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.

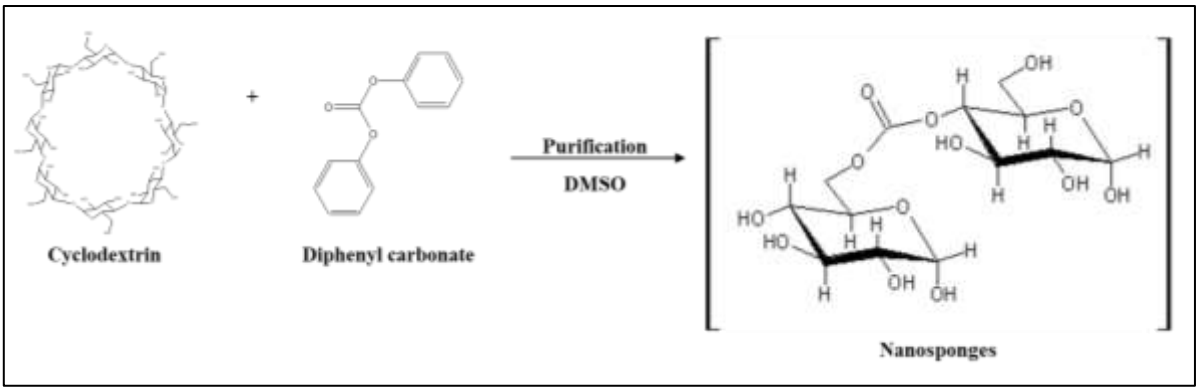

Figure 3.1: Nucleophilic reactions of beta-CD and Diphenyl Carbonate.

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.

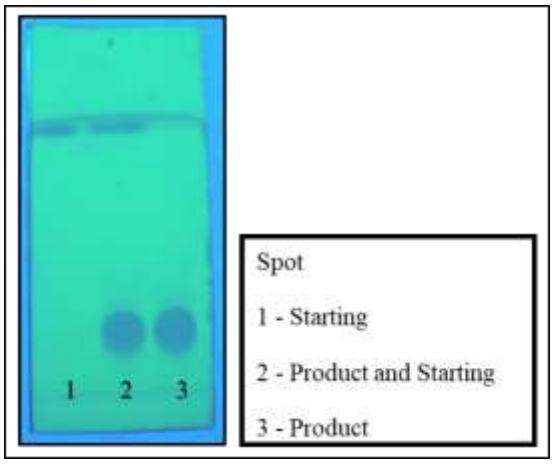

Figure 3.2: TLC Photograph

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

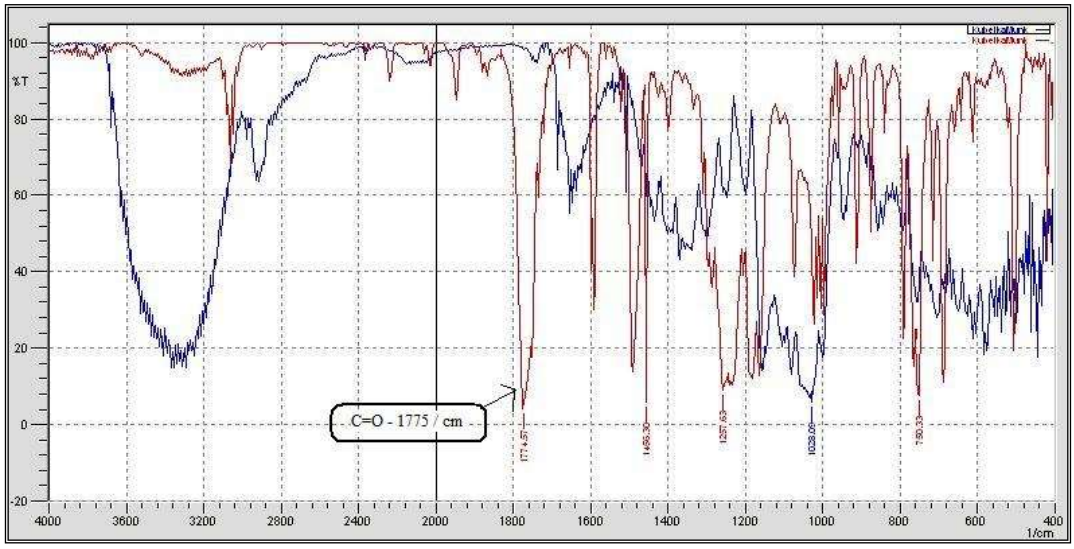

Figure 3.3: FTIR spectra of Nanosponges and Beta-CD

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.

