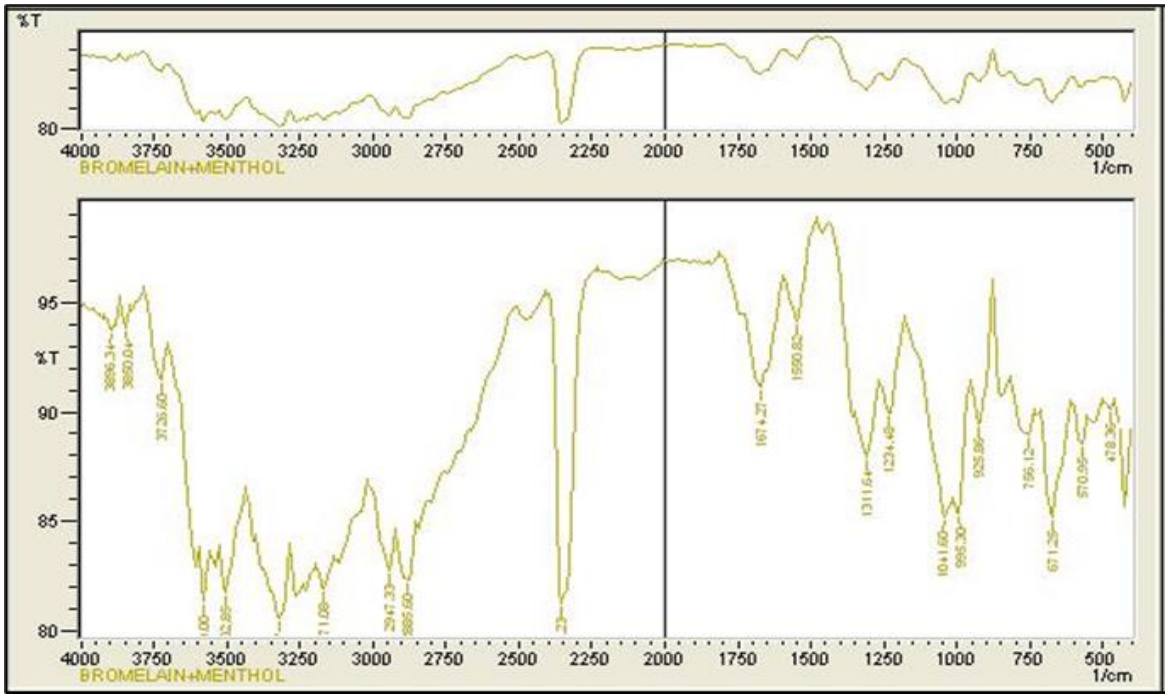

Figure 8: FTIR Studies of Bromelain + Menthol

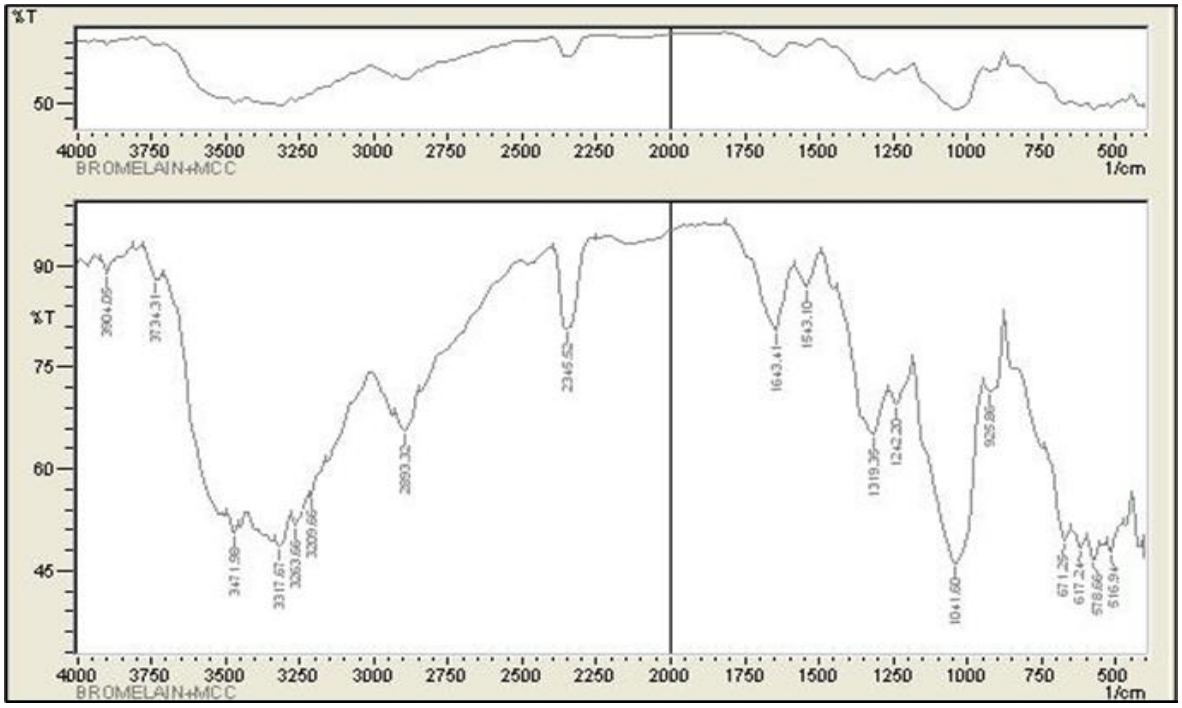

Figure 9: FTIR Studies of Bromelain + MCC

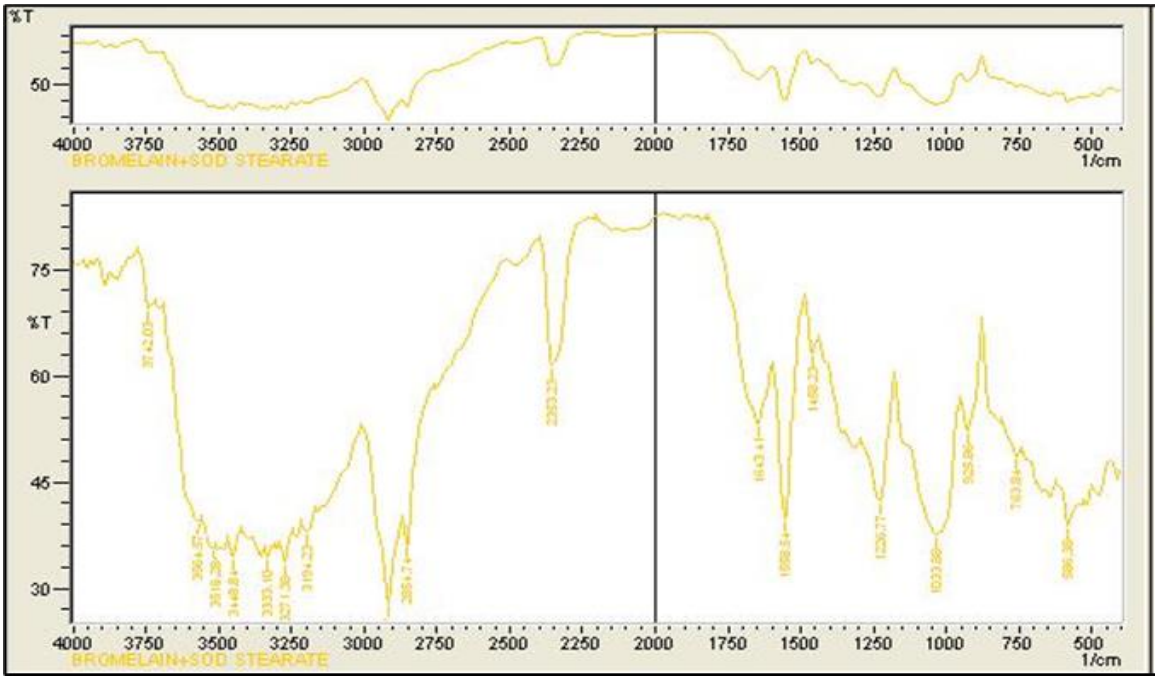

Figure 10: FTIR Studies of Bromelain + Sodium Stearate

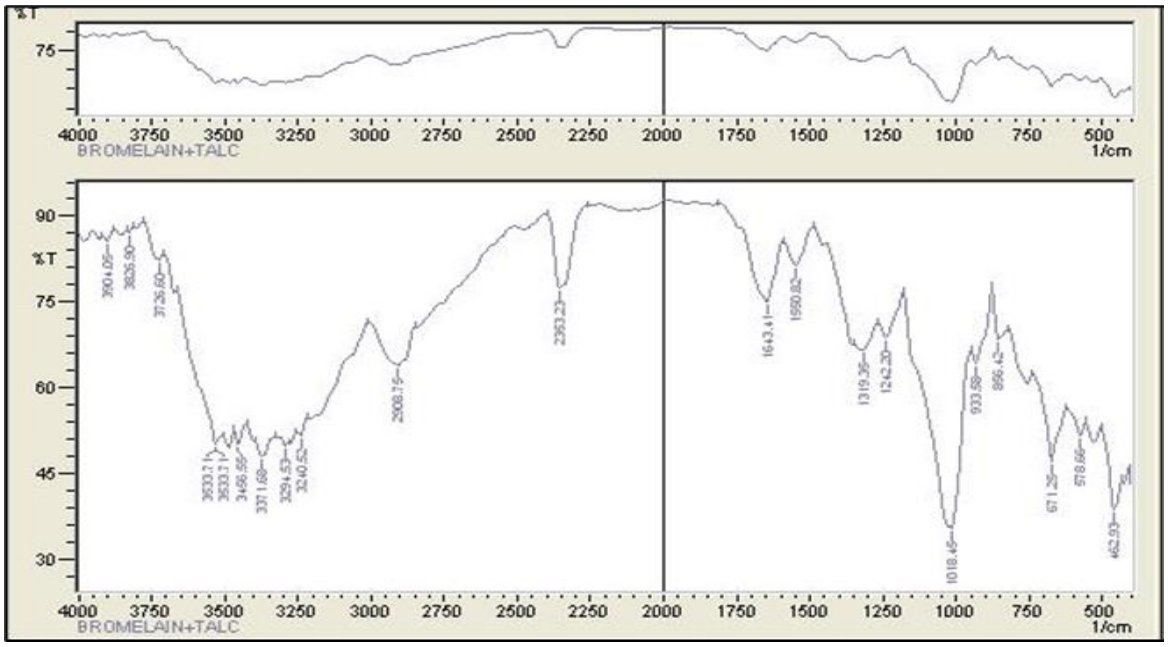

Figure 11: FTIR Studies of Bromelain + Talc

Evaluations:

Mouth dissolving tablets of Bromelain were prepared by a method employing crospovidone and sodium starch glycolate as super-disintegrants at different ratios. A total of six formulations were designed. The flow properties of the powder mixture are important for the uniformity of mass of the tablets; the flow of the powder mixture was analysed before compression to tablets.

Low Hausner`s ratio (≤1.18), compressibility index (≤14.81) and angle of repose (≤ 29.04) values indicated fairly good flowability of the powder mixture (Table 5).

Table 5: Evaluation of Tablet Blend of Mouth Dissolving Tablet of Bromelain.

Batches	Angle of Repose (Ɵ)	Bulk Density (gm/cm^3)	Tapped Density (gm/cm^3)	Housner's Ratio (H_R)	Carr's Compressibility Index
F1	21.78±1.88	0.45±0.12	0.50±0.23	1.18±0.10	10.00±0.20
F2	20.67±0.95	0.43±0.16	0.49±0.09	1.13±0.21	12.24±0.33
F3	23.59±0.47	0.43±0.17	0.48±0.26	1.11±0.20	10.41±0.10
F4	28.42±1.27	0.41±0.10	0.47±0.20	1.14±0.32	12.76±0.63
F5	23.78±1.45	0.45±0.90	0.52±0.21	1.15±0.28	13.46±0.39
F6	29.04±1.14	0.47±0.12	0.54±0.21	1.14±0.18	14.81±0.91

As the tablet powder mixture was free flowing, tablets produced were of uniform weight with acceptable weight variation in the range from 251 mg to 254 mg due to uniform die fill. Hardness (3.2 ± 0.05 - 3.4 ± 0.1 kg/cm2) and friability loss (0.8 ± 0.090 - 0.9 ± 0.117 %) indicated that tablets had good mechanical resistance. Drug content was found to be high (≥ 98.25 %) in all the tablet formulations (Tables 6).

Table 6: Evaluation of Mouth Dissolving Tablets of Bromelain

Batches	Thickness (mm)	Hardness (Kg/cm^2)	Friability (%)	Drug Content (%)	Weight Variation (mg)	Disintegration time (sec)
F1	4.04±0.10	3.26±0.05	0.8±0.05	98.50±0.11	252± 0.93	49±3.28
F2	4.35±0.17	3.36± 0.11	0.8±0.15	98.75±0.01	251±0.32	44±1.41
F3	4.27±0.25	3.26± 0.15	0.9±0.1	98.75±0.13	251±0.70	41±1.41
F4	4.35±0.10	3.36± 0.15	0.9±0.13	98.25±0.06	252± 0.93	50±1.89
F5	4.01±0.17	3.33± 0.25	0.8±0.07	98.70±0.23	251±0.17	48±1.41
F6	4.20±0.10	3.43± 0.10	0.8±0.09	98.75±0.14	254±0.51	45±1.91

The most important parameter that needs to be optimized in the development of mouth dissolving tablets is the disintegration time of tablets. In the present study. The faster disintegration of crospovidone tablets may be attributed to its rapid capillary activity and pronounced hydration with low capacity for gel formation. Thus, these results suggest that disintegration times can be reduced by using a wicking type disintegrant (crospovidone). Thus, disintegration times of tablets with crospovidone were found to be less than those with sodium starch glycolate. IR shows the drug interaction study, indicating that the drug is compatible with all the excipients (Figures 3 to 11).

In vitro, drug release studies were carried out in phosphate buffer pH 6.8 and the dissolution profile is depicted in Table 7 and Figures 12. The drug release from the optimized batch (F3) was 97.67 % at 30 min.

Table 7: In vitro Cumulative % Drug Release from Tablets

Time (In Min)	**Cumulative % Drug Release**					
	F1	**F2**	**F3**	**F4**	**F5**	**F6**
0 min	0	0	0	0	0	0
05 min	30.88±0.97	32.47±1.76	33.34±1.06	30.75±1.46	32.89±1.92	34.55±2.11
10 min	46.76±1.55	41.13±1.23	51.34±1.88	49.98±1.65	42.18±1.54	44.8±0.94
15 min	54.35±1.89	55.04±0.76	61.37±1.46	53.24±2.45	58.6±1.98	61.35±1.56
20 min	75.52±2.63	75.14±1.89	84.21±1.23	75.14±1.33	72.55±1.17	72.55±2.35
25 min	83.29±1.44	83.09±1.27	91.06±0.89	83.29±1.39	81.69±1.43	81.25±1.34
30 min	95.17±1.93	96.59±1.09	97.67±1.98	94.47±1.47	95.88±2.78	96.95±1.89

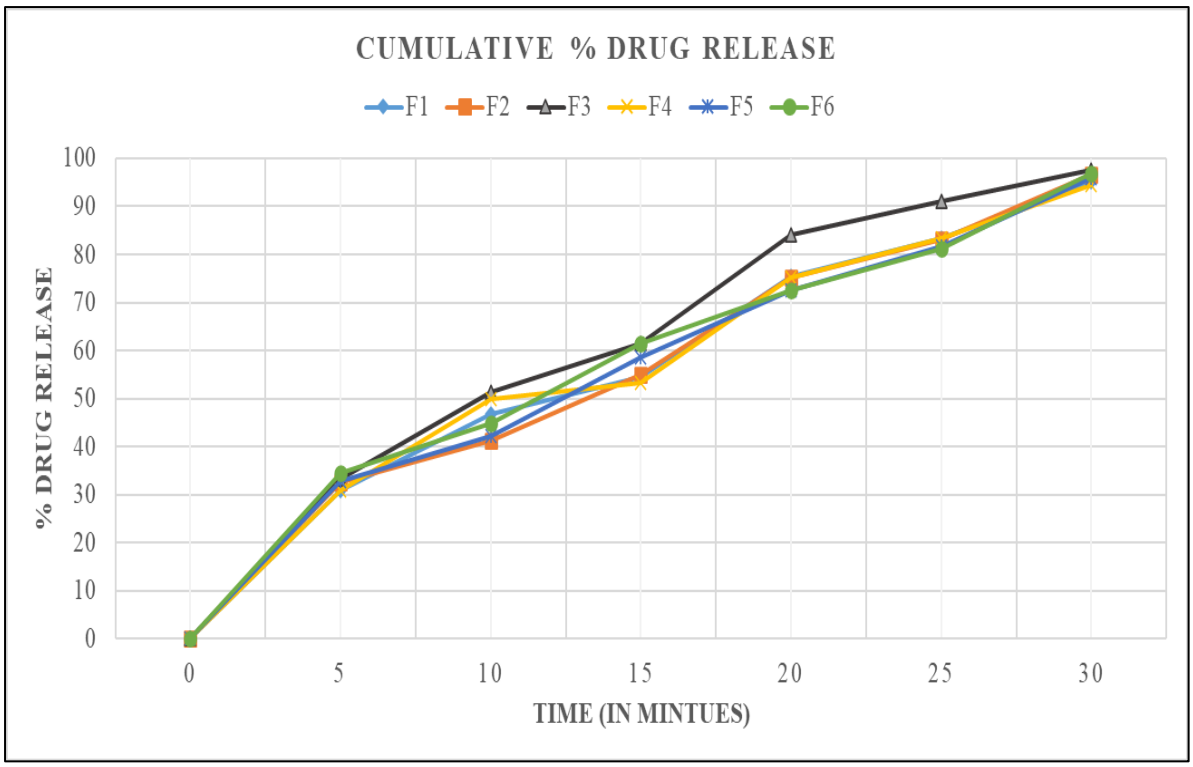

Figure 12: Cumulative % Drug Release

CONCLUSION:

In the present work, mouth dissolving tablets of Bromelain were prepared by direct compression method using superdisintegrants such as sodium starch glycolate and crospovidone. All the tablets of Bromelain were subjected to tests for weight variation, hardness, friability, drug polymer interaction, drug content uniformity, water absorption ratio, wetting time, and in vitro drug release**.**

Based on the above studies, the following conclusions can be drawn:

Tablets prepared by direct compression methods were found to be good and free from chipping and capping.

- The low values of the standard deviation of average weight of the prepared tablets indicated weight uniformity within the batches prepared.
- The hardness of the prepared tablets was found to be in the range of 3.2 ± 0.05 - 3.4 ± 0.1 kg/cm2. The friability values of the prepared tablets were found to be less than 1%.
- IR spectroscopic indicated that the drug is compatible with all the excipients.

- The in vitro disintegration time of Bromelain MDT prepared by the direct compression method was found to be in the range of 41 sec. to 50 sec. fulfilling the official requirements.
- Based on the in vitro disintegration time, formulation F3 (crospovidone) was found to be promising and showed a disintegration time of 41 sec, facilitating faster dispersion in the mouth.
- The drug content of tablets was uniform across all batches, ranging from 98.25 ± 0.06 - 98.75 ± 0.14 %w/w
- The drug release from the optimized batch (F3) was about 97.67 % at 30 min.

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CONFLICT OF INTEREST

All authors declared no conflicts of interest.

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Formulation Development and Evaluation for Sustained Release Tablet Containing Glibenclamide

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ABSTRACT

Objective: The Glibenclamide matrix tablet were prepared using hydrophilic polymers (HPMC complex i.e. HPMC(E4) + calcium phosphate in various proportions as release retarding agent to prolong the drug release and to improve the patience compliance.

Methods: The matrix tablets were prepared by direct compression method. The prepared matrix tablets were subjected to thickness, friability, weight variation test, drug content, hardness, swelling index and in vitro release studies. The drug excipients compatability was evaluated by FTIR studies.

Results: All the formulation showed compliance with pharmacopoeial standards. The in vitro dissolution study shows that F5 formulation was releases the drug in a controlled manner for 12 hours. Among all the formulations, formulation F5 which contains HPMC(E4) calcium complex releases the drugs which follow Zero order kinetics. The FTIR studies was revealed that there was no interaction between drug and excipients.

Conclusion: Hence hydrophilic polymers combination of (HPMC with E4 grade-calcium phosphate complex) in various proportions can be used to prepare matrix tablets of Glibenclamide having prolonged therapeutic effect with enhanced patience compliance.

Keywords: Glibenclamide, HPMC E4-calcium phosphate complex, Sustained release tablet.

INTRODUCTION

Oral sustained release delivery systems are designed to achieve a therapeutically effective concentration of drug in systemic circulation over an extended period of time. Therapeutic benefits of designing SR dosage form include low cost, simple processing, improved efficacy, reduced adverse effects, flexibility in terms of the range of release profiles attainable, increased patient compliance and convenience. Many innovative methods have been developed for obtaining modified drug release. From the practical view point, the least complicated approach for developing a modified release dosage form is the formulation of a hydrophilic matrix tablet [1].

HPMC is the dominant hydrophilic vehicle used for the preparation of oral sustained drug delivery. While HPMC could potentially control the release of a soluble drug, it could also facilitate the release of relatively insoluble drug. In the later case, insolubility of the drug molecule would be the rate limiting step in its release and HPMC's solubilizing effect would facilitate the release. The objective of the present study was to develop a hydrophilic polymer (HPMC – calcium complex) based Glibenclamide matrix sustained release tablet which can release the drug up to time of 12 hours in predetermined rate. The formulation of Glibenclamide matrix tablet was prepared by the polymer combination in order to get required theoretical release profile. The influence of hydrophilic polymer and granulation technique on Glibenclamide was studied. The formulated tablets were also characterized by physical and chemical parameters like drug content, hardness, friability, dissolution rate etc [2].

Diabetes mellitus Type 2 is a long term metabolic disorder that is characterized by high blood sugar, insulin resistance and relative lack of insulin. It primarily occurs due to obesity. Symptoms of high blood sugar include frequent urination, increased thirst and increased hunger [3]. Type 2 diabetes is a progressive condition in which the body becomes resistant to the normal effects of insulin and/or gradually loses the capacity to produce enough insulin in the pancreas. We do not know what causes type 2 diabetes. Type 2 diabetes is associated with modifiable lifestyle risk factors. Type 2 diabetes also has strong genetic and family related risk factors. Type 2

diabetes is diagnosed when the pancreas does not produce enough insulin (reduced insulin production) and/or the insulin does not work effectively and/or the cells of the body do not respond to insulin effectively known as insulin resistance [4]. Over 90% cases of diabetes are type 2 [5,15].

