Chapter 7

Minimatrices of Levofloxacin

7.1 INTRODUCTION

Levofloxacin is a synthetic broad-spectrum antibacterial agent for oral and intravenous administration. Chemically it is a chiral fluorinated carboxyquinolone, is the pure (-)-(S)-enantiomer of the racemic drug substance ofloxacin. Levofloxacin is explored as a newer alternative to the traditional antibiotics in anti H. pylori therapy. Its use along with other antibiotics is widely tested in anti H.pylori therapy (Bytzer and Morain, 2005; Gisbert et al., 2007). Due to its potential activity against H.pylori, delivering levofloxacin through stomach specific drug delivery would definitely improve its effectiveness. Hence aim of the present investigation was to develop levofloxacin minimatrices for sustained delivery of levofloxacin in stomach.

7.2 MATERIALS

Levofloxacin was received as a gift sample from Blue Cross Labs Ltd, (Nashik, India). Sodium alginate (Keltone HVCR) was a gift sample from Anshul agencies (Mumbai, India). Polyox WSR coagulant and HPMC K100M CR were gifted by Colorcon Asia Pvt. Ltd. (Goa, India). Microcrystalline cellulose (Avicel PH102) was obtained from Signet Chemical Corporation (Mumbai, India). Chitosan was provided by Central Inst of Fisheries (Kochi). Xanthan gum, magnesium stearate and PVP K30 were purchased from S.D. Fine Chem (Mumbai, India). Sodium bicarbonate, citric acid, Isopropyl alcohol and hydrochloric acid were purchased from Qualigens Fine Chemicals (Mumbai, India).

7.3 METHODS

7.3.1 Preliminary formulation trials

This exercise was undertaken for selection of suitable excipients and preparation technique. Blend of the drug and excipients can be compressed by simple mixing. This method of direct compression is convenient and cost effective as it involves minimum processing steps. But its implementation is possible only if micromeritic properties of drug and excipient prove suitable for direct compression. Otherwise an additional step of granulation is needed for converting the blend in compressible form. Potential excipients having significance for achieving gastroretention and sustained release feature were selected and mixed with levofloxacin either alone or in combination. Flow properties and compressibility of the blends were evaluated.

As direct compression was not possible, it was decided to proceed further with granulation method. PVP K30 solution (5%w/v) in IPA was used as granulating agent. Experiments L-1 to L-6 were conducted to ascertain role of individual excipients in the formulation (Table 7.1). Formulations prepared in the preliminary trials were evaluated for floating lag time, matrix integrity during drug release study and drug release pattern. These are key parameters for imparting gastroretentive and sustained release capabilities to the formulation. Components playing role in achieving minimum floating lag time and having capability to sustain drug release for extended period of time were identified.

Table 7.1 Composition of preliminary formulation trials

Ingredients (mg/minimatrix)	L-1	L-2	L-3	L-4	L-5	L-6
Levofloxacin hemihydrate*	12.81	12.81	12.81	12.81	12.81	12.81
Sodium bicarbonate	2.625	2.625	2.625	2.625	2.625	2.625
Citric acid	0.875	0.875	0.875	0.875	0.875	0.875
Xanthan gum	16.59	0	0	0	0	0
HPMC K100M CR	0	16.59	0	0	0	0
Polyox coagulant	0	0	16.59	0	0	0
Carbopol 974P	0	0	0	16.59	0	0
Sodium alginate	0	0	0	0	16.59	0
Chitosan	0	0	0	0	0	16.59
PVP K30**	1.75	1.75	1.75	1.75	1.75	1.75
Magnesium stearate	0.35	0.35	0.35	0.35	0.35	0.35
Total wt.	35	35	35	35	35	35

*Equivalent to 12.5 mg of levofloxacin, **Used as 5% w/v solution in IPA

7.3.2 Drug excipient compatibility study

Most appropriate excipients identified in preliminary trials were subjected for DSC studies to ensure their compatibility with active entity levofloxacin. For DSC studies, samples were sealed in an aluminum pan and scanned in temperature range 30° to 300°C at a heating rate of 10°C/min under constant purging of dry nitrogen at 30 ml/min. DSC thermograms of levofloxacin, individual excipients and drug excipient mixture were recorded on an automatic thermal analyzer system (DSC-60, Shimadzu, Japan). Temperature calibration was performed

using indium as the standard. Sealed empty aluminium pan was used as a reference.

7.3.3 Formulation designing by full factorial design

In the present research, 3^2 full factorial design was used as an optimisation tool. Total 9 formulations were designed as shown in Table 7.2. Sodium alginate (A) and HPMC K100M CR : polyox WSR coagulant in ratio 1:1 (B), each at three different levels, were selected as formulation variables. Levels of formulation variables were decided from results of the preliminary studies. Response variables were floating lag time (Y_1), drug release at 1 hour (Y_2), time required for 50% drug release, t50 (Y_3), time required for 95% drug release, t95 (Y_4) and swelling index (Y_5). All the response variables were fitted to quadratic model and regression analysis was carried out to get a quantitative relationship between formulation and response variables that can be given by equation

$$Y_i = b_0 + b_1A + b_2B + b_{11}A^2 + b_{22}B^2 + b_{12}AB \qquad (7.1)$$

where b_0 is arithmetic mean of 9 runs; b_i is an estimated coefficient for factors A and B. All experimental results were computed by statistical software DOE v6.0.5 (Stat-Ease Inc., Minneapolis, MN, USA). Response surface plots and prediction profiler was generated by JMP software v5.1 (SAS Institute Inc., Cary, NC, USA).

Table 7.2 Formulation designing by 3^2 full factorial design

Formulation	Pattern*	Formulation variables	
		A	B
LMT 01	00	0	0
LMT 02	0-	0	-1
LMT 03	++	1	1
LMT 04	+0	1	0
LMT 05	-0	-1	0
LMT 06	+-	1	-1
LMT 07	-+	-1	1
LMT 08	0+	0	1
LMT 09	--	-1	-1

Coded values	Actual values#	
	A	B
-1	7	10
0	10.5	15
1	14	20

* 0,- and + indicate low, medium and high level respectively;
\# Actual values indicate %w/w of final weight of minimatrix

Table 7.3 Composition of the full factorial design batches

Ingredients (per minimatrix)	LMT 01		LMT 02		LMT 03	
	mg	%	mg	%	mg	%
Levofloxacin hemihydrate*	12.81	36.60	12.81	36.60	12.81	36.60
Sodium bicarbonate	2.625	7.50	2.625	7.50	2.625	7.50
Citric acid	0.875	2.50	0.875	2.50	0.875	2.50
Chitosan	1.75	5.00	1.75	5.00	1.75	5.00
Xanthan gum	2.8	8.00	2.8	8.00	2.8	8.00
Sodium Alginate	3.675	10.50	3.675	10.50	4.9	14.00
HPMC K100M CR	2.625	7.50	1.75	5.00	3.5	10.00
Polyox WSR coagulant	2.625	7.50	1.75	5.00	3.5	10.00
Avicel PH102	3.115	8.90	4.865	13.90	0.14	0.40
PVP K-30**	1.75	5.00	1.75	5.00	1.75	5.00
Magnesium stearate	0.35	1.00	0.35	1.00	0.35	1.00
Total Wt	*35*	*100*	*35*	*100*	*35*	*100*