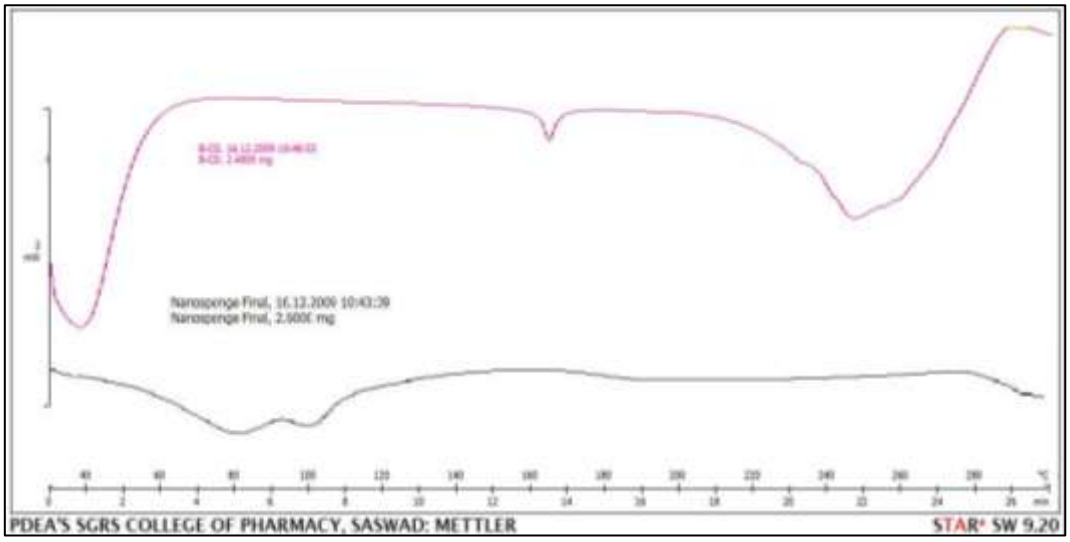

Figure 3.4: DSC graph of Nanosponges and Beta-CD.

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.

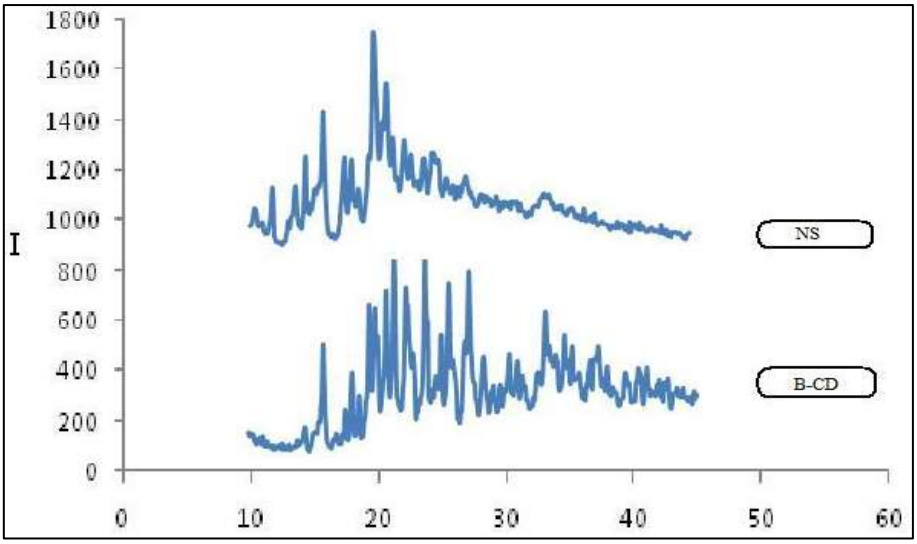

Figure 3.5: PXRD of Nanosponges and Beta-CD

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.