Glibenclamide, also known as Glibenclamide, is an antidiabetic drug belonging to the class of sulfonylureas. Therapy with Glibenclamide is usually initiated with 2.5mg given once daily. The maximal recommended daily dose is 20mg. It has a special status in the treatment of non-insulin-dependent diabetes mellitus because it is effective in many cases which are resistant to all other oral hypoglycemic drugs. It differs from other oral hypoglycemic drugs i.e. more effective during eating than during fasting [6]. It was developed in 1966. It works by binding and inhibiting the ATP-sensitive potassium channels in pancreatic beta cells. It causes cell membrane depolarization, opening voltage-dependent calcium channels. As a result, intracellular calcium level increases in the beta cells and release of insulin is stimulated.

Glibenclamide is a potent sulphonyl urea class which uses an oral hypoglycemic agent. It stimulates insulin release from the beta cells of the pancreas that leads to hypoglycemia. Glibenclamide increases insulin level by reducing the hepatic clearance of the hormone [16]. Glibenclamide belongs to class II (i.e. drugs with low solubility and high permeability) according to the biopharmaceutical classification system [17]. It is practically insoluble in water and consequently, its dissolution has been considered to be the rate-limiting step for absorption. Being weak acid with a pka 5.3, it shows pH dependent solubility and its absorption is expected to be better from the upper part of the gastrointestinal tract (GIT). Plasma half-life of glibenclamide is about 2 to 4 hrs. Various strategies are to be used for the development of oral controlled-release formulations, but the optimum technique should be selected by considering the absorption window of the given drug [5]. The mechanism of action of the drug consists in the inhibition of the ATP-sensitive K+ channels, which leads to depolarization of the cells and insulin secretion[18].

MATERIALS AND METHODS

Material

Glibenclamide was obtained as a sample product from Yarrow chemical products, Mumbai. HPMC E4M was obtained from Ashland Netherlands.co., Magnesium stearate, Talc, Microcrystalline cellulose, was obtained from Research lab Fine chem. Industries, Mumbai. Calcium phosphate was obtained from Yarrow chemical products, Mumbai.

Method

Preparation of HPMC- Calcium Phosphate complex[7]:

- Three biologically relevant calcium phosphates: CaHPO4 ? 2H2O (DCPD), calcium deficient apatite (CDA), and BCP (60% of HA and 40% of b-Ca3(PO4)2) were chosen for the experiments. HPMC (E4M, Dow Chemical), with a molecular weight of 290,000 g/mol, as determined by a laser light diffusion technique, was used.
- One g of each calcium phosphate was mixed with 0.5 g of solid HPMC and 5 mL of doubly distilled water. The mixtures obtained were placed inside glass bottles. The bottles were sealed and kept at 121°C for 48 hours.
- This stage is necessary for simulation of a steam sterilization procedure, widely used in medicine (normally 20 min at 121°C is enough for the sterilization). Later, the bottles were opened and kept for 24 h at 90°C for water evaporation
- No additional treatments were performed with the dry solid composites obtained.
- Chemical and structural analysis of the composites was studied with FTIR (Magna-IR 550, Nicolet) in the range of 400–4000 cm−1 (3–5 mg of the solid composites were mixed with 300 mg of spectral-grade KBr followed by pellet pressing at 12,000 kg/cm2), X-ray diffraction (XRD) (Diffract 5000, Kristalloflex, Siemens) within 2u value of 10–60° (Cu Ka radiation was used), and scanning electron microscopy (SEM) (JSM 6300, JEOL) in the secondary electron mode (acceleration voltage 15 kV).
- Similar measurements were also performed for thermally treated water suspensions of the above calcium phosphates without HPMC (1 g of calcium phosphate and 5 mL of water), and HPMC solution without calcium phosphates (0.5 g of HPMC and 5 mL of water). The latter experiments were used for the control.

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FORMULATION OF SUSTAINED RELEASE TABLET OF GLIBENCLAMIDE

Preparation of Sustained Release Matrix Tablets by Direct Compression Method

Glibenclamide matrix tablets are prepared by direct compression method. The corresponding amount of drug and excipients were accurately weighed and mixedproperly and the matrix is formed. The tablet blends for different batches (F1-F5) areprepared according to Table 1 and further studied for Pre-compression properties.

Table 1: Formulation of Sustain Release Matrix Tablet

Sr. No.	Ingredients	Formulation Codes				
		F1	F2	F3	F4	F5
01	Glibenclamide	5	5	5	5	5
02	HPMC (K4M) complex	50	75	100	125	150
03	Microcrystalline Cellulose	229	204	179	154	129
04	Magnesium Stearate	8	8	8	8	8
05	Talc	8	8	8	8	8
Total		300	300	300	300	300

PRECOMPRESSION EVALUATION OF BLEND OF SUSTAINED RELEASE TABLET OF GLIBENCLAMIDE: [8,9,10,11,12]

Angle of Repose

This is the maximum angle possible between the height of pile of blend powder and horizontal plane. The frictional forces in the lose powder can be measured by angle of repose. The tangent of angle of repose is equal to the coefficient friction (Ɵ) between the particles. Hence the rougher and more irregular the surface of particles the greater will be angle of repose.

$$\Theta = \tan^{-1}(r/h) \qquad \text{.....Eq}^{n}\ (1)$$

Where, H = height of the pile

Table 2: Standards for Angle of Repose

Angle of Repose	Flowability
<20	Excellent
20-30	Good
30-34	passable
>40	Very Poor

Bulk density:

Apparent bulk density (BD) was determined by pouring blend into a graduated cylinder. Weighted quantity of the powder mass (M) was poured into measuring cylinder, then the powder was levelled carefully, and the unsettled apparent volume Vo was noted to the nearest graduated unit. The bulk density was calculated in gm/ml by the formula: The bulk density was calculated using the formula

$$\text{Bulk Density} = M/Vo \qquad \text{----------Eq}^{n}(2)$$

Tapped density: [14]

After determination of the bulk density, the cylinder was tapped mechanically by mounting on a holder in a mechanical tapped density tester that provided a fixed drop of 14 ± 2 mm at a nominal rate of 300 drops per minute. The cylinder was tapped for 500 times initially and the tapped volume Vt was measured to the nearest graduated unit. The tapping was repeated foran additional 750 times and the tapped volume was measured. Final tapped volume was measured and tapped density was calculated by the formula:

Tapped Density = M/Vt. ---------- **Eq^{n} (3)**

Compressibility Index and Hausner's Ratio:

The Compressibility Index and Hausner's Ratio are measures of the propensity of a powder to be compressed. As such, they are measures of the relative importance of inter-particulate interactions. In a free-flowing powder, such interactions are generallyless significant, and the bulk and tapped densities will be closer in value. For poorer flowing materials, there are frequently greater inter-particle interactions, and a greater difference between the bulk and tapped densities will be observed. These differences are reflected in the compressibility index or Carr's index (CI) and the Hausner's ratio (HR) which is calculated using the following formulas

Compressibility Index

The simplest way for measurement of free flow of powder is compressibility, a indication of the case with which a material can be induced to how is given by compressibility index (CI) which is calculated as follows

Carr's Index = [(Tapped density - Bulk density)/ Tapped density] ×100 **------(4)**

Table 3: Standards for Compressibility Index

Carr's Index	Properties
5-15	Excellent

Hausner's ratio	Flow
1.2-1.3	Excellent
1.3-1.4	Good
1.4-1.5	Fair
1.5-1.6	Poor
12-16	Good
18-21	Fair to Passable
23-35	Poor
35-38	Very Poor
>40	Very Very Poor

Hausner's ratio

Hausner's ratio is an indirect index of ease of powder flow. It is calculated by thefollowing formula;

Hausner's ratio (Hr) = [Tapped Density (ρtap)/Bulk Density (ρb)] ------**(5)**

Table 4: Standards for Hausner's Ratio

COMPRESSION OF SUSTAINED RELEASE TABLET OF GLIBENCLAMIDE BY DIRECT COMPRESSION METHOD

Accurate quantity of Glibenclamide and all ingredients were weighed according to formula powders except talc and magnesium stearate was blended homogeneously

in mortar and pestle for 15 minutes. Prepared powder blend was passed through sieve no. 60. Finally, Talc and Magnesium stearate passed from sieve no. 30 added and wasfurther mixed for10 minutes.

Accurately weighed 300 mg homogeneously mixed powder blend was fed manually and compressed with constant compression force and hardness on 16 stations tablet compression machine with 9 mm, breakthrough, and flat faced punches.

Total five formulations were prepared.

EVALUATION OF SUSTAINED RELEASE TABLET OF GLIBENCLAMIDE [8,10,12]

The prepared tablet batches (F1-F5) are subjected to post compression evaluation and evaluation parameters like appearance, weight variation, thickness, hardness, friability, content uniformity, disintegration time, dissolution time was performed and the results are shown in **table 10**.

Appearance:

The tablets were visually observed for capping, chipping and lamination.

Weight Variation:

When a tablet is designed to contain a certain quantity of medication in a specific amount of tablet formula, the weight of the tablet is frequently tested to confirm that the correct amount of drug is included in the tablet. In actuality, ten tablets were consumed and weighed on a digital weighing balance individually.

The average weight of the tablets was determined, and the weight of each tablet was compared to the average. If no more than two tablets are outside the % restriction and no tablet varies by more than twice the percentage limit, the tablet passes the test.

Table 5: Specifications of % weight variation allowed in tablets

Average Weight of Tablet	% Deviation Allowed
80 mg or less	10
More than 80 mg but less than 250 mg	7.5
250 or more	5

Thickness:

The uniformity of tablet size is dependent on the thickness of the tablet. Vernier caliper was used to determine thickness. randomly selected three pills from each formulation were tested to determine it.

Hardness:

The "force necessary to shatter a tablet in diametric compression test" is the definition of hardness. As a result, tablet crushing strength is also known as hardness. The resistancebefore use is determined by the hardness of the material. For each formulation the hardness of 6 tablets was determined using a Pfizer hardness tester. In the hardness tester, tablet was held along its oblong axis in between the two jaws of the tester and the load necessary tocrush it was measured. Then force was applied until the tablet fractured. The value at this point was noted in kg/cm2.

Friability:

This test is used to determine if tablets can survive abrasion while being packed, handled, or transported. Friability is a sign of inadequate tablet ingredient cohesiveness. Friability of the tablets was determined using Roche Friabilator. A total of ten pills are weighed and placed in the Friabilator, which is made up of a circular plastic chamber separated into two or three compartments. The chamber

rotates at 25 revolutions per minute for 4 minutes, dropping the tablets 15 cm away and completing100 rotations. The pills are then weighed for the second time. The weight difference isobserved and given as a percentage difference. It's best if it's less than 1%.

% Friability = (W1-W2)/W1 X 100 ---------- (6)

Where,

W1 = Weight of tablet before test

W2 = Weight of tablet after test

Content uniformity

The Glibenclamide content was estimated as follows.

20 tablets were finely powdered and weight equivalent to 10 mg of Glibenclamide was dissolved in 100 ml of 0.1N HCL and assayed against 0.1 N HCL for drug content using UV-Visible spectrophotometer at 229 nm.

Disintegration test:

Six tablets were placed in each six tubes of the basket and the apparatus operated containing water maintained at 37^0C as the disintegration fluid. The Disintegration time was recorded.

In-vitro Dissolution studies

Dissolution profiles of Glibenclamide tablets were determined using the USP Type II Dissolution test apparatus (paddle) (Electrolab, Mumbai, India). set with a paddle speed of 50 rpm & at temperature 37° C ± 0.5°C. The dissolution media used were 900 mL of 0.1N HCl for first 2 h followed by pH 6.8 phosphate buffer solutions for 12 h. 5 ml samples wereremoved at specified intervals up to 1h and filtered through Whatmann filter paper. An equal volume of fresh medium, prewarmed at 37^0C was replaced into the dissolution medium after each sampling to maintain the constant volume throughout the test. Samples were analyzed by UV spectrophotometer at 229 nm. Drug dissolved at specified timeperiods was plotted as cumulative percent release versus time (h) curve.

Stability Study

The prepared sustained release tablet of Glibenclamide were placed in plastic tubes

containing desiccant and stored at ambient conditions, such as room temperature at $40^0C \pm 2^0C$ /75 % RH ± 5% for period of 90 days. Each tablet is weighed and wrapped in aluminum foil and packed in black PVC bottle and put at above specified condition in a heating humidity chamber for 3 months and evaluated for their physical appearance, hardness, disintegrate time, dissolution testing and drug content at specified intervals of time.

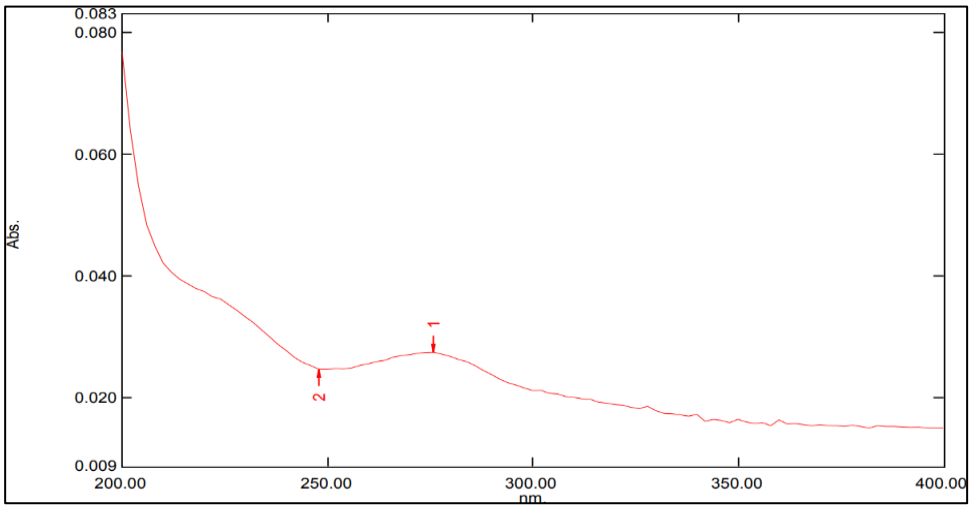
RESULTS AND DISCUSSION

Spectrophotometric Analysis of Glibenclamide UV Spectrophotometric Analysis

Determination of λ max of Glibenclamide in 0.1 N HCL

In UV spectroscopy study, the maximum wavelength (λ max) of Glipizide in 0.1N HCL was found to be 229 nm. The reported λ max value of Glipizide in 0.1N HCL was also 229 nm, so the values similar with the reported value indicates that the given sample of Glibenclamide was in pure form.

Figure 1: UV Spectrum of Glibenclamide in 0.1 N HCl at 229 nm



Preparation of Standard Calibration Curve of Glibenclamide in 0.1N HCl

The Standard curve of Glibenclamide was determined by plotting absorbance Vs concentrationat 229 nm. It was found that there was linear relationship between concentration and absorbance with R^2 value 0.9988. Which reveals that, the drug Glibenclamide obeys the Beers lamberts law.

Table 6: UV Absorbance of Glibenclamide in 0.1 N HCl at 229 nm

Sr.no.	Concentration (µg/ml)	Absorbance
1	0	0
2	10	0.028
3	20	0.057
4	30	0.087
5	40	0.121
6	50	0.14

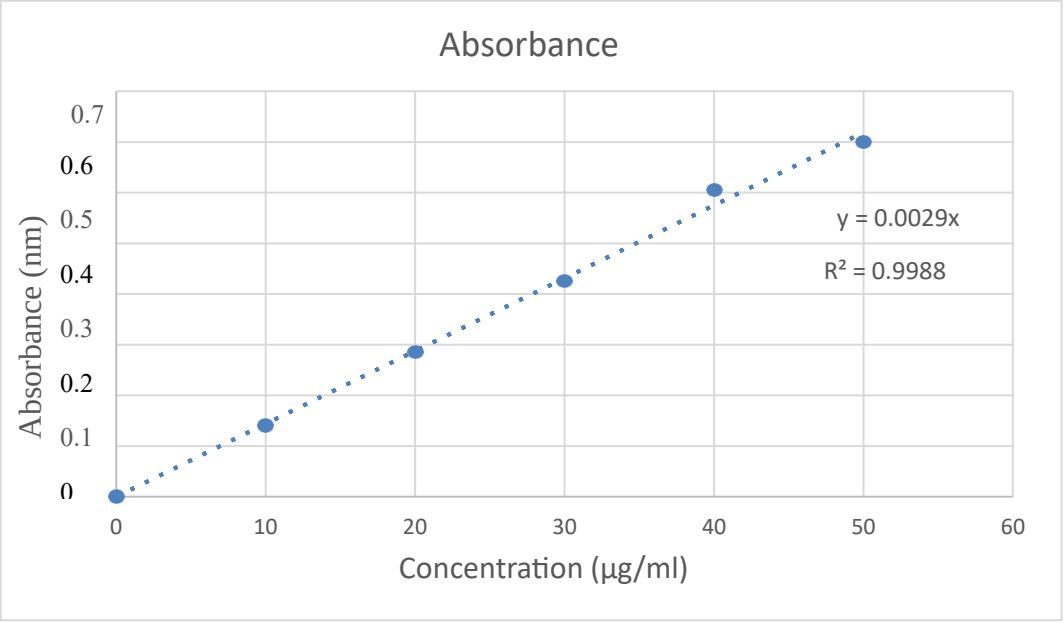


Figure 2: Standard Calibration Curve Graph of Glibenclamide in 0.1N HCL

Determination of λ max of Glibenclamide in 6.8 Phosphate Buffer

In UV spectroscopy study, the maximum wavelength (λ max) of Glibenclamide in 6.8

PhosphateBuffer was found to be 229 nm. The reported λ max value of Glibenclamide in 6.8 Phosphate Buffer was also 229 nm, so the values similar with the reported value indicates that the given sample of Glibenclamide was in pure form.

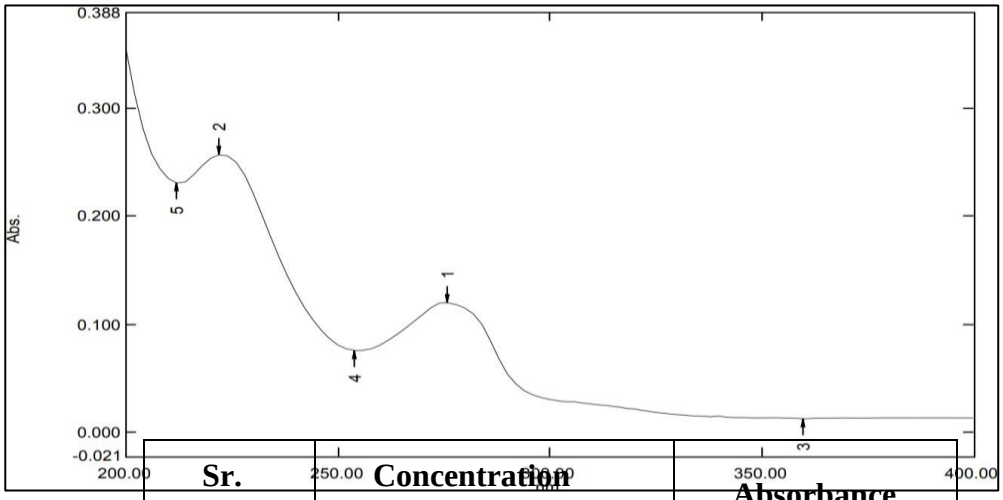


Figure 3: UV Spectrum of Glibenclamide in 6.8 Phosphate Buffer at 229 nm

Sr. no.	Concentration (µg/ml)	Absorbance
1	0	0
2	10	0.12
3	20	0.215
4	30	0.315
5	40	0.4
6	50	0.48
7	60	0.6

Table 7: UV Absorbance of Glibenclamide in 6.8 Phosphate Buffer at 229 nm

Preparation of Standard Calibration Curve of Glibenclamide in 6.8 Phosphate Buffer:

The Standard curve of Glibenclamide was determined by plotting absorbance Vs concentration at 229 nm. It was found that there was linear relationship between concentration and absorbance with R2 value 0.9986. Which reveals that, the drug Glibenclamide obeys the Beers lamberts law.

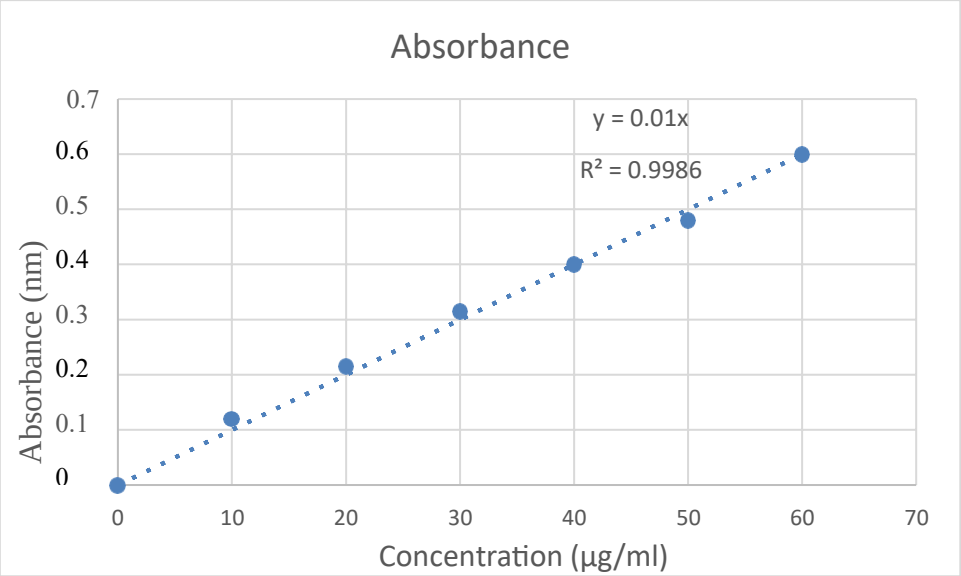


Figure 4: Standard Calibration Curve Graph of Glibenclamide in 6.8 Phosphate Buffer

Drug-Excipient Compatibility Study

Fourier Transform Infra-red Spectroscopy (FTIR) Interpretation of Glibenclamide

The FTIR spectrums of pure Glibenclamide and physical mixtures of drugs and polymers were studied separately as per the excipients used in the formulation. It was observed that there were no major shifts in the main peaks of either drug. This indicates that there were no compatibility problems with the drug with the polymers and excipients used in the formulation. Glibenclamide had peaks at 1658 (C=O amide), 2890 (C=H), 3471 (NH stretch), 1033 (S=O), 1072 (C-O-C).