*Equivalent to 12.5 mg of levofloxacin, **Used as 5% w/v solution in IPA

Table 7.4 Composition of the full factorial design batches

Ingredients (per minimatrix)	LMT 04		LMT 05		LMT 06	
	mg	%	mg	%	mg	%
Levofloxacin hemihydrate*	12.81	36.60	12.81	36.60	12.81	36.60
Sodium bicarbonate	2.625	7.50	2.625	7.50	2.625	7.50
Citric acid	0.875	2.50	0.875	2.50	0.875	2.50
Chitosan	1.75	5.00	1.75	5.00	1.75	5.00
Xanthan gum	2.8	8.00	2.8	8.00	2.8	8.00
Sodium Alginate	4.9	14.00	2.45	7.00	4.9	14.00
HPMC K100M CR	2.625	7.50	2.625	7.50	1.75	5.00
Polyox WSR coagulant	2.2625	6.46	2.2625	6.46	1.75	5.00
Avicel PH102	2.2525	6.44	4.7025	13.44	3.64	10.40
PVP K-30**	1.75	5.00	1.75	5.00	1.75	5.00
Magnesium stearate	0.35	1.00	0.35	1.00	0.35	1.00
Total Wt	*35*	*100*	*35*	*100*	*35*	*100*

*Equivalent to 12.5 mg of levofloxacin, **Used as 5% w/v solution in IPA

Table 7.5 Composition of the full factorial design batches

Ingredients (per minimatrix)	LMT 07		LMT 08		LMT 09	
	mg	%	mg	%	mg	%
Levofloxacin hemihydrate*	12.81	36.60	12.81	36.60	12.81	36.60
Sodium bicarbonate	2.625	7.50	2.625	7.50	2.625	7.50
Citric acid	0.875	2.50	0.875	2.50	0.875	2.50
Chitosan	1.75	5.00	1.75	5.00	1.75	5.00
Xanthan gum	2.8	8.00	2.8	8.00	2.8	8.00
Sodium Alginate	2.45	7.00	3.675	10.50	2.45	7.00
HPMC K100M CR	3.5	10.00	3.5	10.00	1.75	5.00
Polyox WSR coagulant	3.5	10.00	3.5	10.00	1.75	5.00
Avicel PH102	2.59	7.40	1.365	3.90	6.09	17.40
PVP K-30**	1.75	5.00	1.75	5.00	1.75	5.00
Mg stearate	0.35	1.00	0.35	1.00	0.35	1.00
Total Wt	*35*	*100*	*35*	*100*	*35*	*100*

*Equivalent to 12.5 mg of levofloxacin, **Used as 5% w/v solution in IPA

7.3.4 Procedure for preparation of minimatrices

Levofloxacin hemihydrate, xanthan gum, polyox, HPMC K100M CR, sodium alginate, chitosan, sodium bicarbonate and citric acid were weighed in required quantities and passed through sieve no.30 (Jayant Scientific Sieves, Mumbai, India). The blend was properly mixed and granulated using 5% w/v solution of PVP K30 in IPA. Additional quantity of IPA was added, as required, for obtaining properly cohesive mass. Air drying of granulated mass was carried out at room temperature for 15-20 min and then passed through sieve 30. Obtained granules were dried at 40° to 42°C for about 20 min in tray dryer (Shree Kailash Industries, Baroda, India). Dried granules were lubricated by adding magnesium stearate (40#). Lubricated blend was compressed using 4 mm circular multi-tip punches on rotary tablet compression machine (General Machinery Co., Mumbai, India).

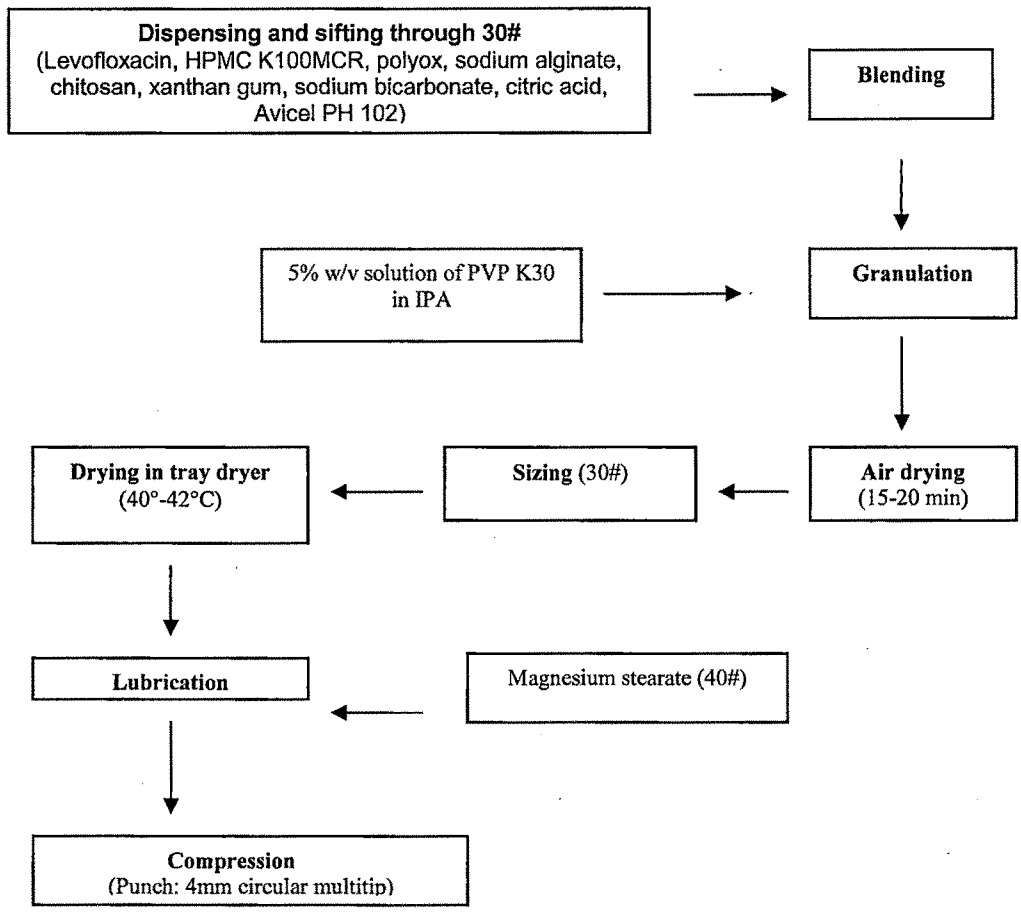

Fig 7.1 Flow diagram for preparation of minimatrices

Lubricated granules were subjected to bulk density testing by using density test apparatus (Electrolab, Mumbai, India). Angle of repose, which is indicator of flow properties, was determined by funnel method. Compressed minimatrices were evaluated for weight variation, thickness, hardness and friability as in process quality control parameters.