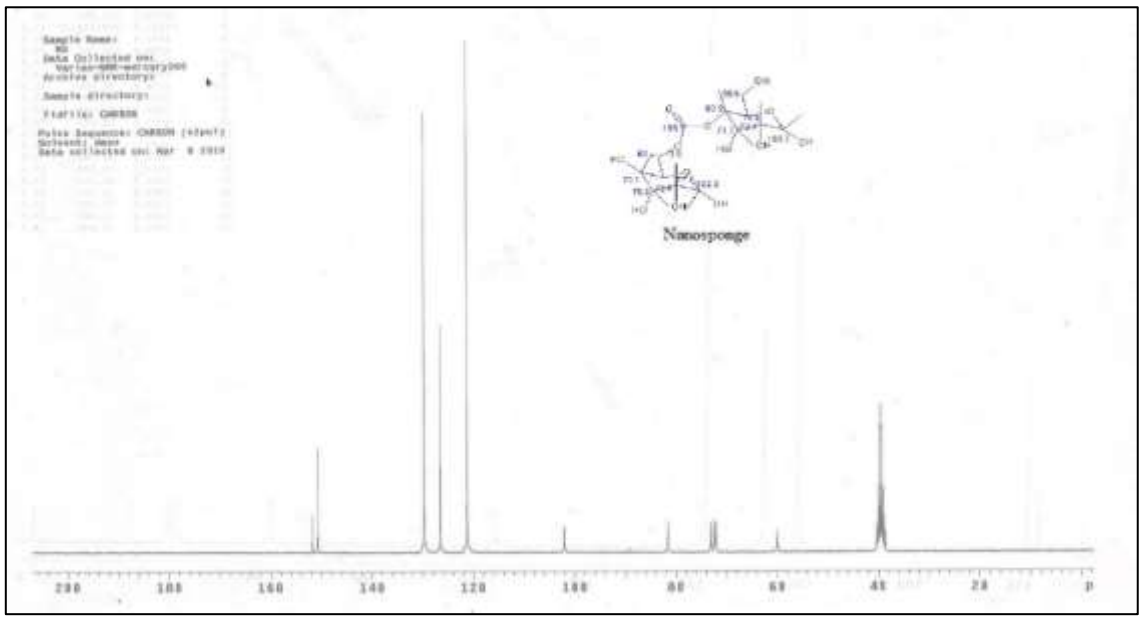

Figure 3.6: NMR of Nanosponges

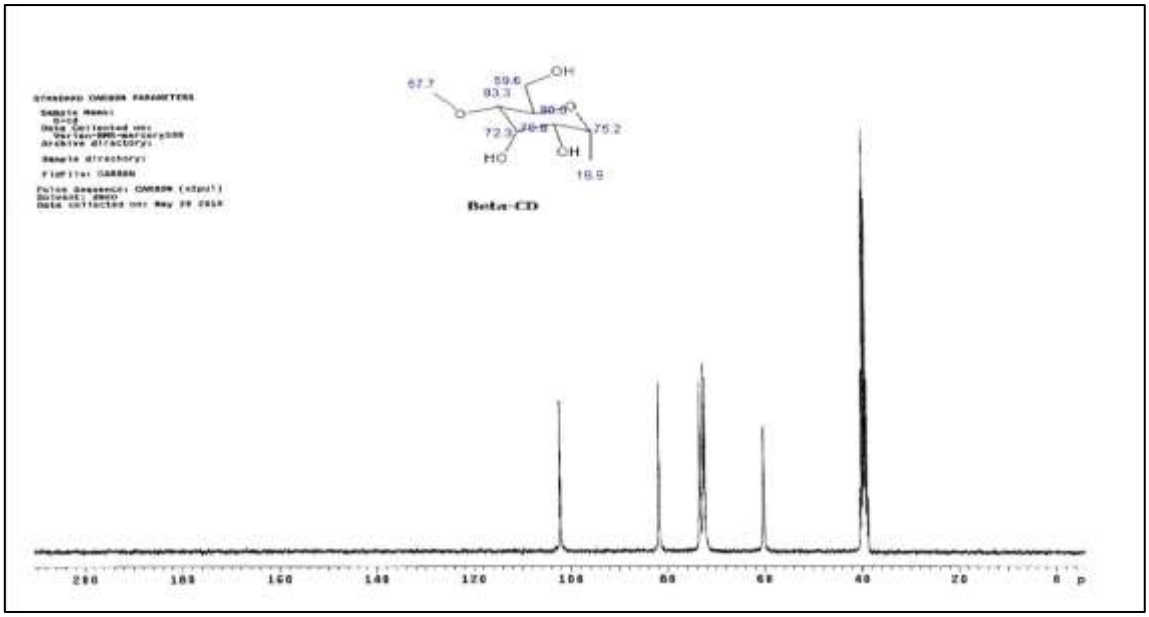

Figure 3.7: NMR of Beta-CD

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.

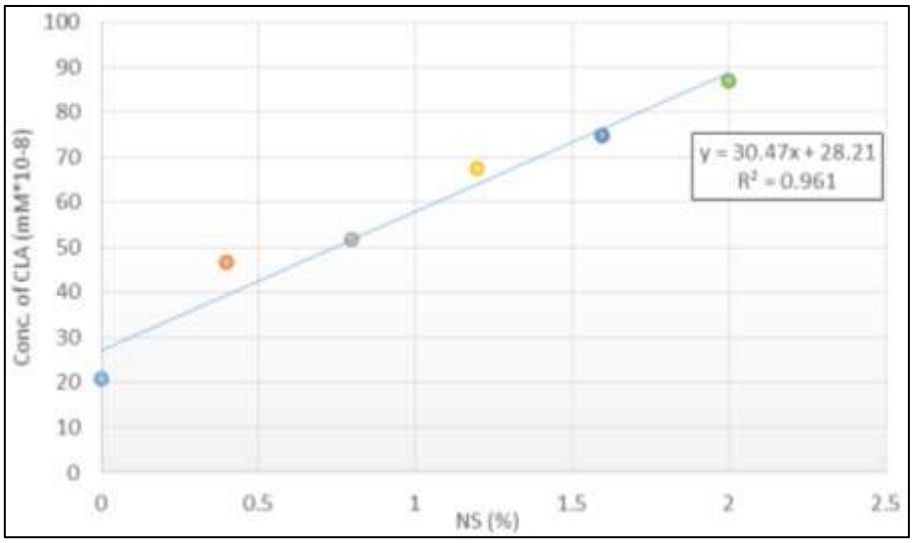

Figure 3.8: Phase solubility study of drug and Nanosponges in 0.1 N HCL

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.

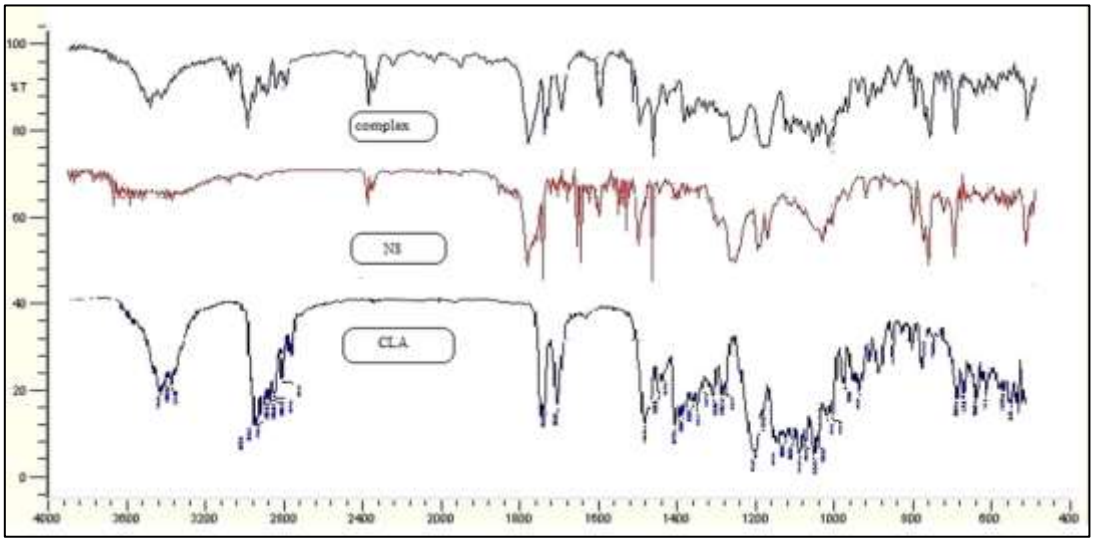

Figure 3.9: FTIR spectra of Nanosponges, Clarithromycin and complex

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.