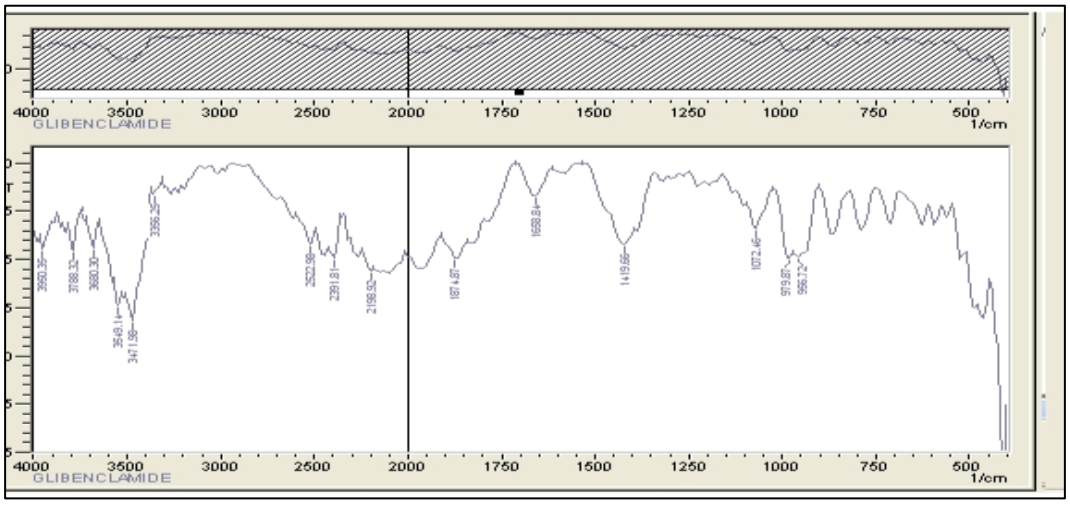


Figure 5: FTIR Spectrum of Glibenclamide

Fourier Transform Infra-red Spectroscopy (FTIR) Interpretation of FTIR of Glibenclamide + HPMC (E4M)- Calcium phosphate (CaPO4) complex

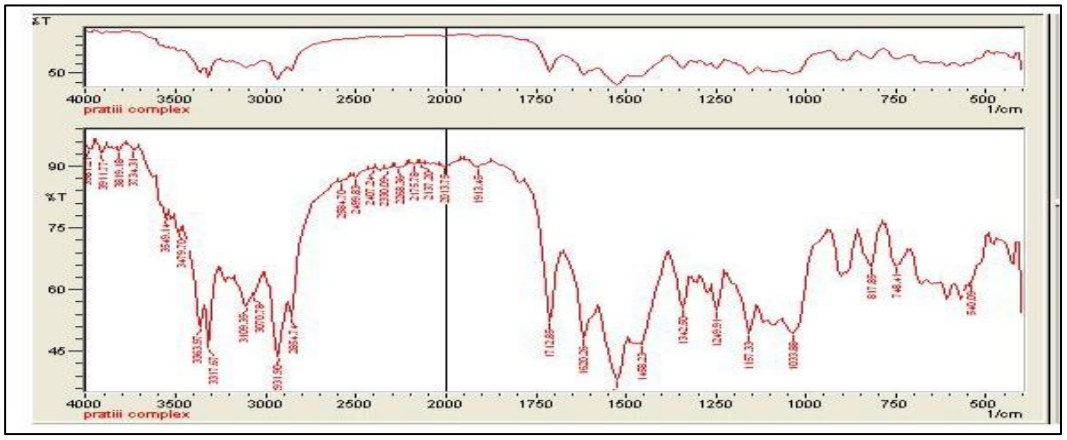


Figure 6: FTIR Graph of Glibenclamide + HPMC (E4M)- Calcium phosphate (CaPO4) complex

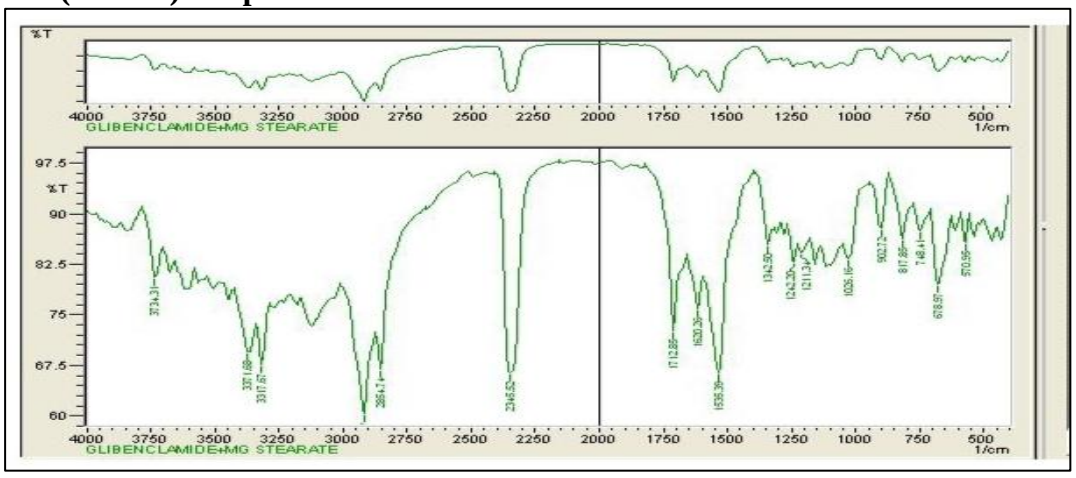


Figure 7: FTIR Graph of Glibenclamide + Mg. Stearate

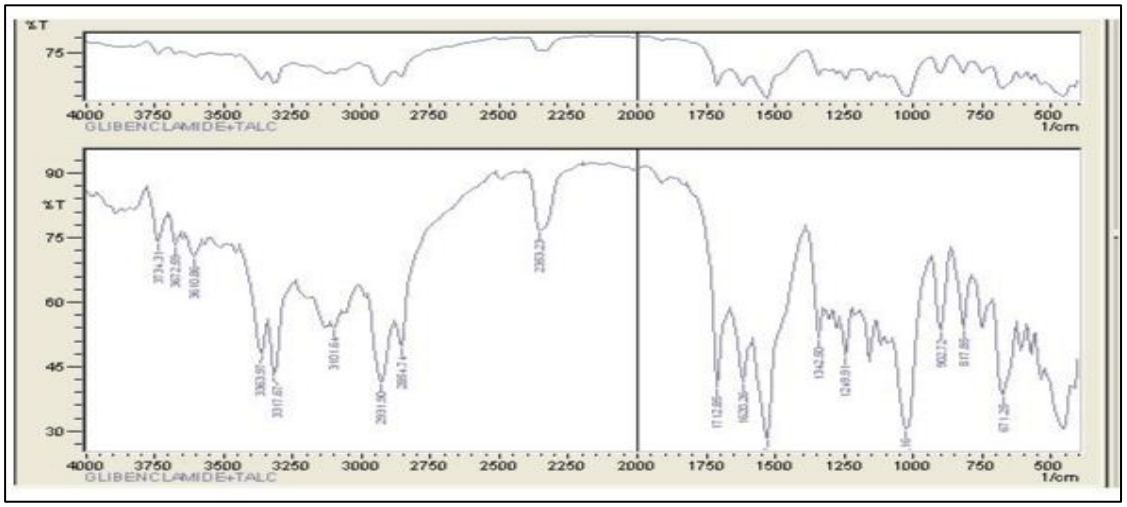


Figure 8: FTIR Graph of Glibenclamide + Talc

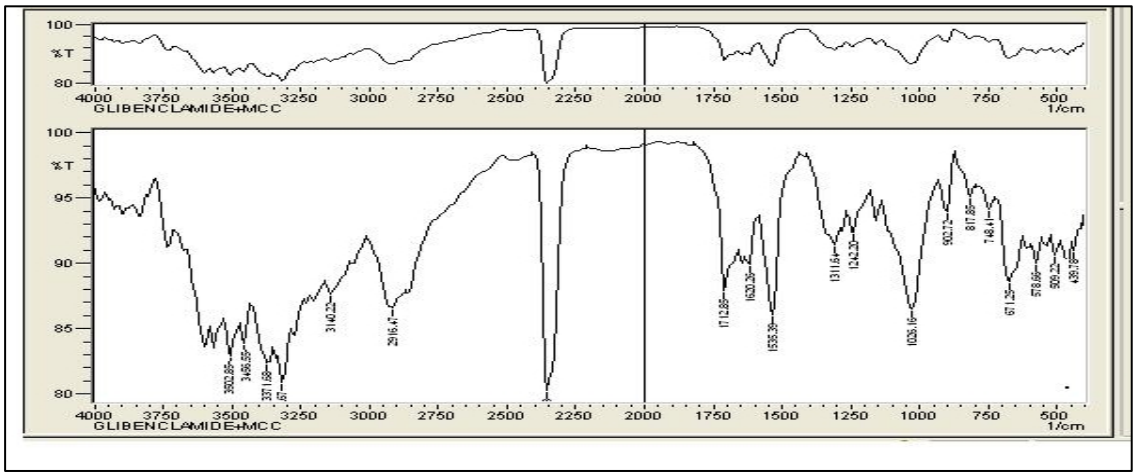


Figure 9: FTIR Graph of Glibenclamide + MCC

PRECOMPRESSION EVALUATION OF BLEND OF SUSTAINED RELEASE TABLET OF GLIBENCLAMIDE:

Sustained release tablets of Glibenclamide were prepared by direct compression method using polymer HPMC-Calcium phosphate complex. A total of five formulations were designed. The flow properties of the powder mixture are important for the uniformity of mass of the tablets; the flow of the powder mixture was analysed before compression to tablets. Low Hausner`s ratio (≤1.18), compressibility index (≤15.68) and angle of repose (≤ 29.39) values indicated fairly good flowability of the powder mixture **Table 8.**

Formulations	Angle of repose (Θ^o)	Bulk Density (gm/cm^3)	Tapped Density (gm/cm^3)	Hausner's Ratio (H_R)	Carr's Compressibility index (%)
F1	28.80±0.8	0.42±0.12	0.49±0.23	1.16±0.10	14.28±0.20
F2	28.21±0.5	0.44±0.16	0.51±0.09	1.15±0.21	13.72±0.33
F3	29.24±0.9	0.45±0.40	0.50±0.06	1.11±0.11	10.00±0.52

F4	29.39±0.5	0.41±0.10	0.47±0.20	1.14±0.42	12.76±0.63
F5	28.80±0.8	0.43±0.90	0.51±0.21	1.18±0.36	15.68±0.39

Table 8: Precompression Evaluation of tablet for sustained release tablets

Results are mean of three dimensions*

EVALUATION OF SUSTAINED RELEASE TABLET OF GLIBENCLAMIDE:

As the tablet powder mixture was free flowing, tablets produced were of uniform weight with acceptable weight variation in the range from 298 mg to 301 mg due to uniform die fill. Hardness (5.7 ± 0.5 – 6.1 ± 0.3 kg/cm2) and friability loss (0.71 ± 0.04 - 0.82 ± 0.03 %) indicated that tablets had good mechanical resistance. Drug content was found to be high (≥ 98.75 %) in all the tablet formulations **Table 9 and Table 10.**

Table 9: Evaluation of Sustained Release Tablet of Glibenclamide

Formulations	**Weight variation (mg)**	**Thickness (mm)**	**Hardness (Kg/cm^2)**	**Friability (%)**	**Drug Content (%)**
F1	299±0.50	3.50±0.10	5.7± 0.5	0.78±0.05	95.25
F2	298±0.58	3.40±0.18	5.9± 0.2	0.75±0.06	96.3
F3	301±0.20	3.45±0.25	6.0± 0.3	0.71±0.04	97.9
F4	298±0.85	3.55±0.10	5.8± 0.4	0.82±0.03	98.5
F5	299±0.65	3.48±0.17	6.1± 0.3	0.78±0.07	98.75

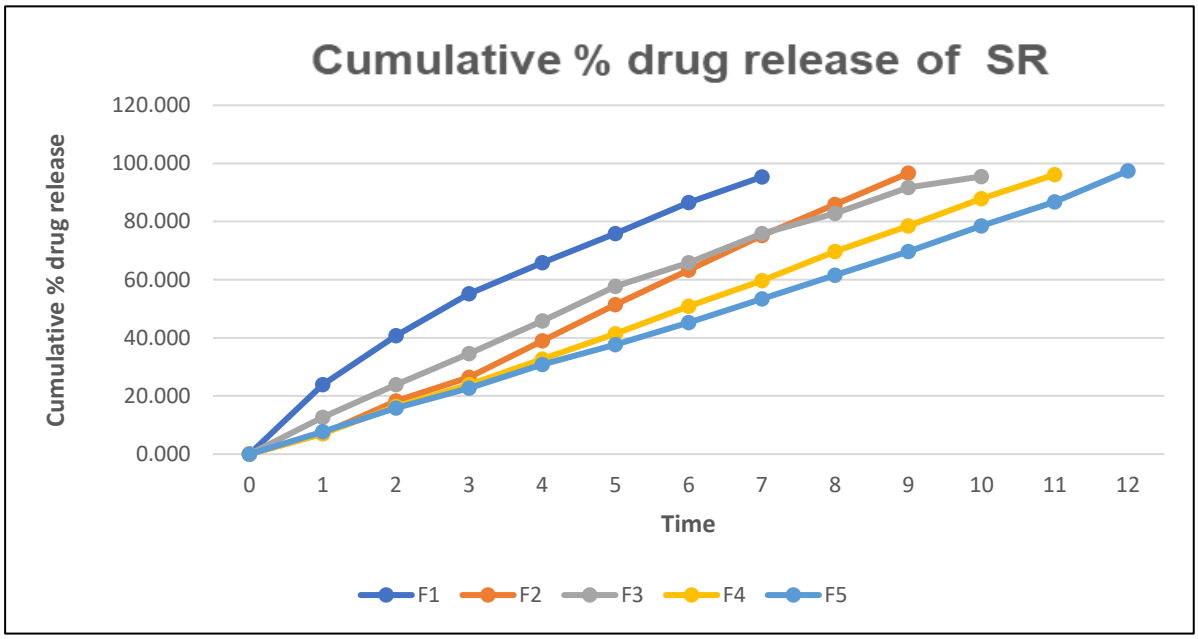
***In vitro* % Drug Release of Drug from Tablet**

All the five tablet batches of fast Sustained release tablet of Glibenclamide were subjected for the in vitro dissolution studies using tablet dissolution test apparatus (USP type II). The

dissolution media used were 900 mL of 0.1 N HCl for first 2 h followed by pH 6.8 phosphate buffersolutions for 12 h.

Table 10: *In vitro* Cumulative % Drug Release from Tablets

Time (Hours)	Cumulative % Drug Release				
	F1	F2	F3	F4	F5
0	0.00	0.00	0.00	0.00	0.00
1	23.99±0.44	7.07±0.48	12.68±0.25	8.94±0.25	9.57±0.55
2	40.76±0.54	18.93±0.52	23.92±0.84	16.43±0.48	15.81±0.35
3	55.15±0.56	33.28±0.85	34.54±0.51	24.56±0.56	22.69±0.65
4	65.80±0.25	47.04±0.55	45.80±0.55	35.80±0.74	31.44±0.26
5	75.85±0.64	58.31±0.84	57.70±0.52	43.95±0.42	40.20±0.45
6	86.53±0.45	68.97±0.52	65.86±0.	53.35±0.57	48.35±0.58
7	95.35±0.48	78.40±0.15	75.91±0.15	61.51±0.65	57.13±0.69
8		86.59±0.45	82.85±0.51	72.18±0.31	65.30±0.22
9		96.66±0.48	91.6±0.45	80.36±0.28	72.85±0.62
10			95.51±0.59	87.93±0.25	81.66±0.44
11				94.88±0.15	89.23±0.64
12					97.43±0.65



STABILITY STUDY

The formulation F5 was selected for stability studies on the basis of their high cumulative % drug release time was studied. The stability studies were carried out at 40˚C±2˚C/75˚C±5% relative humidity for the selected formulation up to two months. For every 1-month time interval the tablets were analysed for drug hardness, content uniformity, % drug release up to two months.

CONCLUSION

In this research work, Preformulation studies of the drug were carried out which includes powder properties and compatibility studies using FTIR. Sustained release tablets were prepared using mixture of hydrophilic polymers such as of HPMC E4M- Calcium phosphate complex. The increasing proportion of polymer in tablet retards the release of drug from tablet. Formulated tablets were evaluated for hardness, friability, thickness, drug content and in-vitro study. F5 batch was selected as optimize batch from the similarity factor, cumulative drug release and drug content study. Then stability study and cumulative release study carried out on optimized batch and compared with marketed product Glynase Xl. Results of present study demonstrated that methodology successfully employed for formulating sustained release matrix tablets of Glibenclamide. The in-vitro dissolution result of batch F5 was fitted best to the kinetic properties as well as showed better similarity to innovator brand Glynase XL (10 mg).

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CONFLICT OF INTEREST

All authors declared no conflicts of interest.

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Formulation Development and Evaluation of a Topical Nanogel Containing Kojic acid

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ABSTRACT- Topical drug administration is a localized method of delivering drugs to specific areas of the body via topical channels. The major route of topical medication delivery is through the skin, which is one of the most easily accessible organs on the human body for topical drug administration. The present investigation involves formulation of topical nanogel using Kojic acid for the treatment of hyperpigmentation, Kojic acid is an effective and well tolerated drug having melanin neutralising activity (Tyrosinase inhibitor). Topical nanogel of Kojic acid was prepared by using High molecular weight water soluble polymer Hydroxy propyl methyl cellulose such as K35M grade and other excipients including methyl paraben, Carbopol 940, glycerine and purified water were reported in the formation of nanogel. In the present investigation nanogel the formulated nanogel was evaluated for pH, viscosity, Spreadability, extrudability, conductivity, particle size, zeta potential, in vitro drug diffusion studies. Among the formulated nanogel batch 4 has met all the specifications and was formed to be optimized Efficient delivery of drug to skin application was found to be highly beneficial in localizing the drug to desired site in the skin.

KEYWORDS- Nanogel, Kojic acid, Particle size, Zeta potential, Drug Release.

INTRODUCTION

Nanogels are defined as nanoscale particles that, either physically or chemically, create crosslinked polymers. In order to transport polynucleotides, cross-linked bifunctional networks of a polyion and a non-ionic polymer were first developed [1]. Although soluble in water, nanogels differ from linear macromolecules with comparable molecular weights in their properties. These structures along with their larger equivalents [2]. Structures along with their larger equivalents [2]. Nanogels are typical formulations that typically range in size from 1000 nm, and their three-dimensional structure can be maintained by altering volume proportion and solvent quality. Nanogels have revolutionized the field of gene therapy because they have made it possible to deliver genes within cellular organelles for gene silencing therapy [3]. Nanogels are composed of ionic or non-ionic polymer chains that are hydrophilic or amphiphilic and

grow into nanoscale structures. Despite its use as a drug delivery system, nanogel has been studied for longer periods in the production of other substances like quantum dots, dyes, and other diagnostic agents [4]. The development of nano-sized microgels and hydrogels as a result of specific delivery system anticipation has been made possible by the wide range of polymer systems and the simple modification of their physico-chemical properties [5]. Transdermal delivery of drug is promising but challenging system is available for local as well as systemic effect of drug. The entry of drug through the stratum corneum may follow the intercellular, transcellular or appendageal route. The intercellular route is the more common pathway of the drug permeation through the skin [6].

Melanocytes create the biological pigment called skin melanin. Skin colour is primarily determined by melanin, which shields human skin from ultraviolet (UV) solar radiation's harmful effects. Hyperpigmentation is the term used to describe melanin over synthesis [7]. For most people, especially women, hyperpigmentation treatment is usually difficult and disappointing [8]. Kojic acid (KA) is a popular hydrophilic tyrosinase inhibitor with natural whitening properties that is used to treat hyperpigmentation. By chelating copper atoms, Kojic Acid inhibits the tyrosinase enzyme and prevents the synthesis of dopachrome. It synthesized several fungi species, including Aspergillus and Penicillium [9]. Despite KA and its derivatives distinctive qualities, the cosmetic industries hardly ever use them. Due to its hydrophilic nature and the two hydroxyl functional groups that make up its chemical structure, Kojic Acid is a hydrophilic component. Its skin absorption is inadequate [10].

The present study was conducted to design and evaluate Kojic Acid nanogel which provides prolonged release, increase the residence time of drug on the skin thereby enhance bioavailability.

MATERIALS AND METHOD

Kojic Acid was purchased from Arti pharmaceuticals, Mumbai. Carbopol 940 Research-lab Fine Chem Industries, Mumbai. HPMC K35M was obtained as a gift sample from Ashlands, Netherlands. Co., Methylparaben and Glycerine was purchased from Research-lab Fine Chem. Industries, Mumbai [11].

METHOD

Preparation of Kojic Acid Nanogel [12]

Preparation of 2% drug solution of KA- weigh 2 gm of drug Kojic acid dissolve in 100ml of distilled water.
2% of prepared kojic acid measure 2ml of solution mix with given quantity of HPMC K35m, mix well then add glycerine. (Organic phase). Weigh carbopol940 accurately, add 10ml distilled water (aqueous phase). Stir aq. phase on magnetic stirrer add organic phase dropwise. Add methylparaben (preservative). Batch B1, B2, B3, B4 was prepared at highest rpm 8000 with variation in composition.

Evaluation Parameters

Appearance: The prepared nanogel bases were inspected visually for clarity, colour and presence of any particles.

Homogenesity

All developed nanogels were tested for homogeneity by visual inspection after the gels have been set in the container. They were tested for their appearance and presence of any aggregates [12].

Measurement of particle size of formulation

Horiba sz-100 windows [z type] were used to investigate the particle size (PS) of the gel. Particle size and zeta potential were measured in triplicates after dilution with distilled water, and the average values ±SD were recorded [13].

pH measurement

The pH measurement was carried out by using calibrated digital type pH meter by dipping the glass electrode and the reference electrode completely into gel system so as to cover the electrodes.

Conductivity

A direct reading digital conductivity meter (Systronics model no. 304) and dipping type conductivity cell [14].