7.3.5 Evaluation of granule properties

Proper flowability the lubricated blend is necessary for its smooth and uniform flow through hopper of tablet compression machine as well as uniform die filling during compression to avoid weight variation. Bulk density is mass per unit volume of loose powder bed. Volume in this case includes space between particles and envelope volumes of the particles themselves. Tapped density is ratio of mass of powder to volume occupied by powder after it has been tapped for defined period of time. It represents random dense packing (Amidon et al.,

2009). Suitably weighed quantity of the lubricated granules was transferred to 25 ml measuring cylinder. Bulk density and Tapped density was estimated as described in section 5.2.5.1 (chapter 5) and compressibility index and Hausner ratio was calculated from equation 5.4 and 5.5

7.3.6 Evaluation of minimatrices

7.3.6.1 Weight variation

Twenty minimatrices were individually weighed and percentage weight variation was calculated.

7.3.6.2 Thickness

Thickness of the minimatrices was measured using vernier caliper.

7.3.6.3 Hardness

Hardness of the minimatrices was measured by using Monsanto type hardness tester.

7.3.6.4 Friability

Twenty minimatrices were weighed and transferred to the drum of friability test apparatus (Electrolab, Mumbai, India). Pan was rotated for 100 revolutions at the speed of 25 revolutions per minute (rpm). Weight of the minimatrices after 100 revolutions was noted. Friability was calculated by equation 5.7.

7.3.6.5 Levofloxacin content

Levofloxacin content was estimated by HPLC method. Twenty minimatrices were finely crushed and powder equivalent to weight of one minimatrix was transferred in 50 ml volumetric flask. About 40 ml of mobile phase was added and sonication (Modern Industrial Corporation, Mumbai, India) was done for 20 min. Final volume was made up by adding mobile phase and filtered using type 1 Whatman filter paper. This filtrate was suitably diluted (0.4 ml to 10 ml) by mobile phase and 20 µl of the finally diluted sample was injected. Levofloxacin content was estimated from calibration plot equation.

7.3.6.6 Floating lag time and total floating time

The time interval between introduction of the minimatrices in the dissolution vessel and the time when these start floating towards the surface of dissolution medium is called floating lag time. It was determined simultaneously during drug

release study. Total duration for which minimatrices were capable to float was noted.

7.3.6.7 Dissolution profile

Dissolution profiling was carried out by using USP type II (paddle type) dissolution test apparatus (VDA 6-DR, Veego Instruments Corporation, Mumbai, India). Dissolution medium was 900 ml of 0.1N HCl maintained at 37±0.5°C and paddle speed was 50 rpm. Eight minimatrices (equivalent to 100 mg of levofloxacin) were introduced in dissolution vessel. Five milliliter sample was withdrawn at 1,2,4,6,8,10 and 12 h. Equal volume of dissolution medium was replenished each time. After suitable dilution, amount of drug released was estimated by UV spectrophotometric method (UV-1700, Pharmaspec, Shimadzu, Japan) at 293.6 nm.

7.3.6.8 Scanning Electron Microscopy (SEM) studies

Sustained release matrix formulations are having drug entity embedded in the polymeric network. When such a formulation comes in contact with dissolution medium, the medium penetrates in the matrix and slowly dissolves the drug molecules. This phenomenon takes place slowly over prolonged time. Drug is released from the matrix network either by diffusion or erosion phenomenon. In the erosion process, matrix shreds layer by layer and releases the drug in surrounding dissolution medium. In diffusion phenomenon, channels are formed in the polymeric network and drug travels from inner core to outer surface through these channels. In such case if diffusion channels are formed, their opening can be observed on the surface in the form of pores. To observe this peculiar phenomenon, SEM studies were carried out. Surface features of the minimatrices exposed to the dissolution medium were observed by scanning electron microscopy. Optimum batch (LMT 03) minimatrices were introduced in dissolution medium and the test was started with the same test parameters as mentioned in dissolution profile study (section 7.3.6.7). Minimatrices were carefully withdrawn after 6 h and excess of dissolution medium was wiped off. SEM images of these minimatrices were taken at different magnifications by Scanning Electron Microscope JSM-5610LV (Jeol Ltd., Japan). Surface features of dry minimatrix were also observed.

7.3.6.9 Kinetic modelling of drug release

In case of sustained and controlled release matrix drug delivery systems, drug is released from the matrix network by either diffusion or erosion phenomenon. The type of release pattern followed by the particular formulation can be predicted by fitting the drug release data to various mathematical equations, which is called as kinetic modelling of drug release. In the present study, in vitro drug release data was fitted to zero-order, first-order, Higuchi, and Ritger and Peppas equations (equation 5.8, 5.9, 5.10 and 5.11 respectively).

7.3.6.10 Swelling index

Polymer hydration increases diffusional path length in the matrix network and hence it is one of the factors determining the degree and velocity of drug release from the swellable matrices. When the polymer swells in contact with an aqueous liquid it forms a gel layer around the whole tablet and controls hydration rate (Miranda et al., 2006). Swelling Index was determined by a new and convenient method using the baskets of dissolution test apparatus. Five minimatrices were placed in a basket and it was placed in a petridish having 100 ml of 0.1N HCl. Baskets were removed every hour, excess of the 0.1N HCl was removed by tissue paper and final weight was measured. The study was carried upto 12 h. Swelling index was calculated by equation 5.12

7.3.6.11 Ex-vivo bioadhesion study

Chitosan was included as one of the formulation component due to its bioadhesive property. Bioadhesive strength of the developed formulations was estimated using Instron tensiometer (Instron 1121, UK). Single minimatrix was stuck on the upper jaw of tensiometer and goat stomach tissue (which was freshly collected from local slaughter house) was fixed on lower jaw. Upper jaw was moved towards lower and minimatrices stuck to it was kept in contact with goat stomach tissue for 20 seconds. Afterwards upper jaw was moved in upward direction at speed of 5 mm/min until the minitablet was completely detached from the tissue. Detachment force in "dynes/cm^2" was measured.

7.3.7 Optimisation of responses by desirability approach

This approach is used to achieve an optimum formula based on desirability functions. In desirability approach, each response is associated with its own

partial desirability function and it varies from 0 to 1 according to closeness of the response to its target value. During optimization of formulations, the responses have to be combined in order to produce a product of desired characteristics. The application of the desirability function combines all the responses in one measurement and gives the possibility of predicting optimum levels for the independent variables. The combination of the responses in one desirability function requires the calculation of the individual desirability (Mashru et al., 2005).