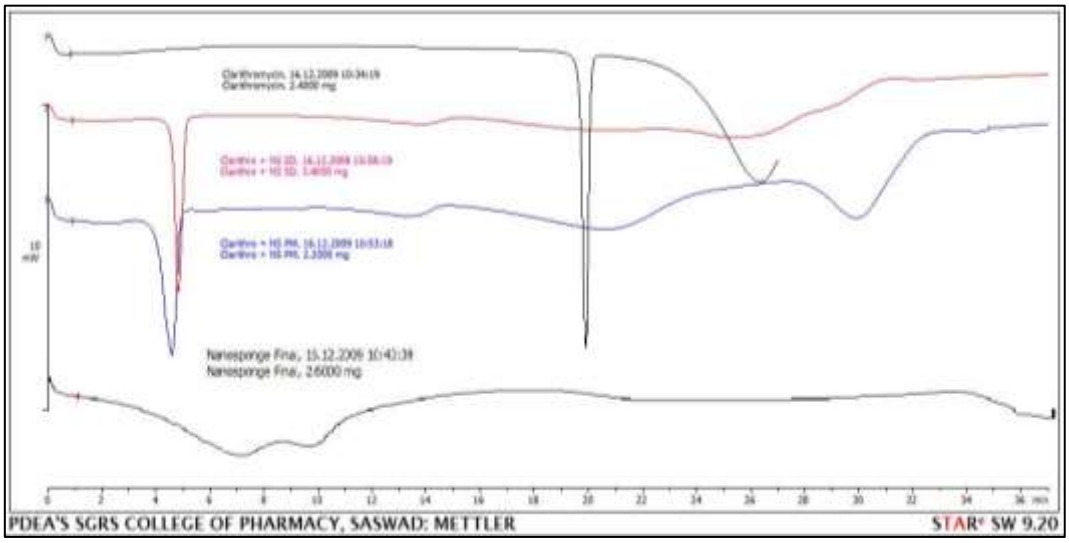

Figure 3.10: DSC graph of pure CLA, complex and Nanosponges

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.

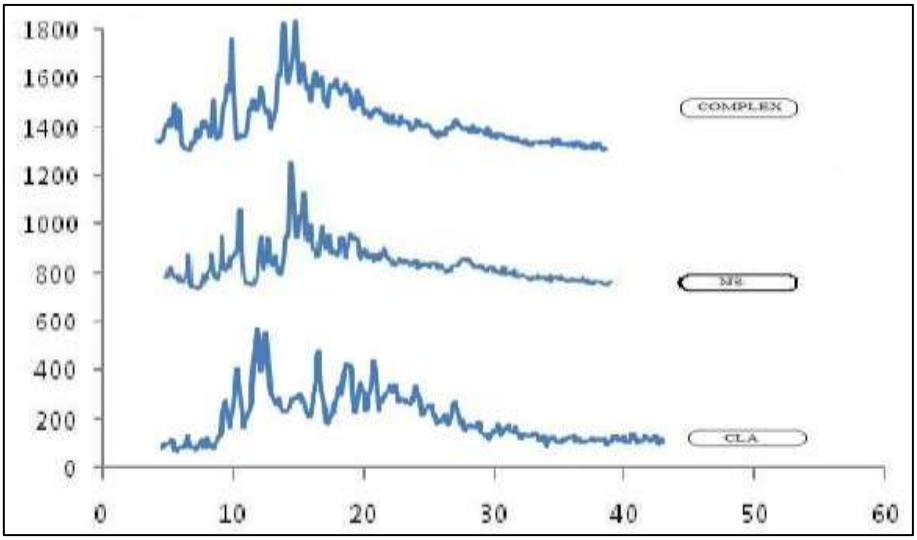

Figure 3.11: PXRD of CLA, NS and Complex

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.