Drug content

For the estimation of the drug in nanogel, kojic acid was extracted from 1 gm of nanogel formulation with 50 ml of distilled water. From this, 2 ml was pipette out and made up to 10 ml. The absorbance of the sample was determined spectrophotometrically at 268 nm. The concentration of Kojic acid was estimated from the calibration curve [12, 24].

In vitro drug Release studies

The drug release from the formulation was determined by using the apparatus known as Franz Diffusion Cell, which consist of a cylindrical glass tube which was opened at both the ends. 1 gm of nanogel equivalent to 4 mg of Kojic acid was spread uniformly on the surface of cellophane membrane (previously soaked in medium for 24 hrs) and was fixed to the one end of tube. The whole assembly was fixed in such a way that the lower end of tube containing gel was just touches (1-2 mm deep) the surface of diffusion medium i.e., 100 ml of pH 7.4 phosphate buffer contained in 100 ml beaker. The assembly was placed on thermostatic hot plate with magnetic stirrer and maintained at temperature 37°±2° the contents were stirred using magnetic bar at 100 rpm for a period of 24 hrs, 5 ml of samples were withdrawn at different time intervals. This 5 ml was diluted up to 10 ml of fresh phosphate buffer (pH 7.4) and sample were analyse at 268 nm in UV-Visible spectrometer for KA. [12, 25-26].

Skin irritation test

Test for irritation was performed on human volunteers. For each gel, four volunteers were selected and 1.0 g of formulated gel was applied on an area of 2 square inch to the back of hand. The volunteers were observed for lesions or irritation [12].

Spreadability

Spreadability is determined by apparatus suggested by Mutimer. It consists of wooden block, which is provided by a pulley at one end. By this method, Spreadability is measured on the basis of "Slip" and "Drag". A ground glass slide is fixed on this block. A sample of 0.1 g of nanogel under study is placed on this ground slide. The gel is fixed on the beach formula was pressed between two slides and a 1 kg weight is placed on the top of two slides and left for about 5 min to expel air and to provide a uniform film of the nanogel between two slides. Excess of the nanogel is scrapped from edges. The top plate is then subjected to pull the weight. With help of string attaches to the hook and the time required by top slide to cover the distance is noted. A shorter interval indicates better spreadability, spreadability was calculated by using the formula,

S=M.L/T,

Where,

S= spreadability, L=Length of glass slide, M=weight tied to upper slide, T=Time taken to separate the slides [13, 15].

Extrudability

Measure the force required to extrude the material from tube. Extrudability was based upon the quantity in percentage of gel and gel extruded from lacquered aluminum collapsible tube on application of weight in grams required to extrude at least 0.5 cm ribbon of gel in 10 seconds [16, 17]

+++ excellent ++ very good + average

Scanning electron microscopy

Scanning electron microscopy (SEM) provides high-resolution imaging that may be used to evaluate diverse materials for surface cracks, defects, contaminants, or corrosion. When a focused stream of secondary electrons interacts with atoms in the sample, multiple signals are produced that include information about the surface topography and sample composition using the Nova NanoSEM NPEP, all pictures were scanned at 10000x with a 5 m dimension scale 303 [18].

Zeta Potential

Zeta Potential of the prepared Nanosuspension was determined using Light Scattering method. The charge on the surface of particles is characterized by the HORIBA Scientific SZ-100 by measuring the zeta potential of a gel. The sample is injected into a disposable cell and a measurement of the particle electrophoretic mobility results in the calculated zeta potential [19-22].

Particle size

Horiba sz-100 windows [z type] were used to investigate the particle size (PS) of the gel. Particle size and zeta potential were measured in triplicates after dilution with distilled water, andthe average values ±SD were recorded [13, 23].

Content uniformity

Identification of pure drug

Identification of pure drug was carried was by Fourier Transform Infra-red Spectrophotometry (Shimadzu 8400s) scanned in the range of 200-400 nm.

Drug-excipient compatibility study

Studies of drug-excipient compatibility are important to ascertain drug and excipients are compatible with each other. IR spectra are used to study drug-excipient compatibility.

FTIR study

FTIR (Shimadzu 8400s) spectrophotometer were used in the range of 400-4000 cm^{-1} using potassium bromide discs (Mixing ratio1:1) The samples were hermetically sealed in aluminium pans and heated at a constant rate of 10°C/ min over a temperature range of 40 to 300°C.

FTIR spectroscopy

The FTIR spectrums of pure Kojic acid and physical mixtures of drugs and polymers were studied separately as per the excipients used in the formulation. It was observed that there were no major shifts in the main peaks of either drug. This indicates that there were no compatibility problems with the drug with the polymers and excipients used in the formulation. Kojic acid had peaks at 1715(C=O stretching), 3549 (O-H str.), 1620 (C-O), 2839 (C-H).

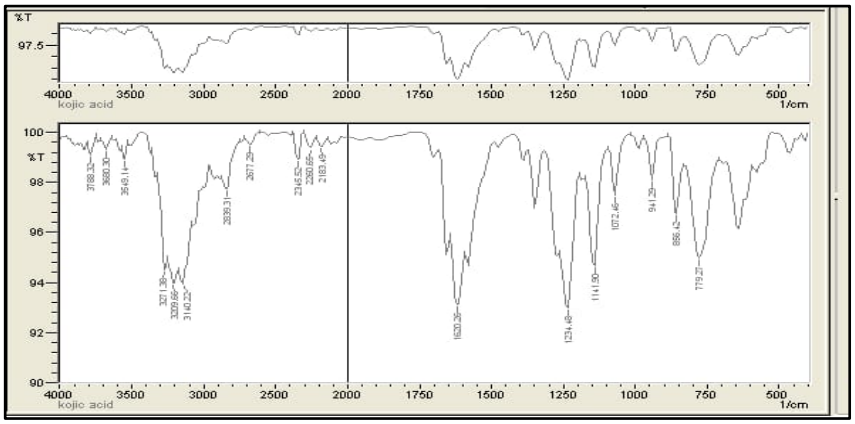
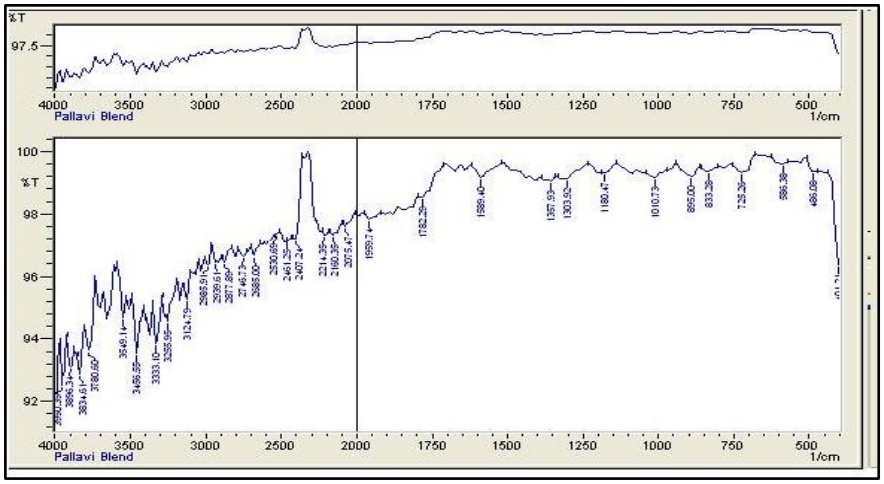


Figure 1: FTIR Studied of Kojic acid



UV spectroscopy:

The linearity of the response of kojic acid was verified at 2–10 µg/ml concentrations. The calibration curve was obtained by plotting the absorbance versus the concentration data and was treated by linear regression analysis. The equation of the linearity curve for kojic acid was y = 0.0646x +0.0036. The linearity curve was found to be linear in the a for mentioned concentrations (the correlation coefficient (r^2) of determination was 0.9978)

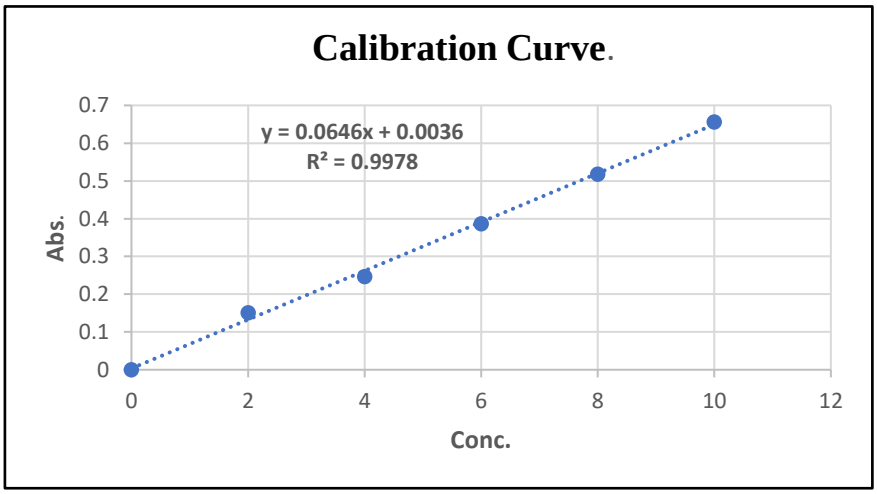


Fig 3: Calibration curve of Kojic acid

Composition of Nanogel: -

Table 1- Composition of nanogel (B1-B4)

Evaluation of prepared nanogel:

Appearance

Appearance of the prepared Nanogel was inspected visually and all the batches were white to Clear, and free from any particulate matters.

Particle Size Determination

Particle size of the prepared Nanogel was determined using Dynamic Light Scattering (DLS) method. Particle size determination results for all the prepared batches kojic acid nano are presented in the Table 3 and all the Graph obtained are reported in the Figure 4-7.

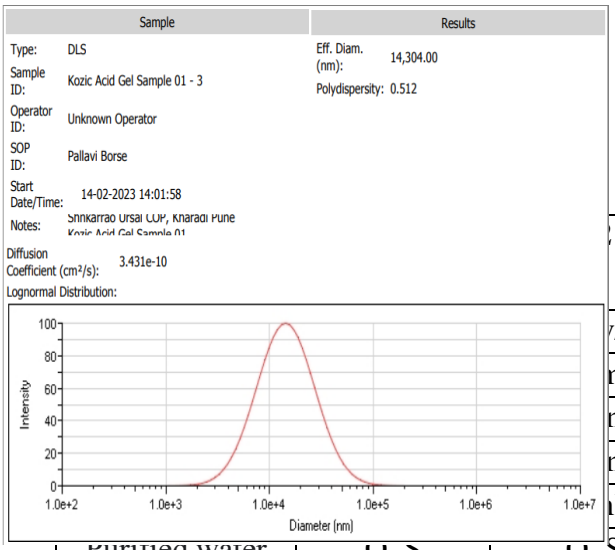


Fig 4 A: Particle size of formulation B1

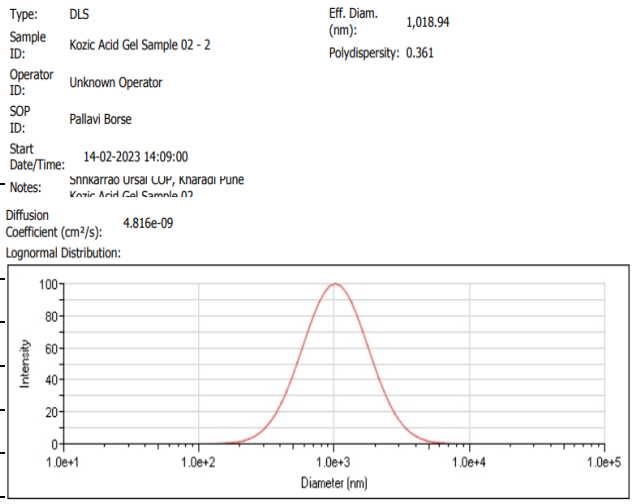
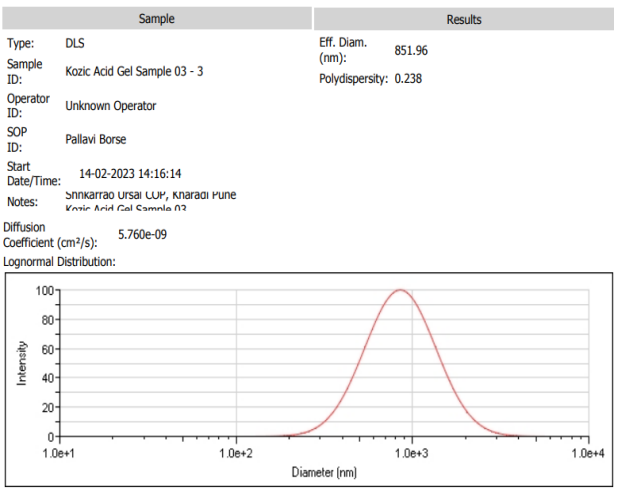


Fig 4B: Particle size of formulation B2

Fig 4D: Particle size of formulation B4



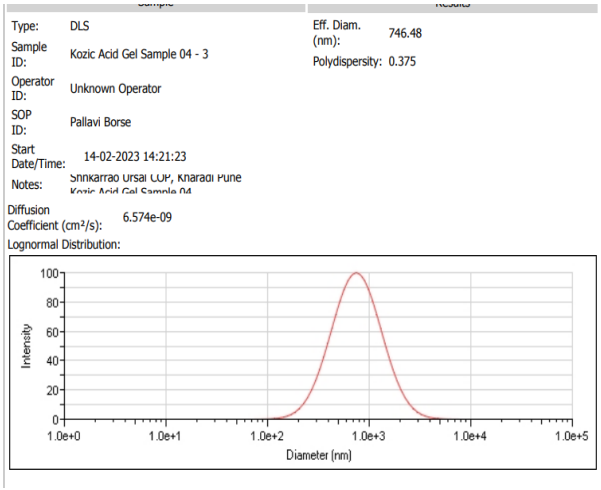
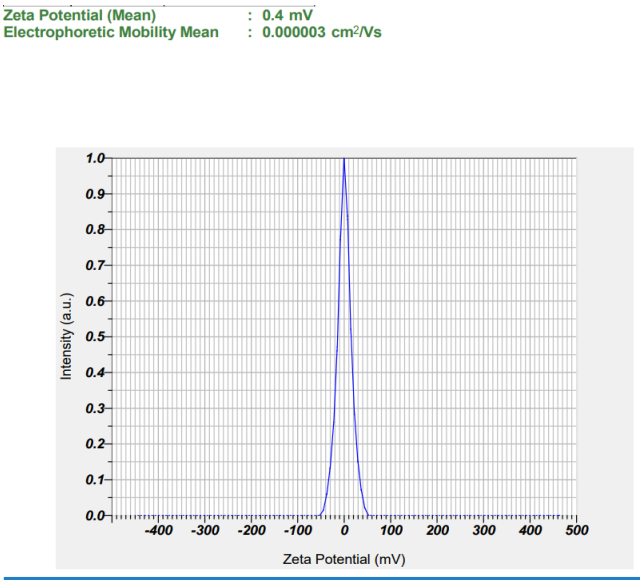


Fig 4C: Particle size of formulation B3

Particle size of batch 4 shows optimise size 746.48nm compare to other 3 batches. Batch4 is having optimum conc. of HPMC K35M and Carbopol 940.

Zeta Potential analysis

From the Graphical representation in following figures, it was observed that when the Nanogel was prepared using maximum conc. Of HPMC polymer compared to other batches is more stable.



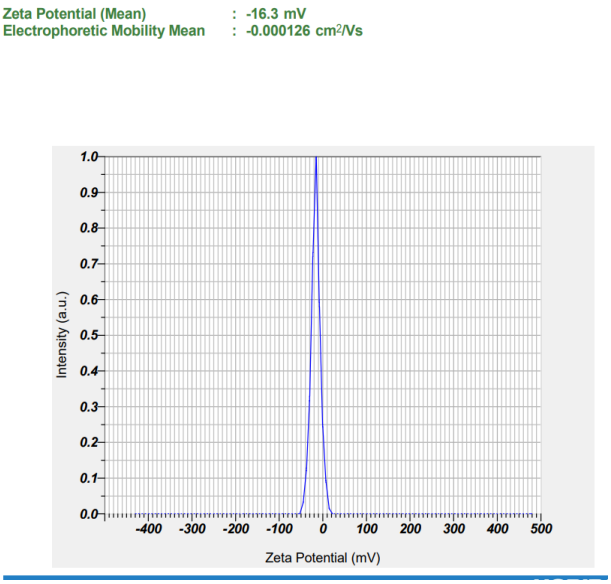


Fig 5A. zeta potential of formulation B1

Fig 5B. zeta potential of formulation B2

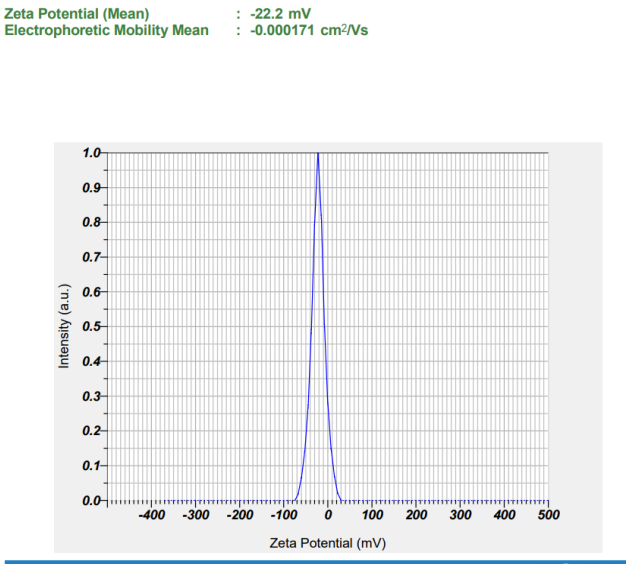


Fig 5C. zeta potential of formulation B3

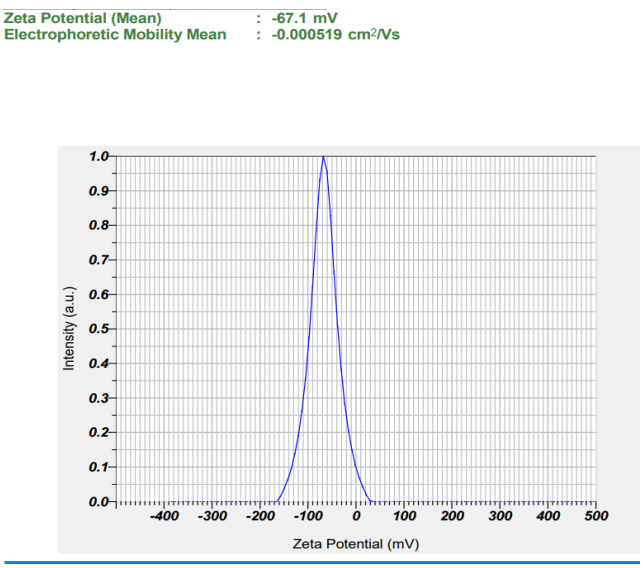


Fig 5D. zeta potential of formulation B4

To stabilize the Nanogel the Zeta Potential must be more that ±20 mV and it was observed from the above figure that batch 4 shows -67.1mV. From the Graphical representation the prepared Nanogel of B4 is more stable.

In-vitro Diffusion studies:

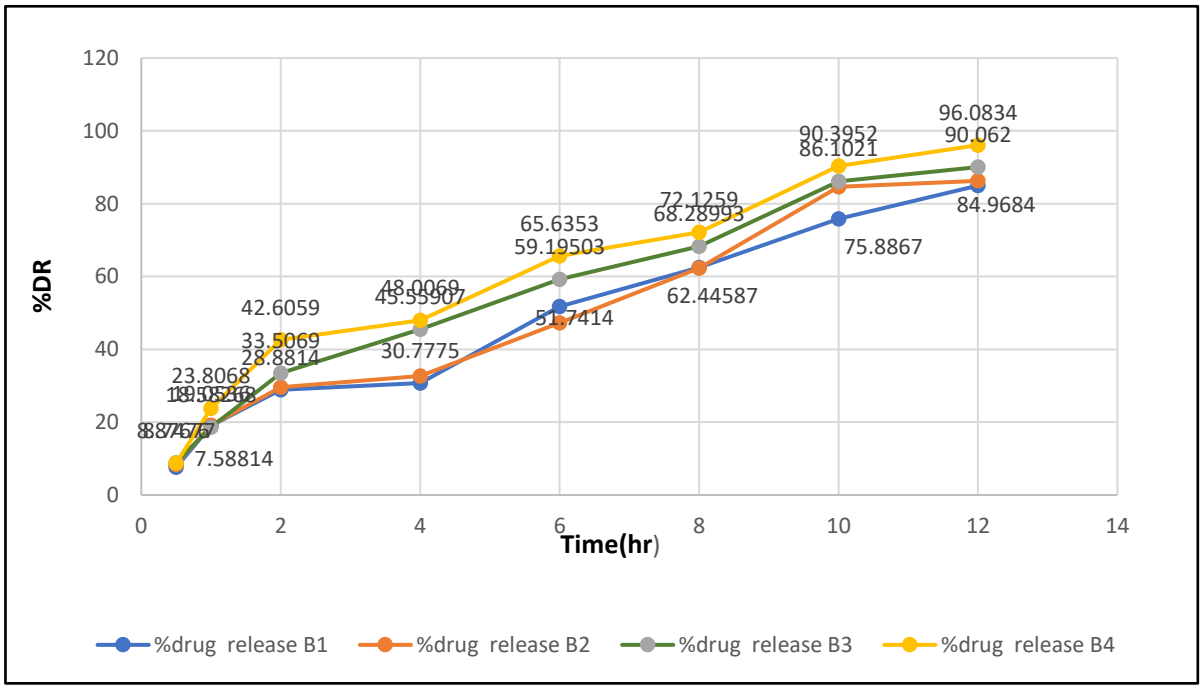


Fig 6: In-vitro drug release profile of nanogel of Kojic acid

From the above graph and % drug released readings batch 1 has 84.96% of drug release, batch 2 shows 86.27% drug release, batch 3 shows 90.06% drug release, batch 4 shows 96.83% drug

release in 12 hours. Formulation batch 4 shows maximum %drug release. Batch 4 have optimum concentration of polymer. Hence batch 4 consider optimise batch among 4 batches.