In the present investigation exercise, optimum formulation should have low floating lag time and low % drug release at 1 h. Hence lowest experimental value obtained for these variables for the factorial design batches was considered as target value. Individual desirability of these responses were calculated using following equation:

$$d_1 \text{ or } d_2 = Y_{max} - Y_i / Y_{max} - Y_{target} \quad \text{for } Y_i > Y_{target} \tag{7.2}$$

$$d_1 \text{ or } d_2 = 1 \text{ for } Y_i < Y_{target} \tag{7.3}$$

where d_1 and d_2 is individual desirability for floating lag time and % drug release at 1h. The Y_{max} and Y_{target} indicate maximum and target (minimum) value of experimental result for respective response variables (Table 7.6). Y_i is the experimental result of the response variable for individual factorial design batch. Optimum formulation should take high time for 50 % and 95% drug release and should have high swelling index. Hence highest experimental value obtained for these variables amongst the factorial design batches was considered as target value. Desirability of these response variables was calculated as:

$$d_3 \text{ or } d_4 \text{ or } d_5 = Y_i - Y_{min} / Y_{target} - Y_{min} \text{ for } Y_i < Y_{target} \tag{7.4}$$

$$d_3 \text{ or } d_4 \text{ or } d_5 = 1 \text{ for } Y_i > Y_{target} \tag{7.5}$$

where d_3, d_4 and d_5 are individual desirability for time required for 50% dug release, time required for 95% dug release and swelling index. The Y_{min} and Y_{target} indicate minimum and target (maximum) value of experimental result for respective response variables. Y_i is the experimental result of the response variable for individual factorial design batch.

The overall desirability values were calculated from the individual values by using the following equation:

$$D = (d_1 d_2 d_3 d_4 d_5)^{1/5} \quad (7.6)$$

7.3.8 In vivo study

Gamma scintigraphy technique was used for evaluating In vivo gastric residence time of the optimum formulation in three healthy human volunteers having age group of 25 to 35 years. There was no history of previous illness in near past. Technetium Tc99m pentetate was used as radiolabelling material. Minimatrices were radiolabelled as described in section 5.3.8 (chapter 5). Four radiolabelled minimatrices were introduced into hard gelatin capsule. Volunteers were fasted overnight and radiolabelled minimatrices introduced in hard gelatin capsules were administered after a standard breakfast along with 250 ml of water. In vivo transit behaviour of the formulation was monitored by capturing the images by Gamma camera (e-cam signature series Siemens). During the study duration, volunteers were allowed to drink sufficient water.

7.3.9 Stability studies

Stability studies were carried out as per ICH guidelines. Minimatrices of the optimum formulation were packed in 0.04 mm aluminium pouch and subjected for stability studies at 40°C/75%RH and 30°C/65%RH in stability chambers (Newtronic, Mumbai). The formulation is said to be stable if there is no significant change in drug content, floating lag time and drug release pattern.

7.4 RESULTS AND DISCUSSION

7.4.1 Preliminary Experiments

An attempt to prepare a formulation by direct compression which is an approach with minimum processing steps, was not successful due to poor flow properties. It may be due to the reason that levofloxacin is amorphous in nature and it occupied major portion in the formulation composition. To overcome this difficulty an attempt was made to obtain properly compressible blend by granulation technique. Non-aqueous solution of PVP K30 (5% w/v solution in IPA) was used as granulating agent. Resultant granules had good strength.

Preliminary trials revealed that HPMC K100M CR and polyox coagulant were excellent polymers for sustaining levofloxacin release. Polyox was also responsible for imparting good floating phenomenon to the formulation. Sodium alginate is efficient in sustaining drug release. It is a naturally derived, gel-forming copolymer of mannuronic and glucuronic acid salts. Systems involving this polymer prevent burst release of highly soluble drugs in stomach by entrapping it in an gelled matrix of the alginic acid formed in the acidic gastric environment (ISP technical bulletin).

Formulations without xanthan gum were unable to maintain matrix integrity for longer duration. Hence it was realized to be an important component for sustaining drug release. Sodium bicarbonate and citric acid react in presence of dissolution medium and the gas bubbles produced in formulation matrix imparted floating feature with reduced lag time. Formulation without this gas generating couple could not float. Chitosan due to its well reported bioadhesive properties was included as one of the formulation components (Dodou et al, 2005). Buoyancy, bioadhesion and sustained drug delivery were important aspects for the formulation intended to be developed in the present investigation. HPMC K100M CR and polyox based matrix network significantly extended drug release while sodium alginate helped in further sustaining drug release. Hence these were selected as formulation variables and effect of different levels was studied by 3^2 full factorial design (Table 7.2). Formulation and response variables were related using the mathematical relationship. Regression analysis results are shown in Table 7.7. Polynomial equations were used to draw conclusions by considering sign and magnitude of the coefficient. High values of coefficient of determination (R^2) indicate good fit.

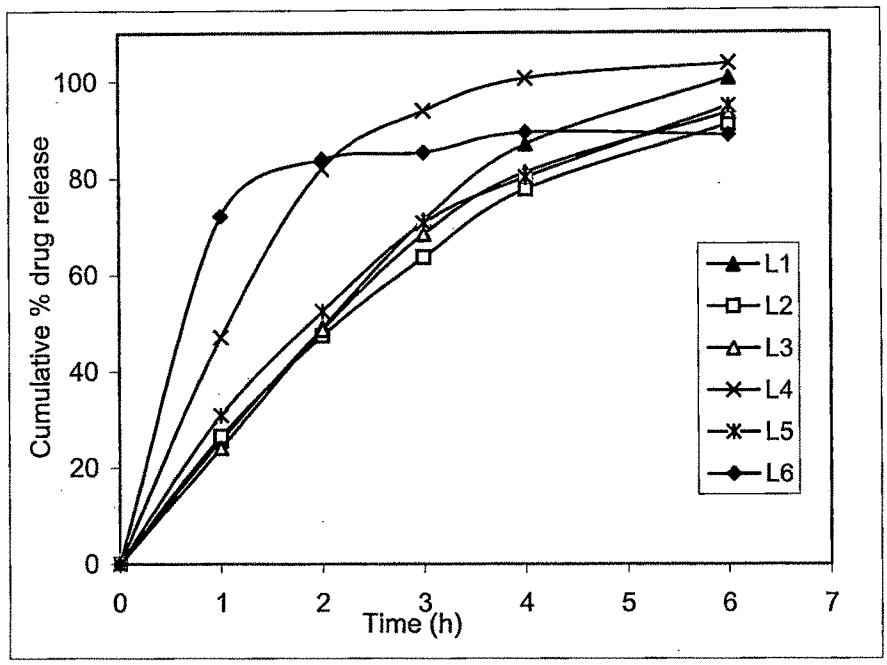

Fig 7.2 Dissolution profile of preliminary trial batches

7.4.2 Drug excipient interaction study

DSC thermogram for levofloxacin showed endothermic peak at 234.02°C (Fig 7.3). The blend of levofloxacin and other formulation excipients also showed an endothermic peak at the same temperature as shown by an individual levofloxacin. It clearly indicated that there is no interaction between drug and the excipients. Additional peak in drug excipient mixture at 70.6°C (Fig 7.3) was due to polyox as it was confirmed by individual polyox thermogram.