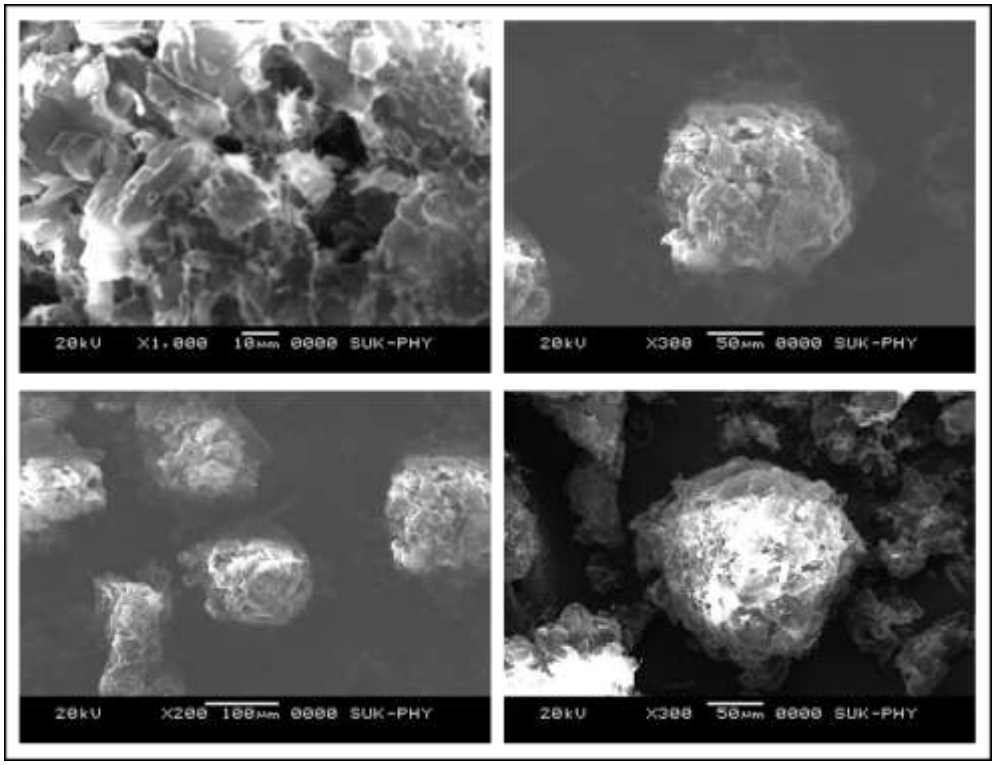

Figure 3.12: SEM Images of NS

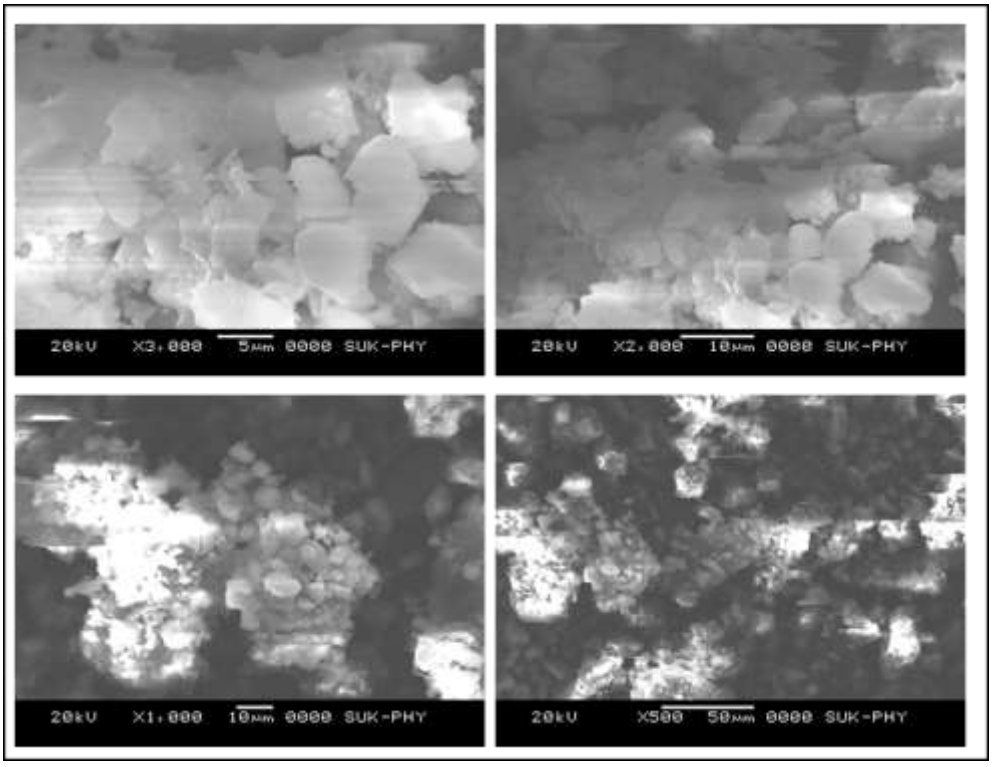

Figure 3.13: SEM Images of Complex

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 %			

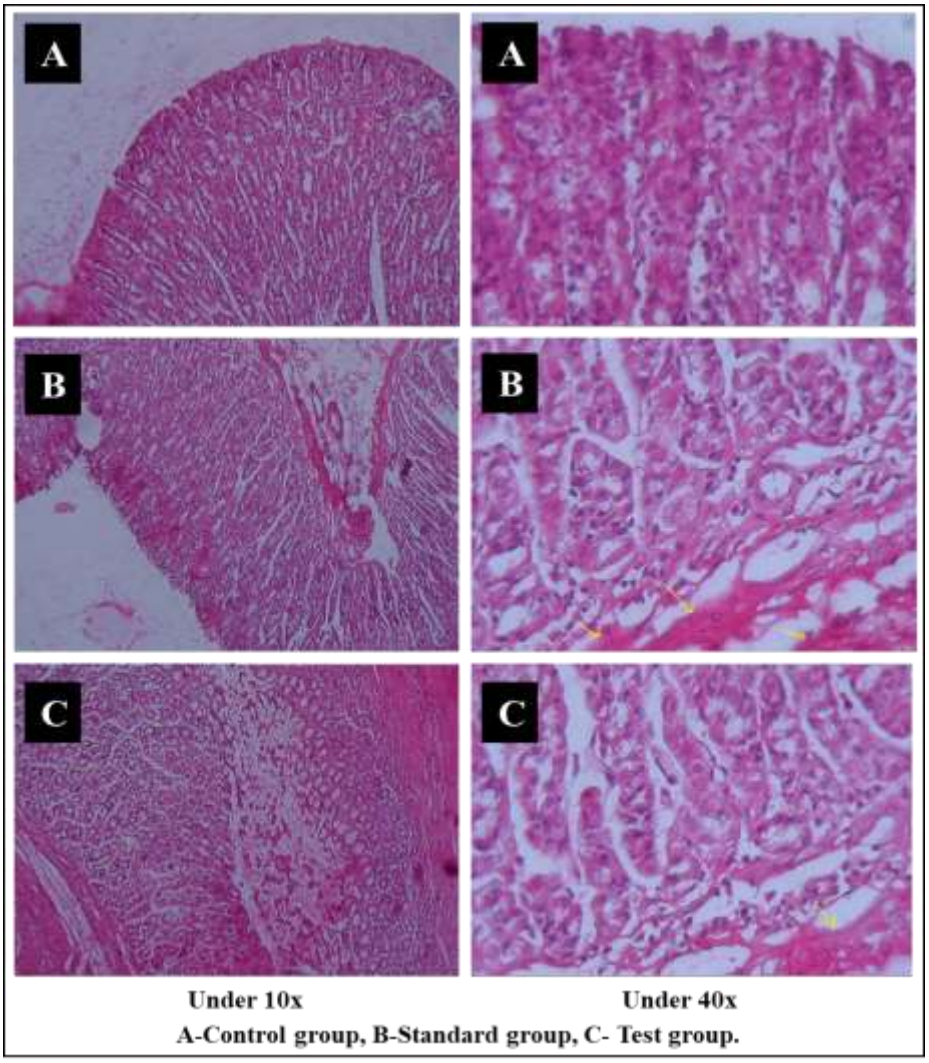

Figure 3.14: Photographs of stomach Tissue

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