Viscosity study: (Spindle number-96)

Table 2: Viscosity results of B1

RPM	Surface (cP)	Middle (cP)	Bottom (cP)
10	27000	27560	28970
20	17530	19310	20480
30	14690	15250	16220
40	10200	10800	10930
50	9328	9469	1547

Table 3: Viscosity results of B2

RPM	Surface (cP)	Middle (cP)	Bottom (cP)
10	21940	21840	25590
20	15230	15940	14160
30	10650	10560	11120
40	7969	7406	7631
50	6688	6531	6625

Table 4: Viscosity results of B3

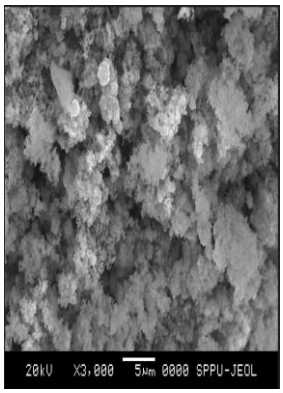
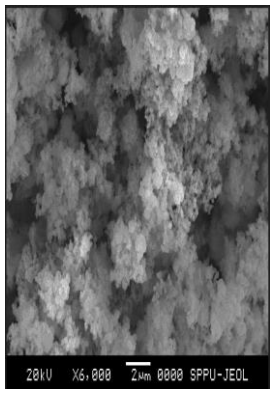
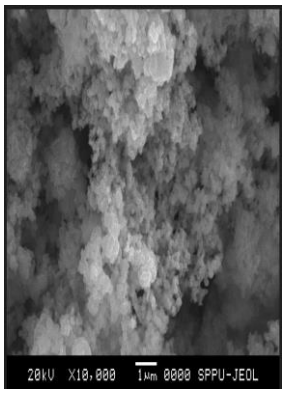
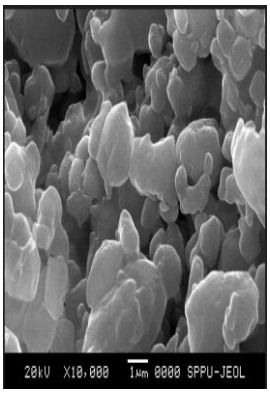
RPM	Surface (cP)	Middle (cP)	Bottom (cP)
10	22220	23110	24000
20	13550	13730	13880
30	10840	11090	11410
40	7388	7575	8231
50	6516	6531	6875

Table 5: Viscosity results of B4

RPM	Surface (cP)	Middle (cP)	Bottom (cP)
10	45000	50440	51000
20	30050	30560	31220
30	23380	23970	24560
40	15960	16560	16610
50	14200	14500	14690

Table 6: Evaluation For all formulated batches (B1-B4)

Particulars	**Batch1**	**Batch2**	**Batch3**	**Batch4**
Appearance	Whitish to colourless	Whitish to colourless	Whitish to colourless	Whitish to colourless
Fill volume (gm)	10gm	10gm	10gm	10gm
pH	5-7.5	5-7.5	5-7.5	5-7.5
Extrudability	++ (very good)	++ (very good)	+++ (excellent)	+++ (excellent)

Conductivity 1)200ms 2)20ms 3)2ms 4)200µs 5)20µs	 0 0 0.03 27 1.0	 0 0.2 0.05 057 1.0	 0 0.0 0.03 021 1.0	 0 0.1 0.12 123 1.0
Zeta potential (mV)	0.4mV	-16.3mV	-22.2mV	-67.4mV
Particle size (nm)	1754.20nm	1018.9nm	851nm	746nm
Spreadability (gm.cm/sec)	4.1 ±0.0264	4.6 ±0.0284	3.9 ±0.057	3.2 ±0.0156
Scanning electron microscopy				
Content Uniformity (%)	94.6%	96.1%	98.4%	98.7%

Conclusion:

It can be concluded from the experimental study carried out that the formulation of a Nanogel containing Kojic acid drug yields a formulation with a spherical and smooth surface, nano in the size range. The prepared nanogel was smooth without any lumps, particles and aggregates. So, all the formulations are homogenous. Based on all the factors the nanogel drug delivery system Batch-4 shows good drug content compared to others. The particle size of the nanogel formulation is optimum and it is less than 1000 nm. So, it concluded that the particles are in the tiny and nano in the size range. All nanogel formulations show pH in the range of 5.5 to 7. Based on the Spreadability diameter study it shows the nanogel is having good Spreadability. Nanogel formulations show a viscosity range from 5000-50000 cps. It concluded that they are stable in nature. Formulation Batch-4 showed the highest percentage of drug release compared to other formulations. In-vitro diffusion studies show Batch-4 formulation shows a controlled release pattern of drug from the formulation. The Zeta potential of batch Batch-4 showed -67.3mV. High zeta potential values show there will be no particles come together and no

flocculation. Hence it can be concluded from the experimental study carried out, that the formulation Batch-4 is an optimized batch with optimum HPMC K35M and Carbopol940.

ACKNOWLEDGEMENT

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CONFLICT OF INTEREST

All authors declared no conflicts of interest.

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FORMULATION, CHARACTERIZATION AND EVALUATION OF MATRIX TYPE TRANSDERMAL PATCH CONTAINING GLIBENCLAMIDE

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Abstract: *Transdermal patches have been one of the effective tools in the delivery of active pharmaceutical ingredients parenterally with greater patient compliance and ease of use with minimum side effects. In the present research matrix type transdermal patches containing Glibenclamide were prepared using different ratios of hydrophilic polymer HPMC and hydrophobic polymer ethyl celluloseby solvent evaporation technique for its sustained release effect and increased bioavailability as compared to oral dosage form. The mixture of methanol and chloroform was used as a solvent and 30%w/w polyethylene glycol 400 (PEG 400) was used as a plasticizer with Dimethyl sulfoxide (DMSO) as a permeation and solubility enhancer. The possible drug and polymer interaction was studied by FTIR spectroscopy. All the prepared patches were subjected to physicochemical studies (Folding Endurance, Thickness, Weight variation, Drug content, Moisture content and Moisture uptake), in vitro permeation studies were done through a cellulose membrane having 45µ pore size.Short term stability studies were carried out to check the shelf life of formulation. The results suggested that there was no interaction between the drug and polymers. Variations in permeation profiles among the Batches were observed. Based on physicochemical and in vitro permeation studies, the formulated Batch 4 with 94.46% drug diffusion met most of the required ideal specifications and was considered an optimized batch. the formulated patches were good in physical strength as well as stable and effective with uniform drug content.Transdermal patches of Glibenclamide are likely to enhance the patient compliance as it would eliminate the need of repeated dosing, enhance the bioavailability and sustain the action of the drug.*

Keywords: *Transdermal Patch, Antidiabetic, Hypoglycaemic, Glibenclamide, HPMC, Ethyl Cellulose, Matrix Type.*

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INTRODUCTION

Diabetes is one of the largest global health emergencies of this century, ranking among the 10 leading causes of mortality together with cardiovascular disease (CVD), respiratory disease, and cancer[1]. Once known as ‘Honey urine’, diabetes was first discovered in 1500 BCE and has been recognized as a calamitous and lethal disease for about 2000 years [2-4]. Despite of availability of various formulations for the treatment of diabetes like Insulin, Tablets, Ayurvedic Syrups, etc. the management of diabetes has been a challenging task. Various approaches for the development of Novel Drug Delivery System have been made and development of such NDDS has been a necessity for the better, easy treatment for the treatment of Diabetes.

Transdermal drug delivery mechanism is most promising one. It has been demonstrated that medicines administered by this method has improved bioavailability with less side effects. It ensures controlled release of drug and deliver it directly to blood circulation. At the same time, the side effects are considerably reduced. As drug does not come in contact with stomach surface, there is no gastrointestinal irritation and side effects thus increasing drug compliance. The process is painless, drug does not go through usual metabolism in liver thus no adverse effects on liver. Most important impact is long availability of required serum levels which may result in decrease frequency of medicine. As a result of all above, TDD is becoming famous method of drug administration for increasing number of medicines [5,6].

A transdermal patch is used to deliver a specific dose of medication through the skin and into bloodstream. Transdermal patches products were first approved in 1981 by FDA. Transdermal delivery systems are currently available containing scopolamine (hyoscine) for motion sickness, clonidine and nitroglycerin for cardiovascular disease, fentanyl for chronic pain, nicotine to aid smoking cessation. Transdermal delivery provides controlled, constant administration of the drug, and allows continuous input of drugs with short biological half-lives and eliminates pulsed entry into systemic circulation. TDDS offers many advantages over conventional injection and oral methods. It reduces the load that the oral route commonly places on the digestive tract and liver. It enhances patient compliance and minimizes harmful side effects of a drug caused from temporary overdose. It is convenient, especially notable in patches which require only once weekly application. Such a simple dosing regimen aids in patient adherence to drug therapy [7,8].

Glibenclamide is a secondgeneration sulphonyl urea oral hypoglycaemic agent, chemically it is 5-chloro-N-[2-[4-(cyclohexylcarbamoylsulfamoyl)phenyl]ethyl]-2methoxybenzamide. It is more potent than first generation sulphonylurea. Glibenclamide is also called as glyburide and its formula is $C_{23}H_{28}CIN_3O_5S$[9]. Plasma ½ life of Glibenclamideis about 3-5 h. Which enhance therapeutic effectiveness and keep steady plasma level. Transdermal application of Glibenclamidecan avoid the hypoglycaemic episode which are concomitant by oral Glibenclamide administration[10]. Glibenclamide has shown several adverse and potential side effect related to hypoglycemia with inaccurate dosing. It is demonstrated that Glibenclamide can cause gastrointestinal side effects including nausea, vomiting, anorexia, and heart burn and increase appetite. It can cause severe hypoglycemia through direct effect on the pancreatic cells of islets to inhibit production of glucagon and increased release of somatostatin[11,12]. The administration of Glibenclamide through Transdermal patch can avoid the above mentioned side effects and can also lead to increased Bioavailability and sustained release effect with better patients compliance.

In the present study and attempt to formulate matrix type transdermal patch was made for Glibenclamide drug by using hydrophilic polymer HPMC and hydrophobic polymer ethyl cellulose with help of plasticizer and permeation enhancer and using a combination of methanol and chloroform as volatile solvents. The possible drug and excipients interaction were checked by FTIR and formulated transdermal patches were thoroughly evaluated on physical and chemical parameters such as appearance,folding endurance,thickness,weight variation,percentage moisture content,percent moisture uptake and *in-vitro* diffusion studies. Short term stability studies were carried out to check the shelf life of formulation. The purpose of this study was to provide the delivery of drug across the skin into the systemic circulation in controlled rate to obtain sustained release effect with reduced side effects and reduced frequency of dosing with enhanced bioavailability as compared to oral dosage form with greater patient compliance.

MATERIALS AND METHODS

Materials

Glibenclamide was procured from Yarrow Chem Pvt. Ltd, Mumbai, India.Hydrophilic polymer HPMC was obtained as gift sample from Ashland, Netherlands. Co. Hydrophobic polymer Ethyl cellulose, Chloroform was used as solvent, Polyethylene Glycol 400 was used as plasticizer was obtained from Research-Lab Fine Chem Industries, Mumbai. Other chemicals such as Dimethyl sulfoxide(DMSO)

which was used as permeation enhancer and solubility enhancer, methanol was used as solvent were procured from COSCO CHEM, Pune.

Drug and excipients compatibility study

The possible Drug and Excipients interaction was checked by FTIR (Shimadzu 8400s)Spectrophotometer. The drug was placed in the sample holder using IR grade potassium bromide as a blank and scanned between the ranges 400–4000 cm^{-1} to determine the characteristic peaks of the drug[13].

The drug and all the Excipients were mixed in 1:1 ratio uniformly and kept for 1 month at room temperature. This mixture of drug and excipients was analysed on FTIR (Shimadzu 8400s) Spectrophotometer.The mixture was placed in the sample holder using IR grade potassium bromide as a blank and scanned between the ranges 400–4000 cm^{-1} to determine the characteristic peaks of the mixture. The obtained spectra of Drug and Mixture was compared for their characteristics peak and checked if any shift in characteristic peak can be seen[14].

Method of Preparation of Transdermal Patch of Glibenclamide

The Matrix type Transdermal Patch containing Glibenclamide was prepared by Solvent evaporation technique. Different concentration of HPMC and Ethyl cellulose were taken keeping total polymer weight 900mg and dissolved in mixture of Methanol and Chloroform (3:2) ratio respectively keeping total volume 30ml. In the above polymeric solution drug was added in small parts for uniform dispersion of drug and subsequent dissolution in polymeric solution. DMSO was added in dropwise manner as solubility enhancer as well as permeation enhancer. It was followed by addition of PEG 400 as plasticizer. The stirring was continued for 1 hour and the polymeric solution containing drug and excipient was poured into petri plate avoiding formation of bubbles and kept for drying for 24hrs. After 24 hours, the petri dish was kept in hot air oven at 50-60^{0}C for half hour to remove excess solvents. Further patch was removed from petri plate and cut into desired size and shape and further evaluated for various physicochemical parameters[15-19].

Table 1: Formulation Table of Transdermal Patch of Glibenclamide

Ingredients	**F1**	**F2**	**F3**	**F4**
Glibenclamide (mg)	125	125	125	125
HPMC K35M (mg)	150	225	300	375
Ethyl Cellulose (mg)	750	675	600	525
PEG 400 (% W/W)	30	30	30	30
Methanol:Chloroform (3:2) (ml)	30	30	30	30
Dimethyl Sulfoxide (DMSO) (ml)	0.6	0.6	0.6	0.6

{*Note: - 1. The total polymer weight was kept 900mg, 2. The total solvent volume was kept 30ml, 3. By above formulation table Patch of 44.15625cm^2area was obtained from which circular patches of 1.76625cm^2 area were cut with each patch containing 5 mg drug}

Evaluation of prepared transdermal patch of Glibenclamide

The formulated Transdermal Patch was evaluated on the basis of various physicochemical parameters such as physical appearance, folding endurance, thickness, weight variation, percentage moisture content, percentage moisture uptake, in-vitro drug diffusion study. Short term stability studies were carried out to check the stability of formulated transdermal patch over time and to check shelf life of the formulation.

Physical Appearance

The prepared transdermal patch was inspected visually for its colour, shape,flexibility, smoothness any possibility of crystal formation of drug[20].

Folding endurance
The transdermal patches were evaluated for mechanical strength by determining the folding endurance. Transdermal patch of 2x2cm size was cut and repeatedly folded at same point until it breaks and the folds were measured. The number of times the patches had to be folded until it breaks was observed is considered as folding endurance[21,22].

Thickness
The thickness of patches was measured by using digital calliper.The mean values and standard deviation were calculated for individual batches[23].

Weight variation
Prepared circular patches of 1.76625cm^2 areawascut and weight of each patch was determined by using digital balance. The average weight of each patch and standard deviation was calculated for individual batches[24].

Percentage moisture content
The prepared films were weighed individually and kept in a desiccator containing fused calcium chloride at room temperature for 24 hrs. After 24 hrs the films were reweighed and the percentage moisture content was determined by using the given formula [25,26].
Percentage moisture content = (Initial weight- Final weight/Final weight) x 100

Percentage moisture uptake
The weighed films were kept in a desiccator at room temperature for 24 hrs containing saturated solution of potassium chloride in order to maintain 84% RH. After 24 hrs the films were reweighed and the percentage moisture uptake was determined by using the given formula[27,28].
Percentage moisture uptake = (Final weight- Initial weight/ Initial weight) x 100

Drug Content
5 Patches were selected randomly and was completely dissolved in methanol. The solution was filtered through filter paper and amount of drug present in the filtrate was determined by using UV spectrophotometer at 228nm. absorbance was taken on UV Spectrophotometer and the drug content was estimated from the standard graph [29,30].

in-vitro drug diffusion
The *in vitro* Diffusion Study was performed using Franz Diffusion having receptor compartment quantity of 50ml using cellulose membrane of 45µ pore size. The patch of predefined size was selected randomly and placed on membrane facing donor compartment and the receptor compartment was filled using phosphate buffer solution (pH 7.4). And the whole assembly was maintained at 37 ± 2 ^{0}C. For diffusion studies cellulose membrane was soaked in the same buffer solution for 12 hrs. before mounting on diffusion cell. The samples were withdrawn after predefined time interval and sink condition was maintained by replacing same amount of phosphate buffer (pH 7.4). Glibenclamide concentration was assayed using UV spectrophotometer for noting absorbance at the lambda max that is 228nm[27-34].

Short term stability studies
To assess the stability the randomly selected patches were kept at room temperature over a period of 45 days. Patches were evaluated at 15th, 30th, 45th day for their physicochemical properties and Drug content[16].

RESULTS AND DISCUSSION

Drug and excipients compatibility study

Drug and excipient interactions play a vital role with respect to release of drug from the formulation amongst others. FTIR techniques have been used here to study the physical and chemical interaction between drug and excipients used.The IR spectral analysis of Glibenclamide (Figure 1) alone showed that the principal peaks were observed at wave numbers of 3357.84cm^{-1}(N-H Stretching), 2931.60cm^{-1} (C-H Stretching), 744.47cm^{-1} (C-Cl stretching), 1712.67cm^{-1} (C=O stretching), 1606.59cm^{-1}(C=O stretching), 1230.50cm^{-1}(C-O-C stretching), 1334.65 cm^{-1}(O=S=O stretching) confirming the purity of the drug as per established standards. In the IR spectra of the physical mixture of Glibenclamide, HPMC and Ethyl cellulose(Figure 2) the major peaks of Glibenclamide were 3357.84cm^{-1} (N-H Stretching), 2935.46cm^{-1}(C-H Stretching), 742.54cm^{-1}(C-Cl stretching), 1701.10cm^{-1} (C=O stretching),1606.59cm^{-1}(C=O stretching), 1228.57cm^{-1}(C-O-C stretching), 1325.01cm^{-1} (O=S=O stretching). However, some additional peaks were observed with physical mixtures, which could be due to the presence of polymers.there were no remarkable changes in these main peaks in IR spectra of mixture of drug and polymers, which show there were no physical interactions because of some bond formation between drug and polymers.

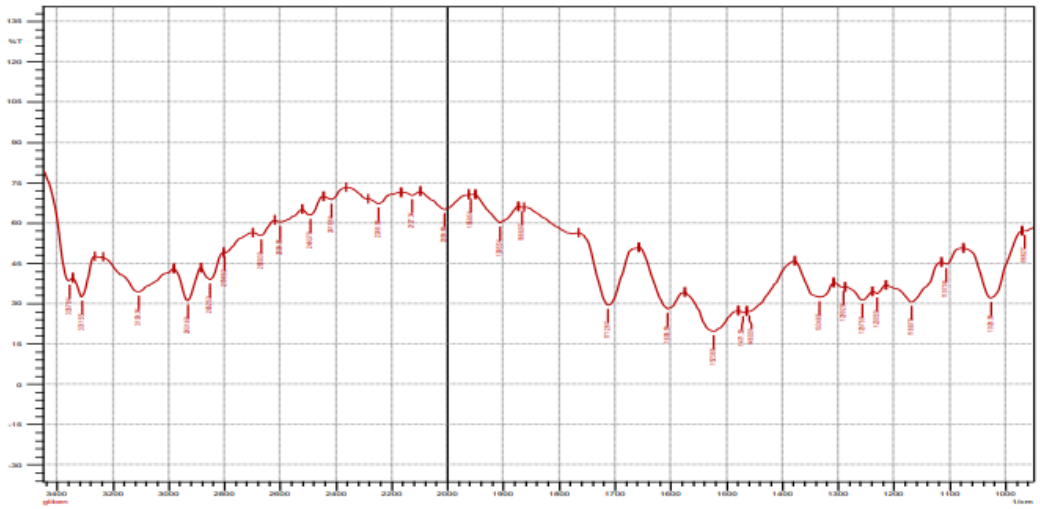

Figure 1: FTIR spectra of pure drug Glibenclamide

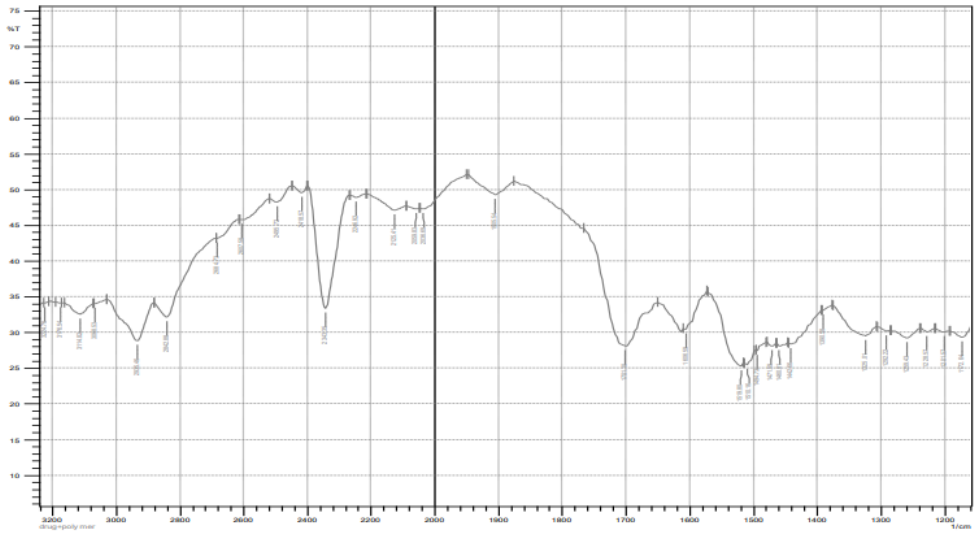

Figure 2: FTIR spectra of Drug and Excipients

Evaluation of prepared transdermal patch of Glibenclamide: -

Physical Appearance

The prepared transdermal patch was inspected visually for its colour, shape,flexibility, smoothness any possibility of crystal formation of drug. All the prepared patches were Uniformly circular, White in colour, Flexible, Translucent and there was no formation of any drug crystals was observed.

Folding endurance

The folding endurance indicated the mechanical strength of transdermal patches. The folding endurance was found to be in the range of 180,193,204,218 folds from batch 1 to 4 Respectively as shown in (Table 2) suggesting that the films produced were found to possess enough mechanical strength.The increased concentration of hydrophilic polymer in the formulation greatly affected the folding endurance as it provided better elasticity.The high value of folding endurance implies that the patch fabricated would maintain its integrity without appearance of any cracks on application of the surface of the skin.