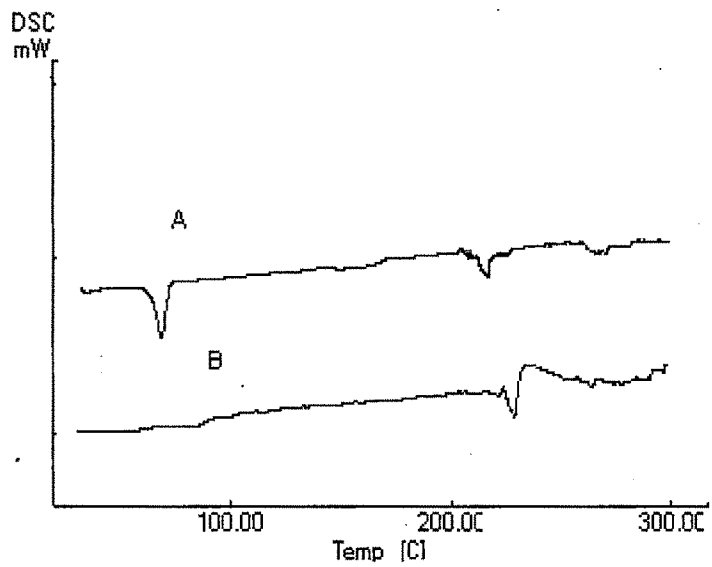

Fig 7.3 DSC thermograms (A) Levofloxacin excipient mixture and (B) pure levofloxacin

7.4.3 Evaluation of granule properties

Lubricated blend was studied for micromeritic properties; bulk density and angle of repose. Bulk and tapped density is an important parameter as it affects die fill and compression and utilized for calculation of compressibility and Hausner ratio which are indicators of flow propeties. Bulk density was found between 0.45 and 0.51 gm/cm^3 and tapped density between 0.53 to 0.57 gm/cm^3 for all the designed formulations. Angle of repose predicts flow property of the granules which is important for weight uniformity during compression of the minimatrices. Angle of repose was observed between 32° and 40° which indicates good flow properties (Marshall, 1987).

7.4.4 Evaluation of minimatrices

7.4.4.1 Physical parameters

The prepared minimatrices were 4 mm in diameter and 2.8 to 3 mm in thickness. Maximum weight variation was 7.2% calculated with respect to theoretical weight. It was found satisfactory as it is less than 10% which is allowable limit for the uncoated tablets having unit weight less than 80mg (IP, 1996). Hardness of the minimatrices was 2-3 kg/cm^2. Though hardness values seem to be low, it imparted sufficient strength to the minimatrices due to their smaller dimensions. Friability was zero indicating that the minimatrices completely withstand the stress during friability test.

7.4.4.2 Levofloxacin content

Levofloxacin content was estimated by HPLC method. The developed minimatrices were found to contain 98.7 to 101.2% of added amount of levofloxacin per minimatrix. Details of the estimation procedure are mentioned in method section 7.3.6.5

7.4.4.3 Floating lag time and total floating time

For retaining the formulation in stomach, its passage into small intestine should be avoided. For achieving this goal, administered dosage form should be kept towards surface of the gastric contents. Floating lag time reflects the minimum time a formulation require, in vitro, to travel towards surface of dissolution medium when it is introduced into the dissolution vessel. Ultimately it shows that after oral administration how fast the administered formulation can achieve buoyancy in vivo and remain towards gastric contents. For the designed

formulations, floating lag time varied from 5 to 8 min. It did not differ significantly amongst various batches which may be due to same amount of the gas generating couple in all of the designed formulations. This observation and results of regression analysis in Table 7.7 indicate that none of the formulation variables have significant effect on floating time. It clearly shows that floating lag time depends solely on gas generating couple and not other formulation variables.

Sodium bicarbonate and citric acid act as gas generating couple and react in presence of acidic dissolution medium to generate carbon dioxide which gets entrapped as bubbles in polymer network. It ultimately decreases density of the minimatrices (Li et al, 2001). Citric acid was included in the formulation to assure an acidic microenvironment that is responsible for continuous generation of gas bubbles in the swollen matrix. Xanthan gum forms viscous network in this matrix minimizing chances of bubbles getting escaped from the polymer network channels. Ultimately this led to floating behavior of the minimatrices for longer duration. All the designed formulations were capable to float until they lost their integrity. It lasted upto 12 h for the formulations containing higher level of polymers which maintained their integrity till that time.