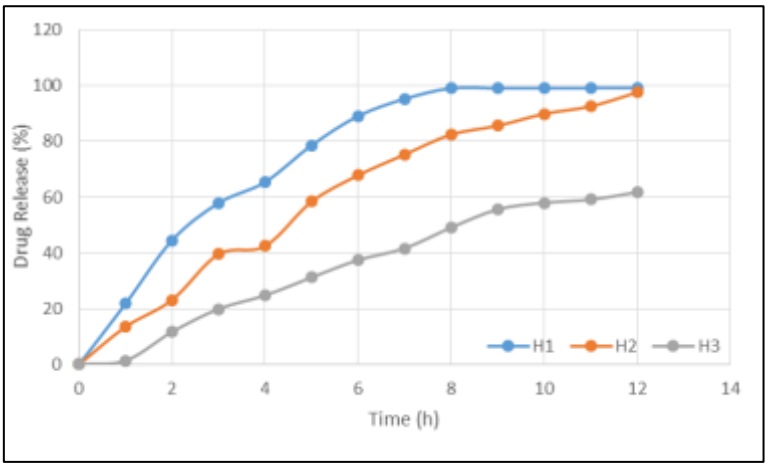

Figure 3.15: % Cumulative Drug release from preliminary Batches H1-H3 in 0.1N HCL

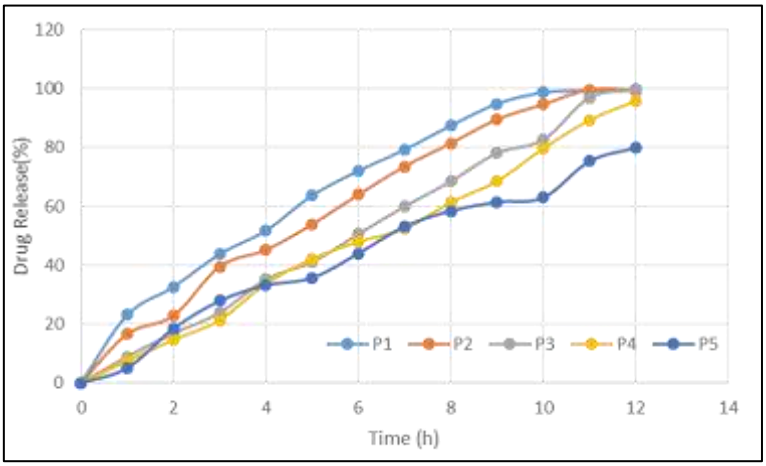

Figure 3.16: % Cumulative Drug release from preliminary Batches P1-P5 in 0.1N HCL

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).