Table 2: Folding Endurance of Formulated Transdermal Patches

Batch No.	Batch 1	Batch 2	Batch 3	Batch 4
Folding Endurance (Folds)	180	193	204	218

Thickness

The thickness of patches was measured by using digital calliper.The thickness ranged between 38 ±2 µm, 36 ±2 µm, 37±2 µm and36±2 µm, from batch 1 to 4 respectively as shown in (Table 3). which indicate that the prepared patches were uniform in thickness.

Table 3: Thickness of formulated patches and standard deviation

Batch No.	Batch 1	Batch 2	Batch 3	Batch 4
Thicknessµm	38 ±2	36 ±2	37±2	36±2

Weight variation

The average weight of patches was found to be in the range of 73 ±5mg, 74 ±5mg, 75 ±5mg, 75 ±5mg for the batches 1 to 4 respectively as shown in (Table 4)which shows the uniformity of weight throughout the formulated transdermal patches.The uniformity of weight indicates that the polymer solution of the drug is well dispersed on a flat surface. However, a little variation in average weight among the formulatedbatch 1 to batch 4 was observed in the range of 73–75 mg which may attribute to the variation in polymeric content. The increase in concentration of Hydrophilic polymer HPMC results into holding of more amount of moisture hence slight increase in weight can be observed.

Table 4: Average weight of Formulated transdermal patches with standard deviation

Batch No.	Batch 1	Batch 2	Batch 3	Batch 4
Average Weight (mg)	73 ±5	74 ±5	75 ±5	75 ±5

Percentage moisture content

Moisture content can influence the mechanical strength and drug release behavior of the transdermal therapeutic systems. Small moisture content in formulation helps them to remain stable and prevent from being completely dry and brittle. The percentage moisture content was found to be 8.96%, 9.26%, 8.34%, 7.28% from batches 1 to 4 respectively as shown in (Table 5). It shows that the concentration of hydrophilic polymer influences the moisture content in the patches. The more concentration of

hydrophilic polymer HPMC in batch 4 holds more amount of moisture and shows less loss in moisture as compared to other batches.

Table 5: Percentage moisture content of formulated transdermal patches

Batch No.	Batch 1	Batch 2	Batch 3	Batch 4
Moisture content %	8.96	9.26	8.34	7.28

Percentage moisture uptake

The absorption of moisture is an imperative aspect which influences the drug diffusion as it extends into the water uptake of the patch from the body tissues as well as from the environment during the application period. It is a vital parameter which helps to maintain the mechanical integrity. The percentage moisture uptake from formulated transdermal patches was reported to be 3.07%, 3.40%, 3.49%, 3.81% from batches 1 to 4 respectively as shown in (Table 6). The formulations with high concentration of hydrophobic polymer Ethyl cellulose displayed the lowest moisture absorption attributes as a result of the decrease in water permeability of the polymer ethyl cellulose. Low moisture uptake prevent formulation from microbial contamination and prevents the bulkiness of the patch.

Table 6: Percentage moisture uptake of formulated transdermal patches

Batch No.	Batch 1	Batch 2	Batch 3	Batch 4
Moisture uptake %	3.07	3.40	3.49	3.81

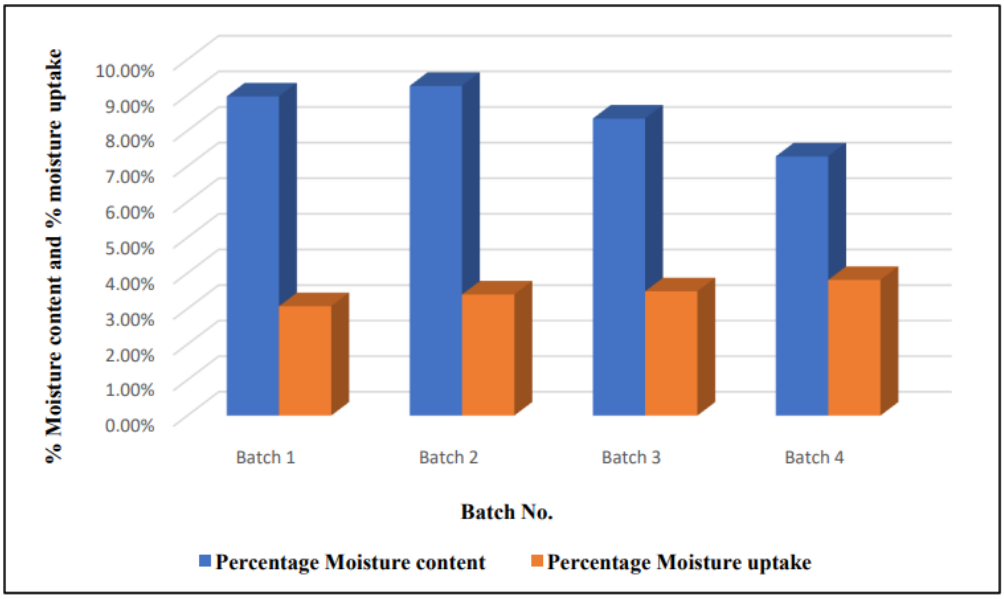

Figure 3: % Moisture Content and % Moisture Uptake

Drug Content

The formulated Transdermal patches were analysed for their drug content and the average drug content from 5 patches of each batch was noted. patches shown drug content from 99.03%, 100.08%, 98.8%, 101.2% from Batch 1 to 4 respectively as shown in (Table 7). The results complies with IP standards which are from 95% to 105%.By this study we conclude that uniform distribution of drug occurred in Formulated Transdermal patches and the expected dose in individual patches that is 5mg was successfully achieved.

Table 7: Percentage drug content in formulated transdermal patches in-vitro drug diffusion

Batch No.	Batch 1	Batch 2	Batch 3	Batch 4
Drug Content %	99.03	100.08	98.8	101.2

The percent drug release in all the 4 batches showed significant difference in pattern of diffusion.The combination of high concentration of Hydrophilic polymer HPMC and low concentration of hydrophobic polymer Ethyl cellulose in Batch 4 gives more Sustained drug release effect as compared to High concentration of Hydrophobic Polymer Ethyl Celluloseand low concentration of Hydrophilic polymer HPMC in Batch1.In thisexperiment, as the concentration of hydrophilic polymer was increased, the amount of drug permeated was increased. This may be a result of the initial rapid dissolution of the hydrophilic polymers when the patch is in contact with the hydrated skin, which results in accumulation of high amounts of drug on the skin surface and thus leads to the saturation of the skin with drug molecules at all times. The rapid dissolution of the aqueous soluble fraction of the film also leads to the formation of pores, and hence, higher release rates[36].The *in vitro* study results showed that with an increase in the concentration of polymers especially hydrophilic, the total amount of drug release increased with increase in sustained release effect. Thus, formulation F4 was considered the best formulation which released 94.46% of total drug in our 24h study.The results obtained are depicted in Figure 4.

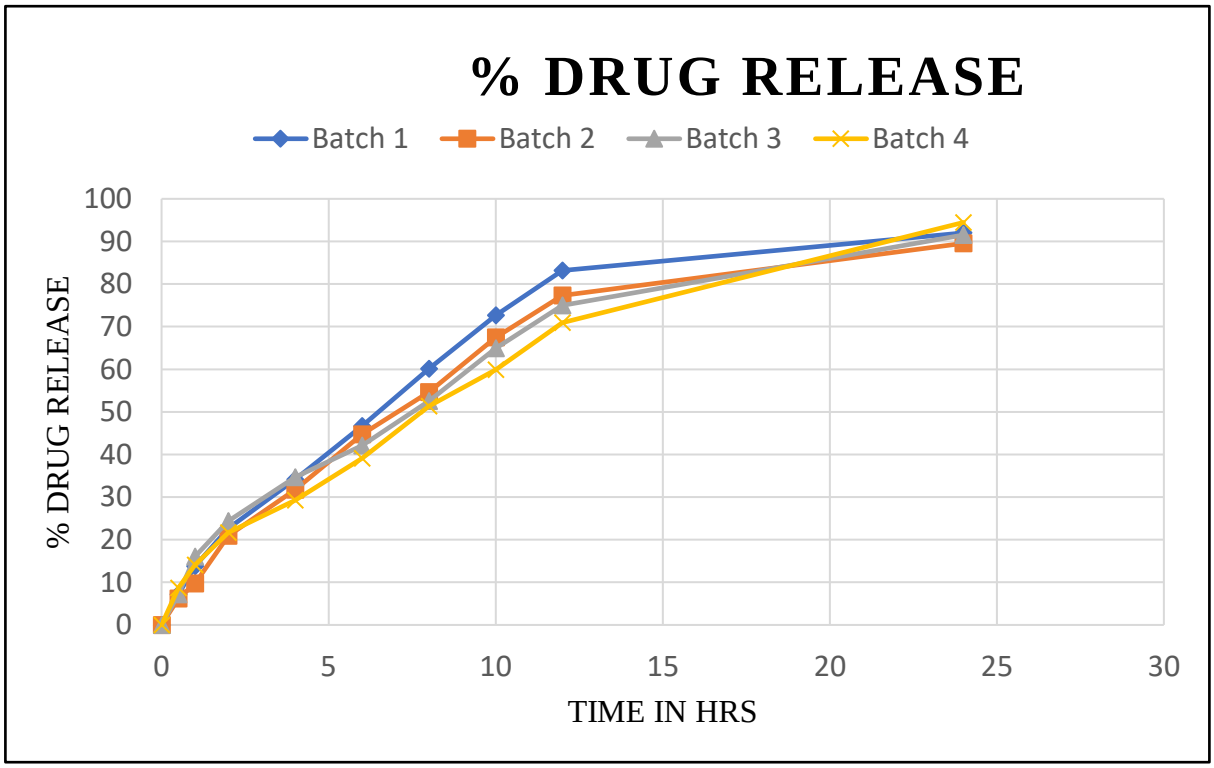

Figure 4: % Drug release VS Time

Short term stability studies

In order to assess the stability of optimized formulation of Batch 4 was subjected to short term stability studies. There was no remarkable change in its colour, shape,flexibility, smoothness any possibility of crystal formation of drug. The patches were analysed for drug content and showed 100.4% average drug

content which was significantly similar to previous drug content. The stability studies results signified that the formulated patches possess adequate shelf life till 45 days.

CONCLUSION

The purpose of the study was to develop,characterize and evaluate the transdermal patch containing Glibenclamide for the treatment of diabetes and the objectives were met. Four batches of Transdermal patch containing Glibenclamide was prepared and evaluated on various physicochemical parameters. Based on the findings of Preformulation studies that is from FTIR studies it was concluded that there is no any interaction between drug and excipients. The uniformity of weight, thickness and drug content between the patches indicates the suitability of procedure for development of transdermal patches.The high value of folding endurance shows thestrength and flexibility of patch to withstand mechanical pressure. The behaviour of patches towards moisture shown that formulated patches were found to contain adequate amount of moisture and significant amount of moisture uptake which will potentially help them for their effectiveness.By the *in vitro* diffusion studies the drug release in optimised formulation that is batch 4 was more sustained as compared to other formulation with maximum drug diffusion of 94.46% across the membrane. The optimised batch also showed satisfactory folding endurance, moisture content as well as drug content with good physical strength. The stability studies results signified that the formulated patches possess adequate shelf life till 45 days.

The objective behind the present work of formulation and evaluation of transdermal patch containing Glibenclamide for treatment of diabetes was achieved and found that the formulated patches were good in physical strength as well as stable and effective.Transdermal patches of Glibenclamide are likely to enhance the patient compliance as it would eliminate the need of repeated dosing, enhance the bioavailability and sustain the action of the drug.

ACKNOWLEDGEMENT

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CONFLICT OF INTEREST

All authors declared no conflicts of interest

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FORMULATION DEVELOPMENT, EVALUATION AND MTT ASSAY OF HERBAL VITAMIN C POWDER.

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Abstract: The present work was aimed at formulation development, evaluation and MTT assay of Herbal Vitamin C Powder. The formulation was prepared by mixing Acerola Extract, Rosehip Extract, Moringa Extract, Amla Powder, Bilberry Extract, Glycine, L- lysine, L- proline, Elaichi Powder, Rock salt, Spirulina Powder and excipients such as Lemon flavor. Initially, the pre-formulation evaluation of each separate ingredient was done by evaluating its Organoleptic characters, Bulk density, Tap density, Hausner Ratio, Moisture Content, pH, and Solid-state stability study. The formulation was prepared after the pre-formulation study was completed and then evaluated for powder characters and MTT assay. Powder evaluation was performed using various physiochemical and microbiological parameters and the cytotoxicity of the formulation was performed by an MTT assay on Human embryonic kidney cells (HEK). From the above study, we can conclude that the stable polyherbal formulation of Vitamin C was prepared and evaluated and did not exhibit cytotoxicity at the daily dose limit.

Keywords: Vitamin C, MTT assay, Cell viability assay, Human embryonic kidney cells

Introduction:

The new single chemical entity is responsible for the medicine's significant therapeutic activity in modern pharmacology and drug development. Still, Ayurveda formulations are founded on different standards: using a single herb and multiple herbs, known as polyherbal formulation. Polypharmacy or polyherbalism refers to the impact of combining several medicinal plants to increase the efficacy of a preparation.[1] Historically, the classic text of Ayurveda, "Sarangdhar Samhita," has emphasized the concept of polyherbalism.[2] Although the bioactive components of individual herbs in many formulations have been well-known, they generally exist in small proportion. They are mostly not sufficient enough to attain the desired therapeutic efficacy. Scientific studies have discovered that when mixed, these herbs of varied effectiveness may yield better results concerning the single or sum of their single effect. This occurrence gives rise to positive herb interaction known as synergism which confers some benefits of the polyherbal formulation. It is evident that with a single multi-constituent formulation, better therapeutic outcomes can be attained.[1] The term "nutraceuticals" can be explained as the food items as a whole or a part which possesses some nutritional value along with medicinal properties.[3] These findings have triggered a series of studies in the nutraceuticals field.[4] There is a controversy over a specific definition and set of regulations to define nutraceutical compounds.[5] However, nutraceutical compounds are health-enhancing products that improve the mental and physical activities of the body. They are commercialized to minimize the risk factors of various diseases. Nutraceutical products are simply a hybrid between drug and food. On the other hand, this terminology is a broader term that includes minerals, vitamins, amino acids, botanicals, or herbs. Therefore, both dietary supplements and fortified foods can be classified as nutraceuticals. The terminology of nutraceutical was defined by the foundation for innovation in medicine in (New York, USA) in 1989. Defelice's definition in 1995 was: "A food or parts of food that provide

medical or health benefits, including the prevention and/or treatment of disease.[5] Nutraceuticals term originated from two terminologies: "nutrition" and "pharmaceutical". [6]

Vitamin C (ascorbate) is an essential water-soluble micronutrient in humans and is obtained through the diet, primarily from fruits and vegetables.[7] Vitamin C is necessary for the development and maintenance of connective tissues. It plays a vital role in bone formation, wound healing, and the care of healthy gums. It helps synthesize and metabolize tyrosine, folic acid and tryptophan and hydroxylation of glycine, proline, and lysine carnitine.[8] It is a cofactor for collagen synthesis and a primary antioxidant and is rapidly consumed post-wounding. Vitamin C could promote wound healing by altering the inflammatory, proliferative and remodelling phases. Vitamin C protects the immune system, reduces the severity of allergic reactions, and helps to fight off infections.[9] Humans cannot synthesize ascorbic acid due to the lack of gulonolactone oxidase enzyme. Hence, ascorbic acid has to be supplemented mainly through fruits and vegetables. It is present in oranges, lemons, grapefruit, watermelon, papaya, strawberries, mango, pineapple, raspberries and cherries. It is also found in green leafy vegetables, tomatoes, broccoli, green and red peppers, cauliflower and cabbage.[10]Vitamin C is a cofactor in the hydroxylation of proline and lysine residues in procollagen, which is vital for the strength and stability of collagen fibers. In addition, ascorbic acid enhances neutrophil function and acts as an antioxidant.[5]Systemic administration of vitamin C plays a vital role in gingival fibroblast proliferation and functions.[6]According to ICMR guidelines, adults' daily allowance (RDA) for ascorbic acid ranges between 70–90 mg daily.[7]The polyherbal formulation comprises Acerola (Malpighia emarginate DC.), known as Barbados cherry or West Indian cherry, which belongs to the Malpighiaceae family. The fruit is known to be one of the world's rich natural sources of ascorbic acid. Apart from containing an exorbitant amount of ascorbic acid, the fruit

also contains several phytonutrients like carotenoids, phenolics, flavonoids, and anthocyanins and possesses numerous bio-functionalproperties.[8] The rosehip is a repository of flavonoids, pectin, vitamins A, B complex, C and E, also minerals like Ca, Fe, Se and Mn. Trace amounts of Mg, K, S and Si have also been discovered.[9] Moringa leaves are an essential source of several nutrients. One hundred grams of dried leaves contain 27.1 g protein, 16.3 mg vitamin A, 17.3 mg vitamin C, 2.0 g calcium, 1.3 g potassium, and 28.2 mg iron, in addition to 19.2 g dietary fiber and several other nutrients.[10] Along with the active vitamin C ingredients glycine, L-proline and L-lysine were added, which metabolize by the vitamin C easily and get maximum benefits.[11]Also, some taste improvements agents such as Elaichi and Rock salt were added to the formulation. The supplementation of spirulina as a potent alternative source of iron and folic acid. Ascorbic acid facilitates iron absorption by forming a chelate with ferric iron at an acid pH that remains soluble at the alkaline pH of the duodenum.[12]

In the present study, the biological effects of vitamin C on cells were investigated in vitro by using the MTT assay. 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide test was used to explore the cell viability in the MTT assay. The MTT colorimetric assay was conducted in a 96-well plate format. The cells may require preincubation in the wells before adding the test drug. The preincubation times may vary from 0-24 hrs. according to the cell line properties. Cells are usually exposed to the drug for 24-96 hrs. depending on the drug activity. MTT solution is then added to the treated cells, where the yellow MTT is reduced to purple formazan by various mitochondrial and cytosolic enzymes that are operational in viable cells. The MTT molecule is not reduced by dead cells, red blood cells (metabolically inactive cells), spleen cells (resting cells) and Stimulated lymphocytes (activated cells). After 3-4 hrs. of incubation with MTT, the formazan absorbance at 550 nm is directly proportional to the number of cells in a range of 200-

50,000 cells per well, and thus very small amounts of cells can be detected. The absorbance indicates the number of viable cells remaining after treatment with the drug and is compared to the absorbance of control cells not exposed to the drug.[13].

Material and methods:

Materials:

Methanol, sulfuric acid, acetic acid, toluene, ethyl acetate, formic acid (Merck made), alpha naphthol, ferric chloride, potassium permanganate, indigo carmine (Loba Chemie made) and polysorbate 80, etc. which are of AR grade and procured from Vijay chemicals, Pune. For microbiological evaluation, chemicals like Soyabean casein digest medium, Gram Negative broth, Rappaport Vassiliadis Salmonella Enrichment Broth, Soyabean casein digest agar, Xylose lysine deoxycholate agar, Bismuth sulphate agar, Cetrimide agar, MacConkey broth & agar, sabouraud dextrose broth & agar (HIMEDIA made) which are of LR grade and procured from Vijay chemicals, Pune. MTT reagent (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide), DMEM media (Gibco), and Fetal bovine serum (Gibco) were procured from genexindia Bioscience.

Instruments:

1. Weighing Balance (Master)
2. Hot Air Oven (Bio-Techniques India)
3. pH Meter (Global)
4. Muffle Furnace (Bio-Techniques India)
5. Mechanical Shaker (Bio-Techniques India)
6. Laminar airflow (Bio-Techniques India)
7. Bacteriological Incubator (Bio-Techniques India)
8. CO_2 incubator
9. Automated microplate reader

Methods:

Pre-formulation studies were done by performing color, odor, taste, bulk density, tap density, Hausner ratio, carr index, angle of repose and solid-state stability. Organoleptic Properties of the formulation like color, odor, taste and powder characteristics like bulk density, tap density, Hausner ratio, carr index and angle of repose, were determined. Physiochemical stability was checked by performing loss on drying, pH, total ash, acid insoluble ash value, water-soluble extractive value, alcohol-soluble extractive value and also the presence of phytochemical constituents like tannin, phenols, flavonoids and carbohydrates was checked.

The microbial evaluation was done by performing the total aerobic bacterial count, total aerobic fungal count and specific pathogen test for Escherichia Coli**,** Staphylococcus Aureus, Salmonella Species and Pseudomonas aeruginosa. The nutritional values were also estimated and the MTT assay was performed to assess the cytotoxicity of the formulation.

A. Pre-formulation Study

Pre-formulation is a phase of the research and development process in which a new medicinal molecule's physical, chemical, and mechanical properties are studied individually and in combination with excipients to generate a stable, safe, and effective bioavailable dosage form.

1. Organoleptic Properties:

Color: Observation was done on bright background with light using drug powder. It was carefully observed by the naked eye.

Odor: Before smelling coffee, beans were used to remove all previous odors. Powders took in between the thumb and 1st finger and smelled it.

Taste: Powders are taken, tasted on the tongue, and examined for the type of taste.

2. Powder characteristics:

Bulk Density:

5 gm of the powdered drug was weighed in the digital balanced weighing machine. These powders were added to the dried 25 ml graduated cylinder. The volume of the cylinder was noted.[15]

Bulk Density = M/ V

Where, M = mass of powder

V = Volume of powder

Determination of tap density:

5 gm of the powdered drug was weighed in the digital balanced weighing machine. These powders were added to the dried 25 ml graduated cylinder. After measuring the initial volume of a cylinder, it was mechanically tapped using a tap density apparatus of BIO TECHNICS INDIA, BTI-08. The final volume was noted.[15]

Tapped density = M/ Vt

Where, M = Mass of powder

Vt = Minimum volume occupied after tapping

Hausner Ratio:

Hausner ratio (HR) indicates the powder's flow characteristics and flowability. The ratio between the bulk densities of compacted and loosely poured powder is called Hausner.[16]

Hausner Ratio = ρ tapped/ ρ bulk

Where, ρ = Density

Carr Index:

Carr index gives an idea indirectly about the flow behavior of a powder. Carr index (Ci) is determined using Hr values as given in Eq. [16]

Hr = 100 - (100/ Hr)

Angle of repose:

In the fixed funnel method, the granular materials are poured from a funnel at a certain height onto a selected base with known roughness properties. The funnel is either fixed or raised slowly while the conical shape of the material heap is forming to minimize the effect of the falling particles. The pouring of the material is stopped when the heap reaches a predetermined height or width. Then, the angle of repose is measured by the inverse tangent (arctan) rule, at which the average radius of the formed conical shape and the maximum height of the heaped material is measured. Then the angle of repose is determined as the arctan of the maximum height.[17]

The following formula can calculate the angle of repose:

$$\tan \theta = h/r$$

So,

$$\theta = \tan^{-1} h/r$$

Where,

θ = Angle of repose

h = height of pile of powder(cm)

r = radius (cm)

Moisture Content:

In the dried petri dish 1.5 gm weighed powder was taken and placed in an oven at 105– 110^0c. After drying and cooling in a desiccator, it was weighed in a digital balanced weighing machine. After drying, the weight was reported and the drying loss was measured.[17]

pH:

In a digital balanced weighing machine, 1 gm of powder was weighed and mixed with 20 ml of distilled water. pH of this solution was calculated using a digital pH meter.[17]

Solid State stability study:

Solid-state reactions are much slower and more difficult to interpret than solution-state reactions, due to a reduced no. of molecular contacts between drug and excipient molecules and to the occurrence of multiple-phase reactions.