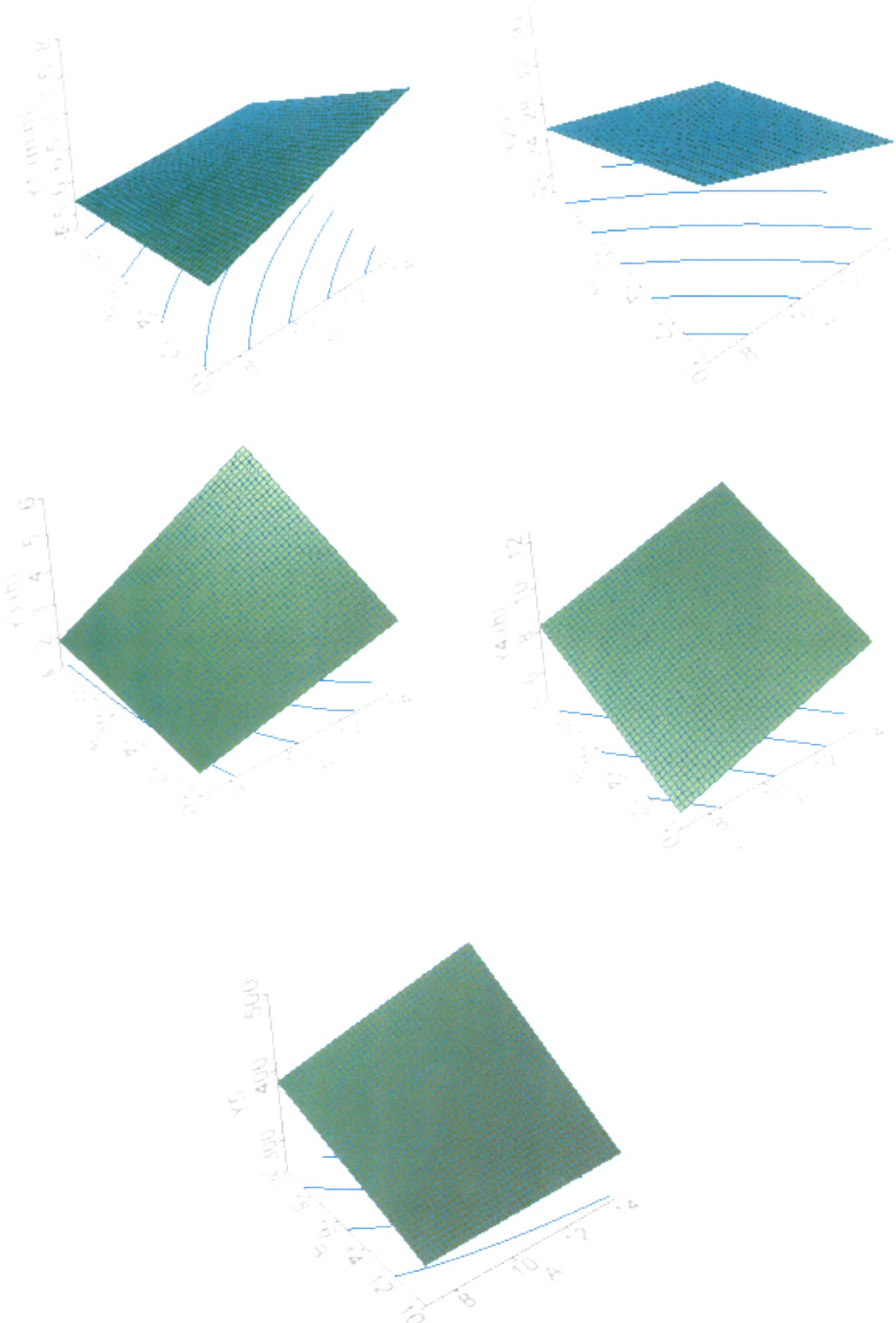

Fig 7.4 Response surface plots showing effect of A and B on response variables

7.4.4.4 Dissolution profiling

H. pylori resides in stomach mucosa necessitating longer exposure of therapeutic moiety for its complete eradication. Keeping in mind this necessity, development aspect in this research work focused on imparting sustained release feature to the formulation (Bardonnet et al, 2006). HPMC is a neutral hydrophilic polymer. The polymer molecular chains of HPMC hydrate in contact with water, entangle and form a gel matrix. HPMC K100M CR and polyox were found to play important role in decreasing drug release at initial hour. Drug release at 1 h varied from 20.4% to 36.2% for the designed experiments (Table 7.6). Formulation LMT 03 and LMT 09 containing high and low level of formulation variables A and B released, respectively, minimum and maximum amount of drug at 1 h. Regression analysis results in Table 7.7 indicate that both variables A and B significantly affect initial hour drug release (Y_2), t50 (Y_3) and t95 (Y_4) as Prob>F values are less than 0.05. Magnitude of estimated coefficient show that effect of variable A is more prominent as compared to B. Sodium alginate is converted to acid at low pH and begins to gel. Thus it might have prevented burst release by entrapping drug in gelled matrix of the alginic acid formed in the acidic gastric environment (ISP technical bulletin). Sodium alginate formulations release drug by swelling and erosion/dissolution (Efentakis et al., 2000).

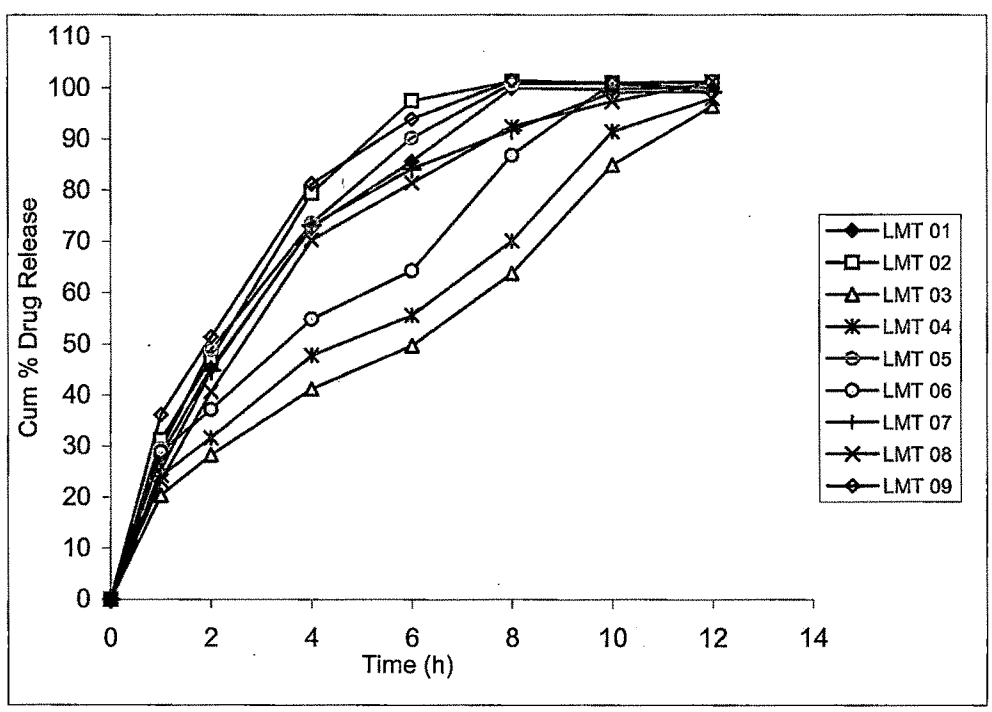

Fig. 7.5 Comparative dissolution profile of factorial design batches

Xanthan gum was included as one of the formulation components as it forms very viscous network in drug diffusion channels formed by polymeric network and ultimately may help in retarding drug release at initial hour. Sodium alginate can be used alone or in combination with other gel forming polymers such as HPMC, xanthan gum or propylene glycol alginate to control drug release from a hydrophilic matrix tablet. In gastric fluid, the hydrated sodium alginate is converted into a porous, insoluble alginic acid skin that suppresses release in the stomach. Once passed into the higher pH of the intestinal tract, the alginic acid skin is converted to a soluble viscous layer that in combination with the gel forming polymer controls release by an erosion mechanism. Formulation LMT 03 containing higher level of the formulation variables A and B was capable to sustain drug release upto 12 h.