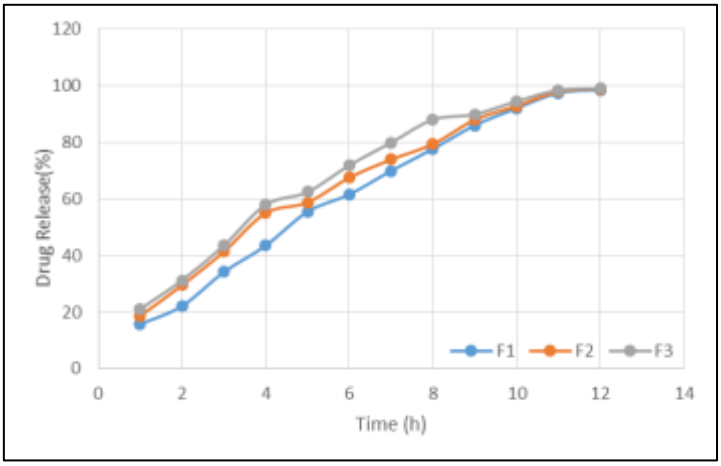

Figure 3.17: % Cumulative Drug release from factorial batches F1-F3

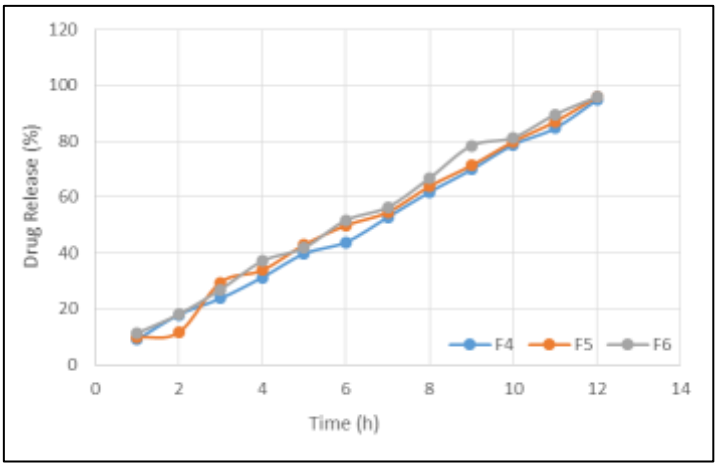

Figure 3.18: % Cumulative Drug release from factorial batches F4-F6

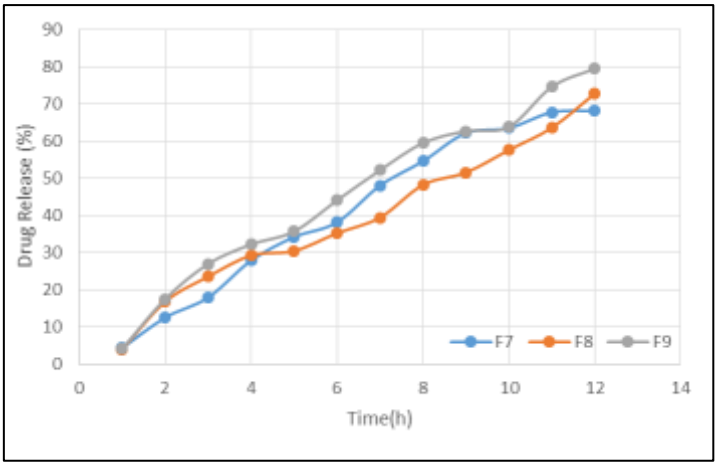

Figure 3.19: % Cumulative Drug release from factorial batches F7-F9

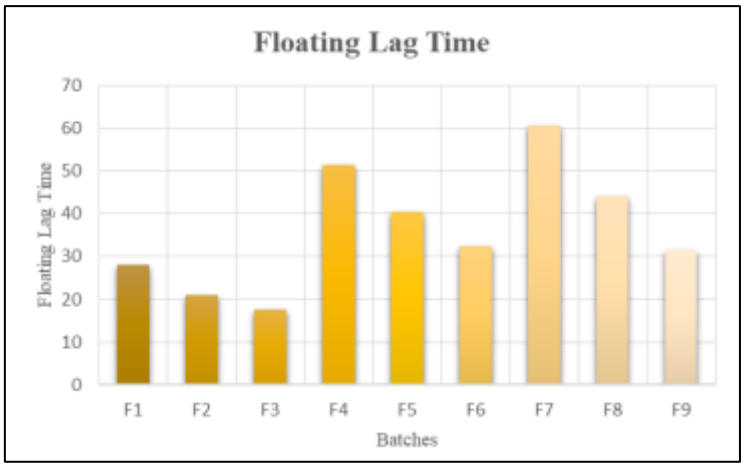

Figure 3.20: Floating Lag Time of Factorial Batches

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.

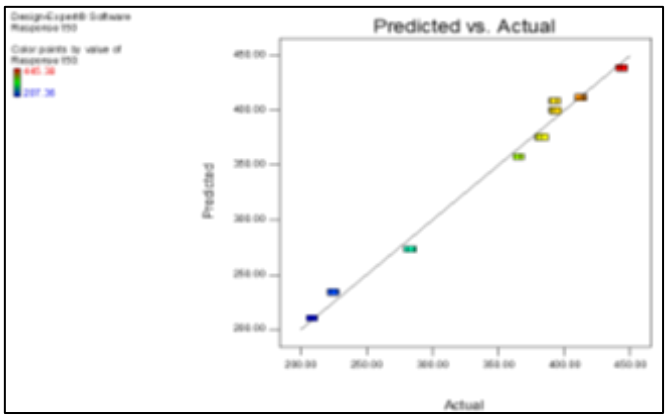

Figure 3.21: Correlation between actual and predicted values for $T_{50\%}$ (Y_1)

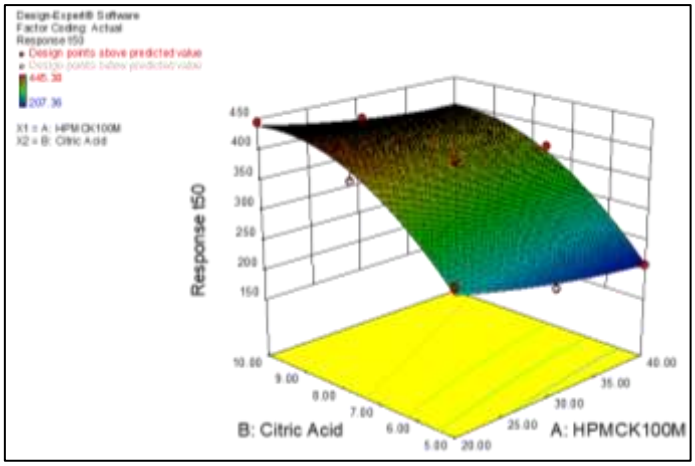

Figure 3.22: Response surface plot showing effect of formulation variables on $T_{50\%}$ (Y_1)

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.