A small mixture of drug and excipient was prepared. The mixture was then placed in the vial. A rubber closure was placed on the vial and the stopper was dipped in the melted Carnauba wax to seal. Then the vials are kept for 1-3 weeks for specified storage conditions. The sample was physically observed for the following.[17]

1) Caking
2) Liquefaction
3) Discoloration

4) Odor
5) Gel formation

B. Formulation Evaluation:

1. Organoleptic Properties:

Color: Observation was done on bright background with light using drug powder. The naked eye carefully observed it.

Odor: Before smelling coffee, beans were used to remove all previous odors. Both Powders took in between the thumb and 1st finger and smelled it.

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So,

$$\theta = \tan^{-1} h/r$$

Where,

θ = Angle of repose

h = height of the pile of powder (cm)

r = radius (cm)

3. Physiochemical parameters:

Determination of loss on drying:

In a dried petri dish, 1.5 gm weighed vitamin C powder was taken and placed in an oven at 105– 1100 c. After drying and cooling in a desiccator, it was weighed in a digital balanced weighing machine. After drying, the weight was reported, and the drying loss was measured.[18]

Determination of pH:

In a digital balanced weighing machine, 1 gm of vitamin C powder was weighed and mixed with 20 ml of distilled water. pH of this solution was calculated using a digital pH meter.[19]

Determination of total ash:

2 gm of vitamin C powder was weighed in a digital balanced weighing machine and was taken as a sample into the silica crucible. It was gradually heated with a burner using a 2 cm high flame and supporting the dish about 7 cm above the flame until vapors almost stopped being produced. The dish was lowered, and the heat increased until all the carbon had burned away. After cooling it in a desiccator, it is placed in a balanced weighing machine. Total ash content was calculated and expressed as % w/w of air-dried material.[20]

Acid insoluble ash value:

After assessing the total ash, it was poured into a 250 ml beaker with no ash loss, and 100 ml of diluted hydrochloric acid was added to it. Using a water bath, this solution was boiled for 5 minutes, followed by filtration of the solution and collecting the insoluble matter on an ashless filter paper (Whatman no.41). This filtrate was washed with hot water till getting the neutral filtrate. Transferred the insoluble matter-containing filter paper to the initial crucible and dried it on a hot plate at 600^0c using a muffle furnace for ignition (until it became white ash). Allowed this residue to cool in suitable desiccators for about 30 minutes and weigh without delay. Repeated the process until a constant weight was obtained. Calculated this acid-insoluble ash concerning the air-dried drug. [20]

Determination of water-soluble extractive value:

A digital balanced weighing machine measured 5 gm of air-dried vitamin C powder. These powders were mixed with 100 ml of distilled water in a glass stoppered conical flask. It was set aside for 24 hours with frequent shaking. Rapidly filter it after 24 hrs. A pipette is used to transfer 25 ml of filtrate into a tarred flat bottom evaporating dish, which is then put over boiling water to evaporate it to dryness. Again, evaporated dish dried at 105^0 c in the oven. The weight of this residue was measured after cooling, and the percentage of water-soluble extractive was determined and expressed as % w/w with reference to air dried sample.[20,21]

Determination of alcohol soluble extractive value:

A digital balanced weighing machine measured 5 gm of air-dried vitamin C powder. These powders were mixed with 100 ml of ethanol in a glass stoppered conical flask. It was set aside for 24 hours with frequent shaking. Rapidly filter it after 24 hrs. A pipette is used to transfer 25 ml of filtrate into a tarred flat bottom evaporating dish, which is then put over boiling water to evaporate it to dryness. Again, evaporated dish dried at 105^0 c in the oven. The weight of this residue was measured after cooling, and the percentage of alcohol-soluble extractive was determined and expressed as % w/w with reference to air dried sample.[20,21]

Determination of phytochemical constituents:

Test for the presence of tannin and phenol:

- A test tube was filled with 2 ml of vitamin C powder aqueous extract and a few drops of 5 % FeCl3 solution. Deep blue-black color was observed at the end.
- A test tube was filled with 2 ml of vitamin C powder aqueous extract. It was treated with a few drops of acetic acid solution. The red color persists at the end.

- A test tube was filled with 2 ml of vitamin C powder aqueous extract and a few drops of dilute potassium permanganate solution. Discoloration of the solution was observed at the end. This test was performed for the 1st, 2nd, 3rd and 6th months and was found present each month.[22]

Test for the presence of Flavonoids:

- Shinoda test: We took 2 ml of vitamin C powder aqueous drug extract and mixed it with 5 ml of 95% ethanol. Then it was mixed with a few drops of concentrated HCl and 0.5 gm of magnesium. The pink color was observed.
- Adding an increasing amount of sodium hydroxide to the residue shows yellow coloration, which decolorizes after adding acid. [22]

Test for the presence of carbohydrates:

- Fehling's Test: Take 2 ml of the sample solution in a clean test tube. Add 2 ml of Fehling's solution A and Fehling's solution B. Keep the solution in a boiling water bath for about 10 minutes. If a red precipitate is formed, then the presence of carbohydrates is confirmed.
- Molisch's Test: Take 2 ml of the sample solution in a clean test tube. Add 2-3 drops of Molisch reagent slowly. Now add concentrated sulfuric acid along the sides of the test tube. The acid layer forms a layer at the bottom. Note the junction of the two layers. If there is a formation of the violet ring, then the presence of carbohydrates is confirmed.[22]

Determination of total tannin:

For blank:300 ml distilled water and 25 ml, indigo carmine solution was transferred to a 500 ml conical flask and thoroughly mixed. This solution was titrated against 0.02 M KMnO4 solution until stable golden-yellow color developed and the burette reading was noted.

For sample: Air-dried vitamin C powder was weighed 0.05 gm in the digital balanced weighing machine. This sample was poured into a 500 ml conical flask, and 50 ml of distilled water was added until the sample was fully dissolved. 250 ml sterile water was carefully added to this solution and thoroughly mixed. 25 ml of indigo carmine solution was added to it and mixed well. Carefully titrated this solution against 0.02 M KMnO4 until stable golden-yellow color persisted. The burette reading was noted. The percentage of total tannin was calculated using the following factor. 1 ml of 0.02 M KMnO4 is equivalent to 0.00415 gm of tannin.[23]

4. Microbiological parameters

Determination of total aerobic bacterial count:

Before starting microbial analysis LAF (Laminar Air Flow) and UV (Ultra Violet) lights were switched on for about 30 minutes. Before starting microbial analysis under LAF, UV lights were switched off and daylight was switched on. 10 gm of air-dried vitamin C powder were dissolved and suspended in 90 ml sterile soybean casein digest medium with 4% polysorbate 80 (Tween -80). 1 ml of the solution was pipette out from the above solution using a micropipette and poured into each of the two sterile petri plates. Micropipette tips were discarded in a beaker containing disinfectant in use. Hands were wiped with 70% IPA immediately. 15-20 ml of sterile molten soybean casein digest agar (40-45^{0}c) was poured into the two plates for the total aerobic bacterial count. Plates were swirling slowly to give them uniform dispersion of sample by taking

care that media did not touch the plate lid. Negative control was prepared by pouring 15-20 ml of each used media in a separate empty sterile petri plate and allowing it to solidify at the end of the analysis. Soybean casein digest agar plates were inverted and incubated at 30- 35^0c for 120 hours.[24]

Determination of total aerobic fungal count:

Before starting microbial analysis LAF (Laminar Air Flow) and UV (Ultra Violet) lights were switched on for about 30 minutes. Before starting microbial analysis under LAF, UV lights were switched off, and daylight was switched on. 10 gm of air-dried vitamin C powder were dissolved and suspended in 90 ml sterile soybean casein digest medium with 4% polysorbate 80 (Tween -80). 1 ml of the solution was pipette out from the above solution using a micropipette and poured into each of the two sterile petri plates. Micropipette tips were discarded in a beaker containing disinfectant in use. Hands were wiped with 70% IPA immediately. 15-20 ml of sterile molten sabouraud dextrose agar (40-45^0c) was poured into the two plates for the total aerobic fungal count. Plates were swirling slowly to give them uniform dispersion of sample by taking care that media did not touch the plate lid. Negative control was prepared by pouring 15-20 ml of each used media in a separate empty sterile petri plate and allowing it to solidify at the end of the analysis. Sabouraud dextrose agar plates were inverted and incubated at 20-25^0 c for 120 hours.[24]

Determination of specified microorganisms:

Test for Escherichia coli:

100 ml of Soyabean Casein Digest Broth was prepared and autoclaved for 15-20 minutes at 121^0 c and 15 psi pressure. 10 gm of air-dried vitamin C powder were dissolved and suspended in a sterile 90 ml Soyabean casein digest medium with 4% polysorbate 80 (Tween80). (Solution

A). 100 ml MacConkey's broth was prepared and autoclaved for 15-20 minutes at 121^0 c and 15 psi pressure and then add 1 ml of solution A was added. The above solution was incubated at 43-45^0 c for about 24 hours. Then 100 ml of MacConkey's agar was prepared and sterilized by autoclaving. After sterilization, the agar solution was cooled and poured into the sterile petri dish. Wait until it solidifies. Incubated broth solution was removed and inoculated into solidified agar using an inoculation loop under the laminar airflow. These agar plates were incubated at 37^0 c for about five days. Growth of E- coli was compared with the standard. [24]

Test for pseudomonas aeruginosa:

100 ml of Soyabean Casein Digest Broth was prepared and autoclaved for 15-20 minutes at 121^0 c and 15 psi pressure. 10 gm of air-dried vitamin C powder were dissolved and suspended in a sterile 90 ml Soyabean casein digest medium with 4% polysorbate 80 (Tween80). (Solution A). The above solution was incubated at 43-45^0c for about 24 hours. 100 ml Cetrimide agar was then prepared and sterilized by autoclaving. Wait until it solidifies. Incubated broth solution was removed and inoculated into solidified agar using an inoculation loop under the laminar airflow. These agar plates were incubated at 37^0c for about five days. The growth of Pseudomonas aeruginosa was compared with the standard.[24]

Test for Salmonella species:

100 ml of Soyabean Casein Digest Broth was prepared and autoclaved for 15-20 minutes at 121^0 c and 15 psi pressure. 10 gm of air-dried vitamin C powder were dissolved and suspended in a sterile 90 ml Soyabean casein digest medium with 4% polysorbate 80 (Tween80). (Solution A). The above solution was incubated at 43-45^0c for about 24 hours. 100 ml Rappaport Vassiliadis Salmonella Enrichment Broth was prepared, and 1 ml solution A was added. Again,

the above solution was incubated at 30-35^0 c for 24 hrs. 100 ml of Bismuth sulfate agar was prepared and sterilized by autoclaving. Incubated broth solution was removed and inoculated into solidified agar using an inoculation loop under the laminar airflow. These agar plates were incubated at 37^0 c for about five days. The growth of salmonella species was compared with the standard.[24]

Test for shigella boydii:

100 ml of Soyabean Casein Digest Broth was prepared and autoclaved for 15-20 minutes at 121^0 c and 15 psi pressure. 10 gm of air-dried vitamin C powder were dissolved and suspended in a sterile 90 ml Soyabean casein digest medium with 4% polysorbate 80 (Tween80). (Solution A). The above solution was incubated at 43-45^0 c for about 24 hours. 100 ml of GN broth was prepared, 1 ml of solution A was added, and it was incubated at 30-35^{0}c for 24 hours. 100 ml of Xylose Lysine Deoxycholate agar was then prepared and sterilized by autoclaving. Incubated broth solution was removed and inoculated into solidified agar using an inoculation loop under the laminar airflow. These agar plates were incubated at 37^0 c for about five days. The growth of shigella boydii was compared with the standard.[24]

5. **Estimation of Nutritional Value:** We tested vitamin C powder's nutritional value from the TUV lab in Pune. All **(Table 3)**
6. **Cell viability assay/ MTT assay:**

Human embryonic kidney (HEK293) cells were obtained from the National Centre for Cell Science (Pune). HEK293 cells were cultured in DMEM. The culture media were supplemented with 10% Fetal Bovine Serum, grown in a humidified incubator with 5% CO_2.

Cell viability was assessed by MTT assay. Briefly, HEK293 cells (1×10^4) were seeded into 96-well microplates (flat-bottom), treated with vitamin C powder at 25-1100 μg/ml concentrations, and incubated for 24 h. After 24 h. MTT (0.5 mg/ml) solution was added to each well and incubated for 24 hr. at 37^oC. MTT solution was carefully aspirated and isopropanol was added to dissolve formazan crystals, and the optical density of formazan solutions was recorded at 570 nm using an automated microplate reader (EPOCH2; Bio Tek Instruments, Highland Park, VT, USA). All experiments were done in biological triplicates.

Result and discussion:

Result:

Table 1: Table: Organoleptic Properties of Raw Material

Parameters	Acerola Extract	Rosehip Extract	Moringa Extract	Amla Powder	Bilberry Extract
Colour	Light Red	Light Brown	Brown	Light Brown	Brown
Odour	Characteristics	Characteristics	Characteristics	Characteristics	Characteristics
Taste	Characteristics	Characteristics	Characteristics	Slightly bitter and sour	Characteristics

Table 2: Powder Characterization of Raw Material

Parameters	Acerola Extract	Rosehip Extract	Moringa Extract	Amla Powder	Bilberry Extract
Bulk Density	0.62	0.64	0.63	0.64	0.62
Tapped Density	0.70	0.71	0.70	0.72	0.70
Hausner Ratio	1.12	1.11	1.11	1.12	1.12
Carr Index	11.50	9.90	9.90	11.50	11.50
Angle of repose	40.06	37.23	39.35	40.03	39

Table 3: Solid-State Stability Study Parameters

Sr. No.	Test Parameters	Observations
1.	Caking	Absent
2.	Liquefaction	Absent
3.	Discoloration	Absent
4.	Odor	Absent
5.	Gel formation	Absent

Table 4: Powder characterization of Vitamin C powder formulation

Sr. No.	Test parameter	Result
1.	Color	Light Brown
2.	Odor	Characteristics
3.	Taste	Bitter

Table 5: Powder characterization of Vitamin C powder formulation

Sr. No.	Test parameter	Result
1.	Bulk density(gm/ml)	0.63 ± 0.02
2.	Tapped density(gm/ml)	0.70± 0.02
3.	Hausner Ratio	1.11 ± 0.01
4.	Carr Index	10.86 ± 0.02
5.	Angle of repose	39.39^{0} ± 0.20

Table 6: Physicochemical characteristics of Vitamin C powder formulation

Sr. No.	Test parameter	Result
1	Loss on Drying	8 %
2	Ph	3.87
3	Total Ash	5.73
4	Acid Insoluble Ash	0.43 %
5	Water soluble extractive value	67 %
6	Alcohol soluble extractive value	25 %
7	Determination of Phytochemical constituents	
	a) Tannin	Present
	b) Flavonoid	Present
	c) Phenol	Present
	d) Carbohydrates	Present
8	Essay	
	a) Determination of Total Tannin	21.24 %

Table 7: Microbiological parameters of Vitamin C powder formulation

Sr. No.	Test parameter	Result
1	Total Aerobic Bacterial Count	12 cfu /gm
2	Total Aerobic Fungal Count	7 cfu /gm
3	Specific Pathogens	
	a) Escherichia Coli	Absent
	b) Staphylococcus Aureus	Absent
	c) Salmonella Species	Absent
	d) Pseudomonas Aeruginosa	Absent

Figure 1: Total aerobic bacterial count in SCDA medium

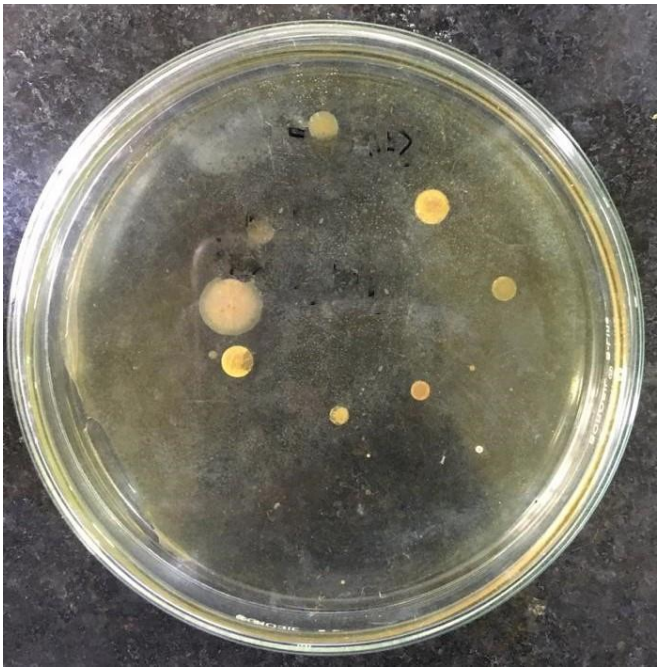

Figure 2: Total aerobic fungal count in SDA medium

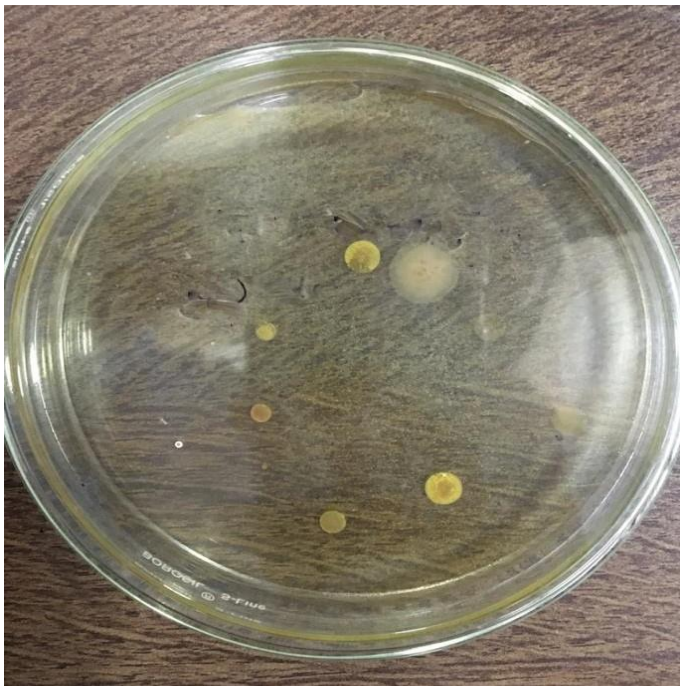

Figure 3: Specific Pathogen test of Escherichia coli in MacConkey agar

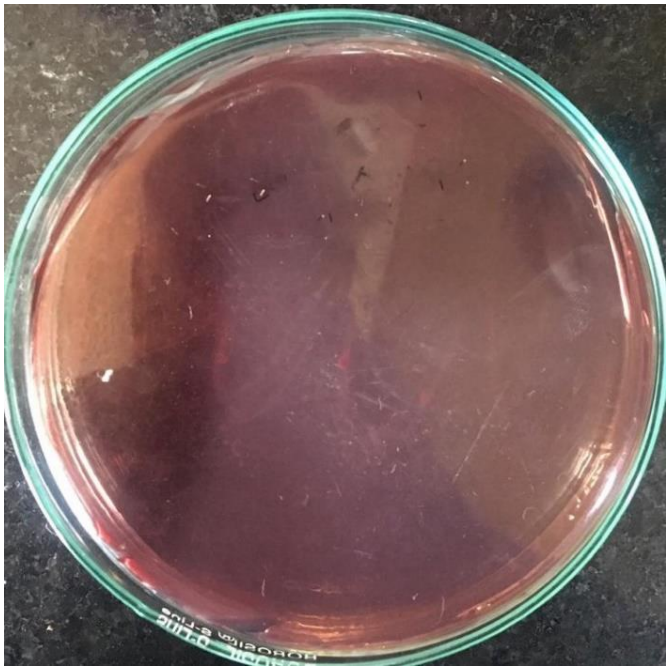

Figure 4: Specific Pathogen test of pseudomonas aeruginosa in cetrimide agar

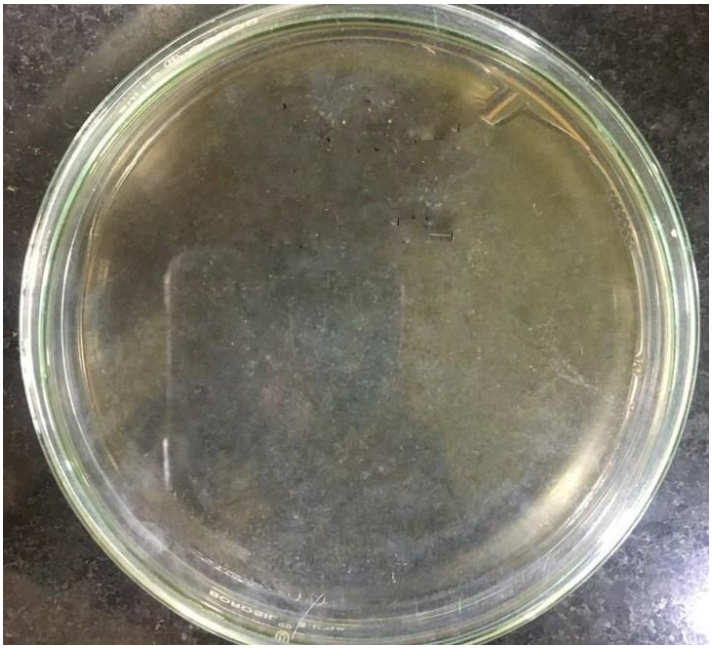

Figure 5: Specific Pathogen test of salmonella species in Bismuth sulphate agar

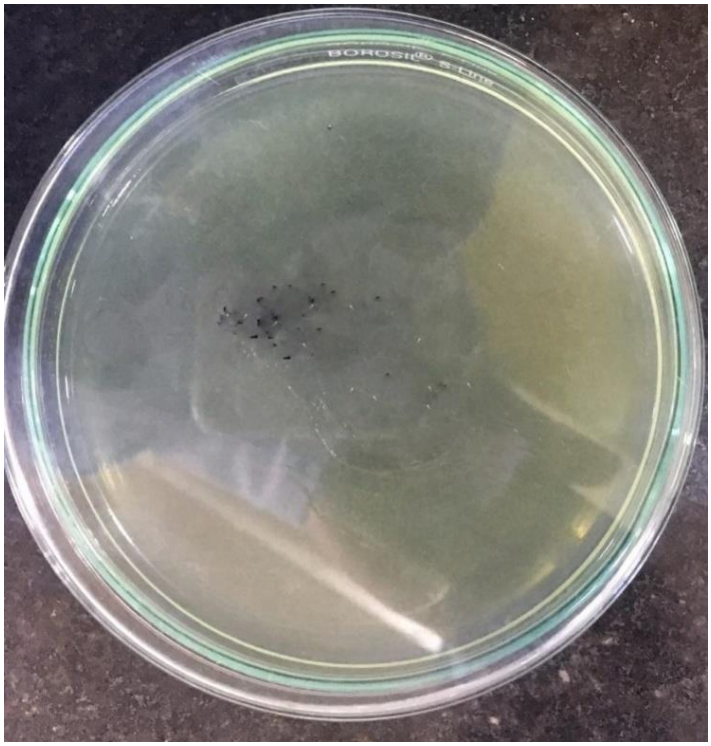

Figure 6: Specific Pathogen test of shigella boydii in Xylose Lysine Deoxycholate agar

Table 8: Estimation of Nutritional Value of Vitamin C powder formulation: -

Nutritional Facts			
NUTRIENTS	**APPROX PER**		**% RDA***
	100 g	**6g**	
Energy (Kcal)	383	22.98	1.03 %#
Total Protein (g)	7.15	0.43	0.94 %
Carbohydrate (g)	79.27	4.76	**
Total Sugar (g)	16.85	1.01	**
Added Sugar (g)	0.00	0.00	**
Dietary Fibre (g)	17.59	1.06	**
Total Fat (g)	0.27	0.02	0.06%#
Cholesterol (mg)	0.00	0.00	**
Vitamin C (mg)	1050.64	63.04	96.98 %*
Potassium (mg)	693.22	41.59	1.19 %*
Sodium (mg)	1270.14	76.21	3.81 %*
Zinc (mg)	0.67	0.04	0.40 %#

* %RDA values established as per ICMR Guidelines-2020.