Table 7.6 Experimental result of the response variables

		Response variable				
Formulation	**Pattern***	**Y_1 (min)**	**Y_2 (%)**	**Y_3 (h)**	**Y_4 (h)**	**Y_5**
LMT 01	00	7	28.2	2.4	7.4	370.9
LMT 02	0-	7	31.2	2.2	5.7	314.9
LMT 03	++	6	20.4	6.1	11.8	485.7
LMT 04	+0	6	24.2	4.6	11.1	384.9
LMT 05	-0	7	29.4	2.1	6.9	359.7
LMT 06	+-	8	28.9	3.5	9.1	325.8
LMT 07	-+	5	25.4	2.4	8.9	385.9
LMT 08	0+	6	23.2	2.7	9	411.1
LMT 09	--	6	36.2	1.9	6.2	303.8

Table 7.7 Results of regression analysis

Term	Y_1		Y_2		Y_3		Y_4		Y_5	
	EC	Prob > F	EC	Prob > F	EC	Prob > F	EC	Prob > F	EC	Prob > F
b_0	6.889	--	27.344	--	2.367	--	7.378	--	366.056	--
A	0.333	0.4102	-2.917	0.0053	1.300	0.0029	1.667	0.0034	24.500	0.0284
B	-0.667	0.1522	-4.550	0.0014	0.600	0.0255	1.450	0.0050	56.367	0.0028
A^2	-0.333	0.6199	-0.117	0.8765	1.000	0.0283	1.633	0.0169	8.667	0.4760
B^2	-0.333	0.6199	0.283	0.7088	0.100	0.7168	-0.017	0.9637	-0.633	0.9564
AB	-0.250	0.5999	0.575	0.3235	0.525	0.0595	0.000	1.0000	19.450	0.0819
R^2	0.647	--	0.984	--	0.976	--	0.981	--	0.973	--

EC indicates Estimated Coefficient; The terms having Prob > F values very small (< 0.05) indicate that these have significant effect on the response variables.

7.4.4.5 SEM studies

Fig 7.6a shows intact surface of the minimatrix in dry state. SEM Images for the minimatrices exposed to dissolution medium for 6h were captured at 25x, 50x and 100x (Fig 7.6 b, c and d). These images clearly show the pore formation, which are openings of the drug release channels on minimatrix surface.

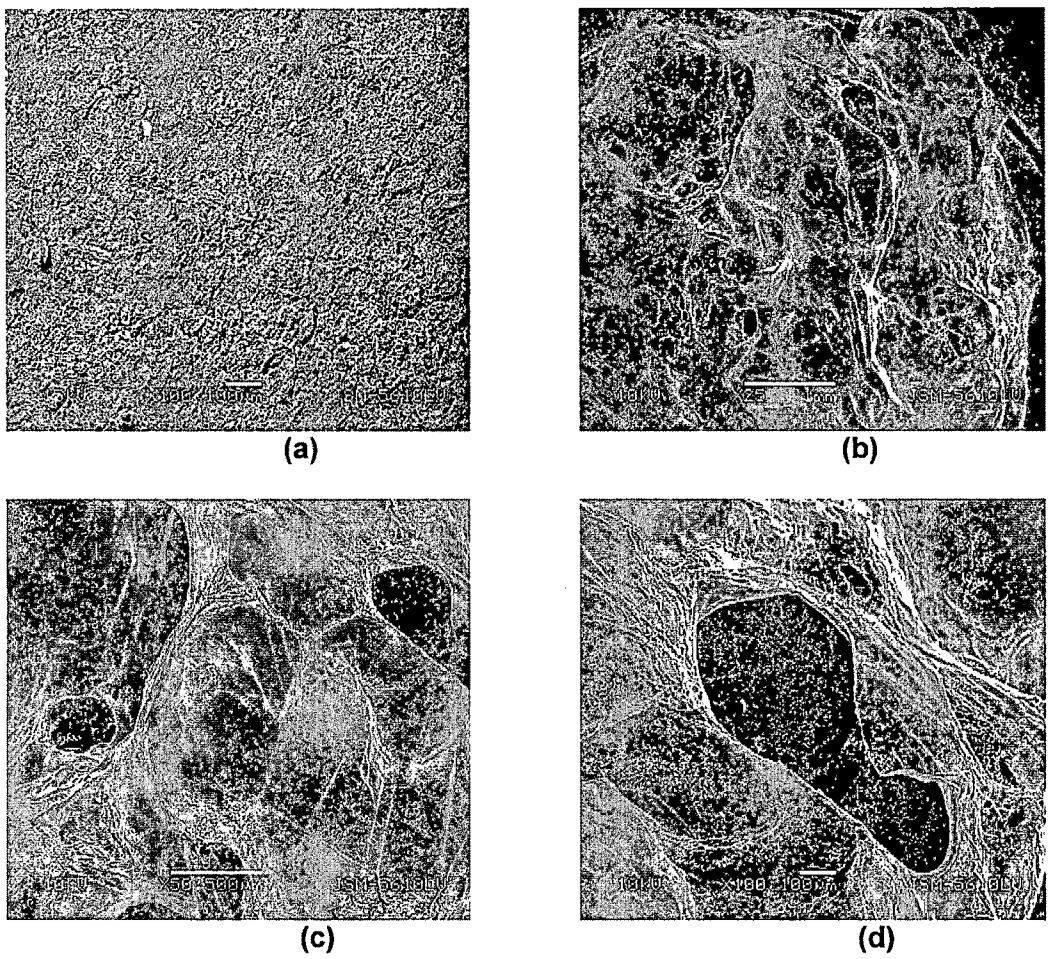

Fig 7.6 Scanning Electron Micrographs of formulation LMT03 (a) Surface of dry minimatrix at 50x; (b) (c) and (d) minimatrix surface after 6h exposure in dissolution medium at 25x, 50x and 100x magnification

7.4.4.6 Kinetic modelling of drug release

In vitro drug release data was analysed by zero-order, first-order, Higuchi and Ritger and Peppas equations. Optimum formulation LMT 03 best fitted to zero order release kinetics as maximum R^2 value of 0.979 was observed for this release kinetics.

Table 7.8 Comparative characteristics of different drug release kinetic models

Formulation	Zero order		First order		Higuchi		Ritger & Peppas		
	R^2	K_o	R^2	K_1	R^2	K_H	R^2	K_R	n
LMT 01	0.838	7.951	0.759	0.104	0.967	31.701	0.962	0.309	0.684
LMT 02	0.784	7.903	0.709	0.096	0.939	32.108	0.935	0.338	0.597
LMT 03	**0.979**	**7.383**	0.969	0.136	0.953	27.047	0.973	0.195	0.503
LMT 04	0.967	7.558	0.957	0.125	0.972	28.132	0.978	0.228	0.481
LMT 05	0.817	7.879	0.738	0.099	0.959	31.699	0.952	0.329	0.661
LMT 06	0.941	8.050	0.931	0.116	0.983	30.544	0.981	0.267	0.463
LMT 07	0.844	7.814	0.741	0.107	0.970	31.090	0.953	0.292	0.819
LMT 08	0.874	8.055	0.764	0.117	0.977	31.625	0.964	0.258	0.811
LMT 09	0.764	7.509	0.718	0.084	0.938	30.880	0.939	0.393	0.506