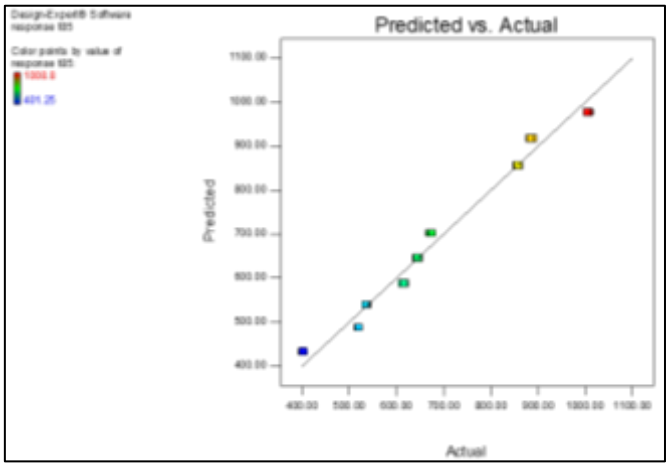

Figure 3.23: Correlation between actual and predicted values for $T_{85\%}$ (Y_2)

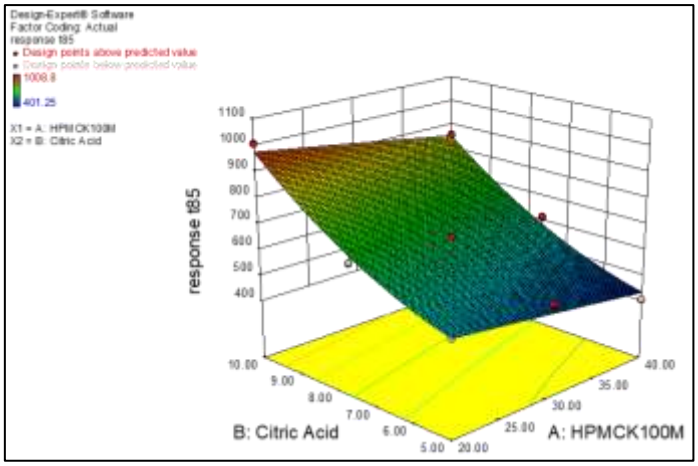

Figure 3.24: Response surface plot showing effect of formulation variables on $T_{85\%}$ (Y_2)

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.

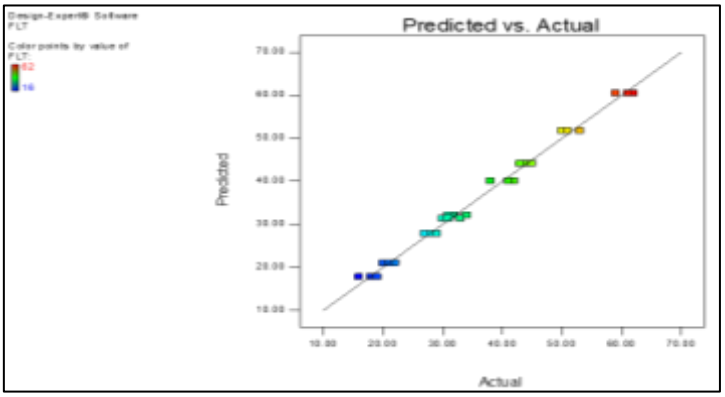

Figure 3.25: Correlation between actual and predicted values for FLT (Y_3)

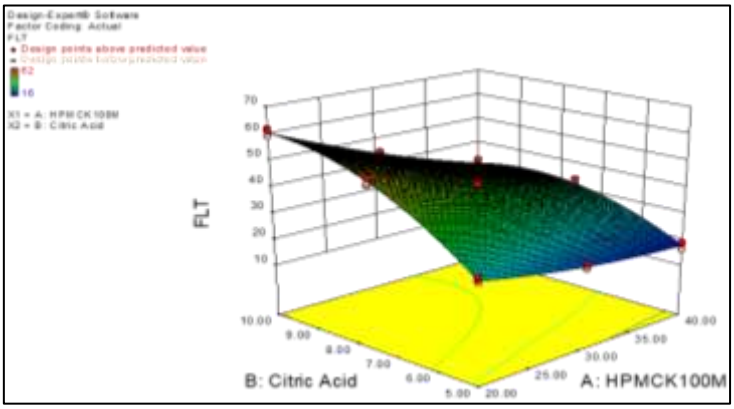

Figure 3.26: Response surface plot showing effect of formulation variables on FLT (Y_3)

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.

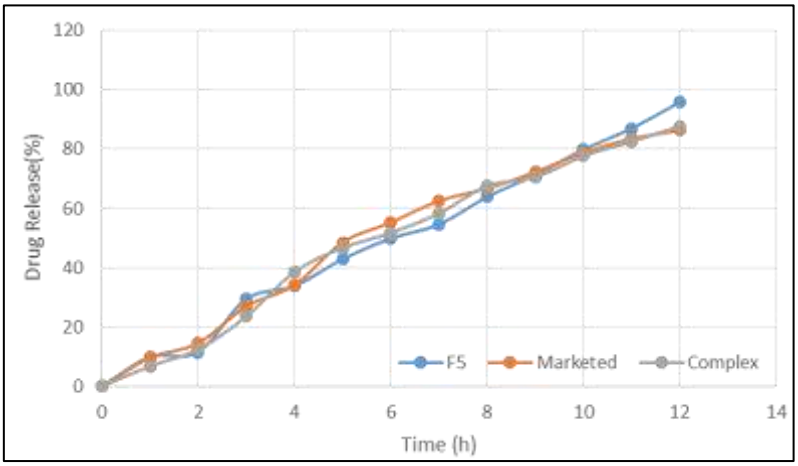

Figure 3.27: Dissolution Profile of marketed formulation with capsule fills with complex and formulated tablet in 0.1N HCL.

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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