** %RDA values not established.

%RDA values established as per ICMR Guidelines-2010

Cell viability assay/ MTT assay:

The cell viability of the vitamin C was determined by MTT assay (Fig 7). The IC50 (Inhibitory concentration) value for the MTT assay is 850 mcg/ml, meaning that 50% of cells die at this concentration. It demonstrates that we can give the maximum daily dose of 850 mcg/ml.

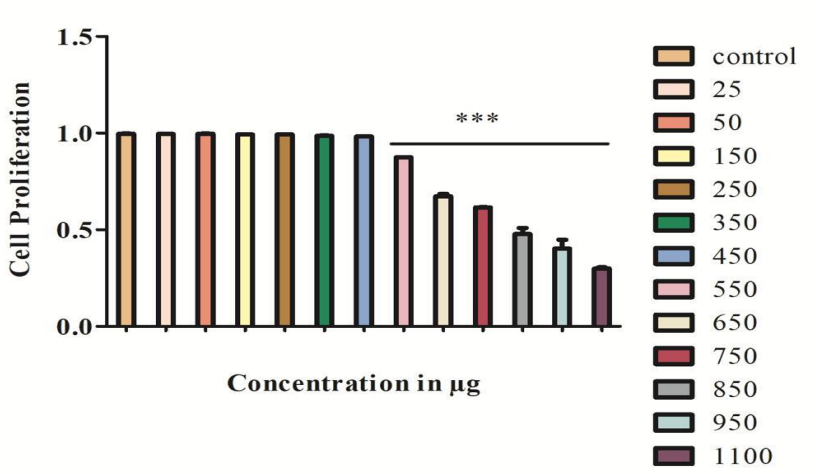

Fig 7. Cell viability for HEK293.

Discussion:

The physical characteristics of the powder were evaluated. The color of the powder was light brown with characteristics of odor and bitter taste. The powder characterization of powder is illustrated in (Table 1). The mean values of bulk density, tapped density, Hausner's ratio, Carr Index, compressibility index, and angle of repose were 0.633 ± 0.02, 0.70± 0.02, 1.11 ± 0.01, 10.86 ± 0.02, 10.25 ± 0.22 and 39.39^{θ} ± 0. 20 respectively. The mean weight loss percentage on drying of vitamin C powder is 8 %. (Table 2) The pH of vitamin C powder was found to be 3.87. A high ash value indicates contamination, substitution, adulteration, or carelessness in preparing the drug or drug combinations for marketing. The total ash value of vitamin C powder was found to be 5.73% w/w. The acid-insoluble ash value of vitamin C powder was 0.43% ± 0.01. The water-soluble and alcohol-soluble extractive values of vitamin C powder were 67% and 25%, respectively. (Table 2) Phytochemical constituents' tannins, flavonoids, phenols, and carbohydrates were present on qualitative estimation tests. The overall tannin percentage was found to be 21.24%. The total aerobic bacterial and fungi count below the permitted range, while specific pathogens were reported to be absent during the study.

The percentage of the recommended daily allowance (%RDA) for nutritional parameters such as energy (Kcal), total protein (g), carbohydrate (g), added sugar (g), dietary fiber (g), total fat (g), cholesterol (mg), potassium (mg), sodium (mg) and zinc (mg) in vitamin C powder formulations is not exceeded, according to ICMR guidelines. Also, it fulfills the daily Vitamin C (Table 3) requirement of adults.

The in vitro cellular cytotoxicity test of vitamin C powder showed no significant cytotoxicity against the HEK293cell line.

Conclusion:

In the present study, a stable vitamin C powder formulation was prepared and evaluated physiochemically and microbiologically. Also, the MTT assay proved that it does not indicate cytotoxicity at the daily dose limit and fulfills adults' daily Vitamin C requirement.

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Analytical Method Development And Validation Of Econazole Nitrate by using RP-HPLC

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Abstract

A simple and accurate method was developed for the determination and validation of the Econazole Nitrate. HPLC system used was JASCO system equipped with model PU 4180 RHPLC pump, Rheodyne sample injection port (20 µl), JASCO UV-4075 UV-VIS detector and ChromNAV CFR chromatography software (version 2.0). Separation was carried out on HiQSil C18 (250 mm × 4.6 mm, 5 µm) column using Acetonitrile:Methanol (85:15v/v) as mobile phase at flow rate of 1.2 mL/min. Samples were injected using Rheodyne injector with 20 µL loop, Detection was carried out at 225nm.

The HPLC linear regression analysis results for calibration plots demonstrated a good relationship with (R^2 = 0.9994). The method has been validated for its accuracy, Recovery, Robustness and documented. The LOD and LOQ were found to be 1.12 & 2.59 µg/m/ respectively.

Keywords- Econazole nitrate, Method Development, Validation,

Introduction

Econazole Nitrate is an imidazole derivative and broad-spectrum antimycotic agent with fungistatic properties. Econazole nitrate inhibits biosynthesis of ergosterol, thereby damaging the fungal cell wall membrane and altering its permeability which leads to a loss of essential intracellular components. Additionally, Econazole Nitrate inhibits the biosynthesis of

triglycerides and phospholipids as well as oxidative and peroxidative enzyme activity, which may aid in cell necrosis and death. It is also active against some gram-positive bacteria. The treatment of different dermatomycoses uses this antifungal agent.

Econazole nitrate is an antifungal drug containing imidazole ring which interacts with 14 demethylase a cytochrome P-40 enzyme which converts to lanosterol to ergosterol. Econazole inhibits the ergosterol synthesis which is the essential component of fungal cell membrane, as a result of increased cellular permeability, fungal cells die because cellular components leak out of the cells. Econazole Nitrate is incompletely absorbed after being administered orally due to it's low solubility. Additionally, it can be used topically to treat skin infections like tinea and cutaneous candidiasis.

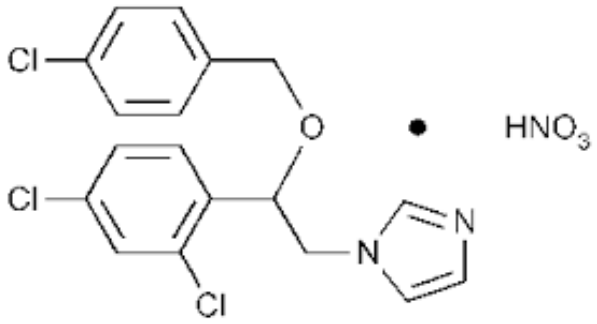

Figure. Structure of Econazole Nitrate

Analytical method development

Determination of Lambda maximum

Preparation of stock solution of Econazole nitrate

Econazole nitrate (100 mg) in a 100mL volumetric flask and 100 mL of methanol to it and it was vortexed (Eltek) for 2 minutes. This was the main stock accounting for concentrations of 1000 μg/mL. A diluted solution was used to scan in UV-Spectrophotometer in the range of 200-400nm, taking methanol as blank.

The lambda maximum for Econazole Nitrate was found to be 225nm.

Instrumentation and Chromatographic Conditions

HPLC system used was JASCO system equipped with model PU 4180 RHPLC pump, Rheodyne sample injection port (20 µl), JASCO UV-4075 UV-VIS detector and ChromNAV CFR chromatography software (version 2.0). Separation was carried out on HiQSil C18 (250 mm × 4.6 mm, 5 µm) column using Acetonitrile: Methanol (85:15v/v) as mobile phase at flow rate of 1.2 mL/min. Samples were injected using Rheodyne injector with 20 µL loop, Detection was carried out at 225nm. All weighing were done on Shimadzu balance (Model AY-120)

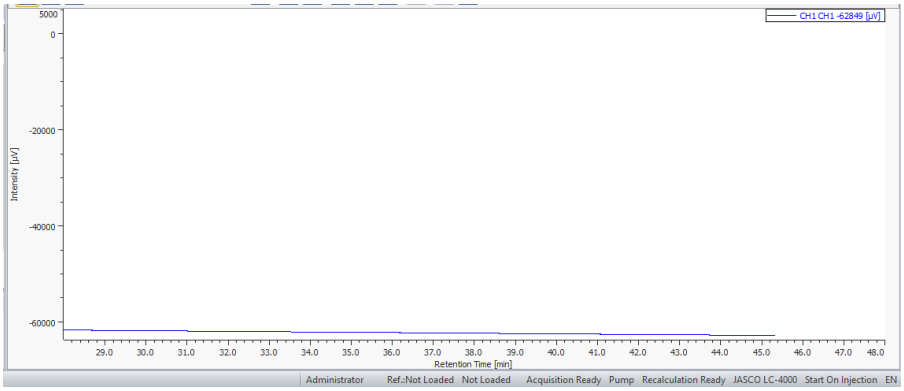

Figure 1: HPLC chromatogram of blank.

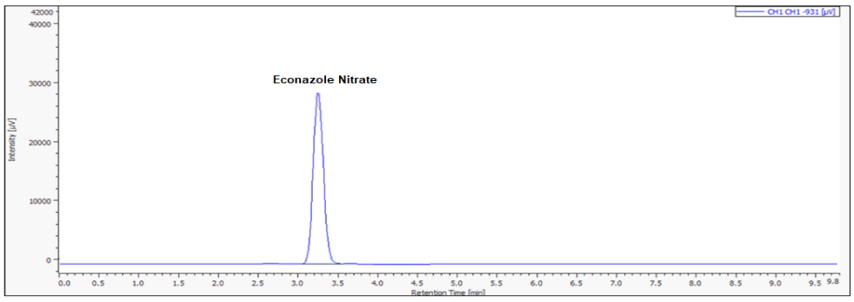

Figure 2: HPLC chromatogram of standard Econazole nitrate.

The retention time was found to be 3.32 with distinct peak.

MATERIALS AND METHODS

Material

Econazole nitrate standard is procured from Solanki Suppliers (Pune, India). Chemicals utilized for method development are of HPLC grade includes Methanol, water were purchased from Merck (India) Ltd.

Preparation of mobile phase

The preparation of mobile phase was done by mixing methanol with ACN in the ratio of 85:15 v/v. Filtered the solution through 0.45µ filter.

Diluent preparation

Mobile phase used as diluents.

Preparation of standard stock solution

100mg of Econazole nitrate standard was transferred into 100ml volumetric flask, dissolved & make up to volume with mobile phase. Further dilution was done by transferring 1 ml of the above solution into a 10mlvolumetric flask and make up to volume with mobile phase and performed the subsequent dilutions.

Preparation of test solution

100mg equivalent of Econazole nitrate API standard was transferred into 100ml volumetric flask, dissolved & make up to volume with mobile phase. Further dilution was done by transferring 1 ml of the above solution into a 10ml volumetric flask and make up to volume with mobile phase and performed the subsequent dilutions.

Selection of analytical wavelength

It is the characteristic of a compound which helps to provide the electronic structure of the compound or analyte. The structural analysis of Econazole nitrate was carried out under UV ranging from 200-400nm using the standard solution.

Method Validation

Linearity:

The linearity of the developed method was studied over the concentration ranges between 10-30µg/ml. The aliquots of 5, 10, 15, 20, 25 and 30µg/ml were prepared by diluting standard stock solution of 0.1, 0.2, 0.3, 0.4, 0.5 and 0.6 ml with mobile phase. The obtained concentrations were injected into the chromatographic system. Calibration curve of

Econazole nitrate was constructed by plotting peak area versus used concentration of Econazole nitrate. To assure the concentration range studied is linear the regression equation and correlation coefficient were evaluated.

Accuracy

Accuracy was carried out by % recovery studies at three different concentration levels. To the pre-analysed sample solution of Econazole nitrate, a known amount of standard drug powder of Econazole nitrate was added to 80, 100, 120% level.

Precision method

By studying the changes in the inter-day and intra-day determined the precision of the method. In the intra-day studies, six repeated injections of standard solution was made and % RSD were calculated. In the inter-day variation studies, six repeated injections of standard solution were made for six consecutive days and %RSD were calculated.

Limit of Detection and Limit of Quantitation

Based on the standard deviation of response of the calibration curve the LOD and LOQ of the drug was determined separately.

Robustness

Robustness of the method was tested by small but deliberate variations of flow rate, mobile phase composition and wavelength.

RESULTS AND DISCUSSION

Selection of wavelength maxima

The solution of Econazole nitrate was scanned between ranges 200- 400nm. UV spectra of the drug show maximum absorbance at 225nm.

Method development

The proposed chromatographic method was found to be suitable for effective separation of Econazole nitrate with good resolution, peak shape given in the figure. The mobile phase composed of Acetonitrile: Methanol in ratio of 85:15 % v/v, at a flow rate of 1.2 ml/min was selected as it gave well resolved peaks of standard Econazole nitrate. The optimum wavelength 225nm selected for detection and quantitation.

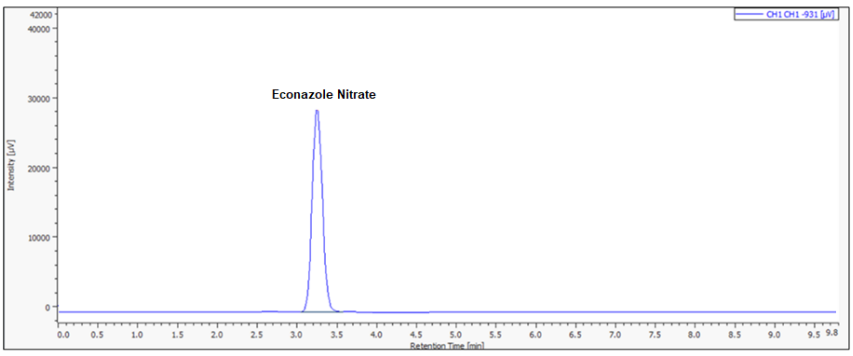

Figure 3: HPLC Chromatogram with resolved peak of Econazole nitrate

Method validation

Linearity

The calibration curves were found be linear for the concentration range of 5-30ppm. The standard working curve equation for drug was found to be y = 2768.4x + 670.53 with correlation coefficient value R^2 = 0.9994. The results of linearity are given in Table and Figure.

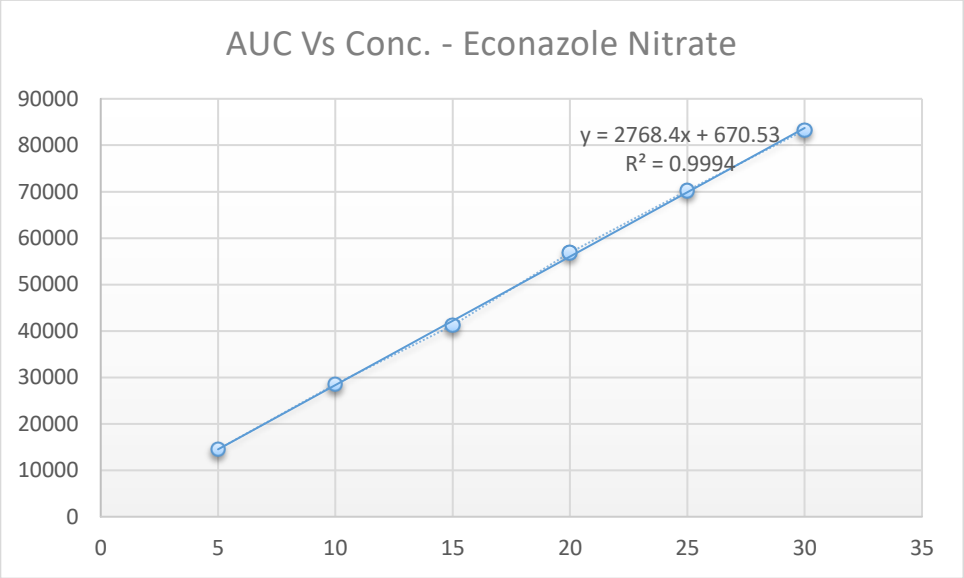

Figure 4: Linearity curve of standard Econazole nitrate

Table 1: Linearity data of Econazole nitrate

Concentration µg/mL	Area
5	14512
10	28567
15	41215
20	56897
25	70254
30	83256

Recovery studies

The mean % recovery at 80, 100, 120 % of the test concentration along with its statistical validation for drug Econazole nitrate given in Table. The % recovery at 80, 100, and 120 % was found to be 101.25, 99.8, and 101.25. It was confirmed that the developed method was accurate as the percent recovery was in the range of 100%.

Table 2: Recovery data of Econazole nitrate

Level (%)	Drug Conc (mg)	Amt recovered (mg)	% Recovery
80%	8	8.1	101.25
100%	10	9.98	99.8
120%	12	12.15	101.25

Precision

The repeatability of sample application and measurement of peak area were expressed in terms of % RSD and was found to be less than 2.0%. The % RSD of intra-day precision was found to be 0.009, 0.006 and 0.004 % RSD of interday precision was found to be 0.011, 0.009 and 0.004. The results of precision studies are shown in Table.

Table 3: Precision study (intra- day) of Econazole nitrate

Conc µg/mL	Area	AVG	%RSD
10	28745	28624.6667	0.48911778
	28658		
	28471		
15	42156	42370.3333	0.50863294
	42587		
	42368		
20	56874	56624.3333	0.94181701
	56987		
	56012		

Conc, Concentration; AVG, average; RSD, Relative standard deviation

Table 4: Precision study (inter-day) of Econazole nitrate

Conc µg/mL	**Area**	**AVG**	**%RSD**
10	28456	28608	0.50714043
	28623		
	28745		
15	41545	41711.6667	1.87815881
	41025		
	42565		
20	56897	56486	0.63400044
	56241		
	56320		

Conc, Concentration; AVG, average; RSD, Relative standard deviation

Limit of Detection (LOD) and Limit of Quantification (LOQ)

This data showed that the sensitivity of method to determine the drug Econazole nitrate. The Minimum concentration level at which the analyte can be reliable detected (LOD) &quantified (LOQ) were found to be 1.12 & 2.59 µg/m/ respectively.

Robustness

Robustness of method was measured by multiple injections of a homogenous sample containing Econazole nitrate by changing flow rate 1.0 mL/min and 1.4 mL/min, mobile phase composition ACN: Methanol ratio 84:16 and 86:14, wavelength i.e., 224nm and 226nm. The method was found to be robust in the range of deliberate changes made.

Table 5: Robustness study with change in flow rate of Econazole nitrate

Flow rate mL/min	Conc µg/mL	Area	AVG	%RSD
1.0	20	55421	55836.67	1.565403
1.0		55248		
1.0		56841		
1.4	20	55789	55411.67	0.590198
1.4		55210		
1.4		55236		

Conc, Concentration; AVG, average; RSD, Relative standard deviation

Table 6: Robustness study with change in concentration of mobile phase of Econazole nitrate

Mobile phase (Methanol: 01% OPA)	Conc µg/mL	Area	AVG	%RSD
84:16	20	56321	56542.67	0.545612
84:16		56895		
84:16		56412		
86:14	20	56321	56367	0.171372
86:14		56478		
86:14		56302		

Conc, Concentration; AVG, average; RSD, Relative standard deviation

Table 7: Robustness study with change in Wavelength of Econazole nitrate.

Wavelength nm	Conc µg/mL	Area	AVG	%RSD
224	20	56320	56334	0.251217

224		56482		
224		56200		
226	20	56874		
226		56231	56376	0.786936
226		56023		

CONCLUSION

A HPLC method developed has been validated as per ICH guidelines in terms of accuracy, precision, linearity, robustness, limit of detection and limit of quantitation, for the determination of Econazole Nitrate API. A good linear relationship was observed in concentration ranges of 5 and 30µg/ml. The correlation coefficient was 0.9994. The inter day and intraday precision results were good enough to say that the method developed is precise and reproducible. Accuracy studies revealed that mean recovery after spiking experiments was 100.7%, an indicative of accurate method. Accordingly, it can be concluded that the developed method is accurate, precise, linear, and robust.

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