7.4.4.7 Swelling index

After coming in contact with dissolution medium, HPMC and polyox absorbed water and the matrix gets hydrated. This phenomenon starts from surface and slowly travels towards matrix core. It leads to formation of channels in the polymer and simultaneously drug dissolution. Dissolved drug travels through the channels towards surface and finally gets released. Fluid uptake increases matrix volume due to swelling and it causes increase in channel length. The time required for the drug moiety to travel through lengthy channel is of course more. It ultimately extends drug release phenomenon for longer duration. (Michailova et al, 2001; Siepmann and Peppas, 2001). HPMC and polyox have the property to absorb water and get hydrated. Fluid uptake capacity depends on the amount of these components present in the formulation. Sodium alginate (A) and HPMC polyox ratio both significantly affected swelling index but effect of B was more prominent than A. Increase in level of these variables increase swelling index. Formulation LMT 03 containing highest amount of formulation variables was having swelling index 485.7 while formulation LMT 09 containing least amount has value of 303.8. Significance of the effect of formulation variables on swelling index can be interpreted from regression analysis values in Table 7.7. Designed formulations also contain xanthan gum as one of the formulation components which has water holding and viscolysing properties. Viscolysing property is important for maintaining matrix integrity for longer duration.

7.4.4.8 Ex-vivo bioadhesion study

Gastroretentive phenomenon of a formulation can be enhanced by imparting mucoadhesive feature along with floating property. To impart mucoadhesive feature, chitosan was included as one of the formulation components. Chitosan is a natural polycationic hydrophilic polymer, derived from polysaccharide chitin. It exhibits strong mucoadhesive properties due to the formation of hydrogen and ionic bonds between the positively charged amino groups of chitosan and the negatively charged sialic acid residues of mucin glycoproteins (Dodou et al, 2005). HPMC, a long-chain and non-ionic polymer, has limited bioadhesive property. It could be due to formation of physical or hydrogen bonding with the mucus components. Due to presence of chitosan in the formulation, it was having mucoadhesive strength. For the factorial design formulations, it varied between 6.7 to 7.1 dynes/cm^2. For optimum formulation LMT 03, it was 6.9 dynes/cm^2.

7.4.5 Optimisation of responses using desirability function

Desirability approach was used to select an optimum formulation. Formulation with maximum desirability is said to be optimum. The Y_{max} and Y_{target} values were 8 and 5 for floating lag time and 36.2 and 20.4 for % drug release at 1 h. Respective Y_{min} and Y_{target} values were 1.9 and 6.1 for time required for 50% drug release, 5.7 and 11.8 for time required for 95% drug release and 303.8 and 485.7 for swelling index. Overall desirability was calculated by equation 7.6. As shown in Table 7.9, formulation LMT 03 was having maximum desirability of 0.92 and hence it was selected as an optimum formulation.

Table7.9 Overall desirability values

Formulation	Desirability
LMT 01	0.29
LMT 02	0.00
LMT 03	**0.92**
LMT 04	0.66
LMT 05	0.21
LMT 06	0.00
LMT 07	0.45
LMT 08	0.51
LMT 09	0.00

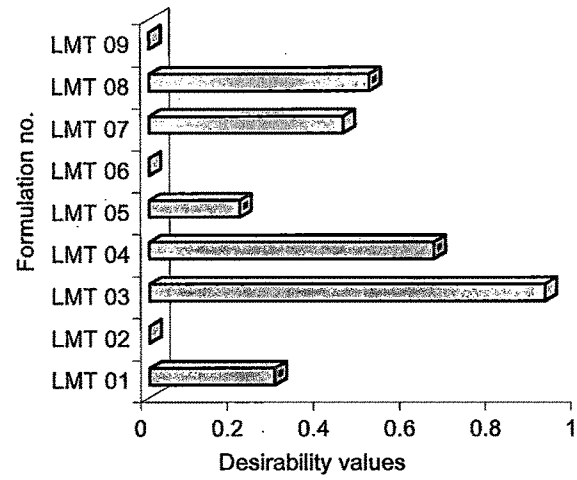

Fig 7.7 Overall Desirability

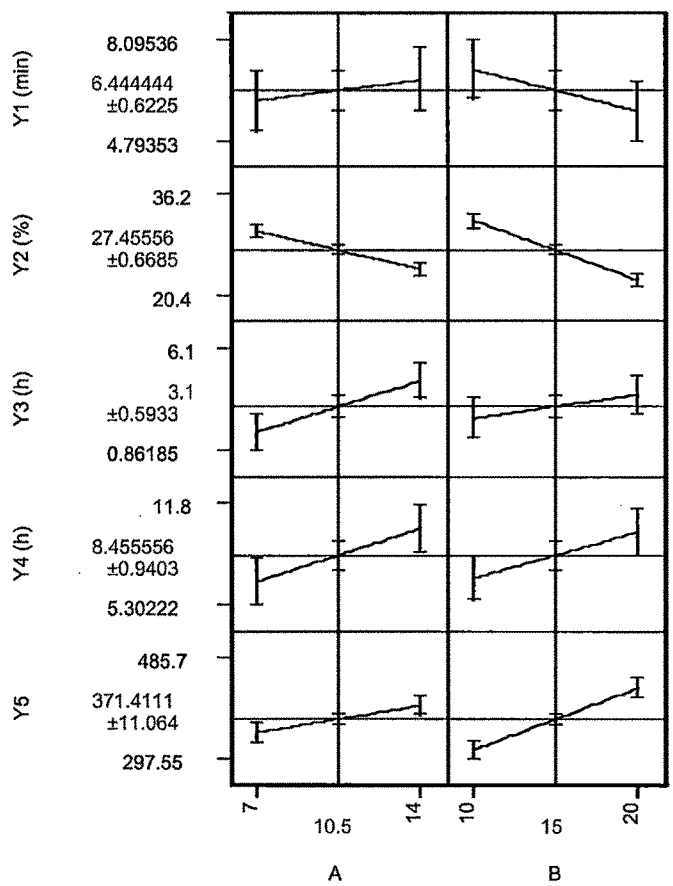

Fig 7.8 Prediction profiler showing effect of formulation variable levels on response variables

7.4.6 In vivo study by gamma scintigraphy

Radioactive material Tc99m pentetate emits low intensity gamma rays which can be detected by ultrasensitive gamma camera. In vivo transit of the Tc99m radiolabelled formulation was monitored by detecting these gamma rays which ultimately predicted location of the formulation. Image captured at 0 h, i.e. immediately after administration shows single entity as the minimatrices were administered by putting them in capsule which was intact at that time. In vitro studies had already proved that the capsule gets disintegrated within 5 min. Image captured at 2 h clearly shown the individual minimatrices which proved that capsule shell did not interfere in functioning of minimatrices. Images were captured every hour to ascertain location of the minimatrices. The images captured at 0, 2, 6, 9 and 10 h are shown here. Minimatrices were observed in stomach till 9 h and disappeared as indicated by the image captured at 10 h. it proves capability of the developed minimatrices to remain in stomach till 9 h.

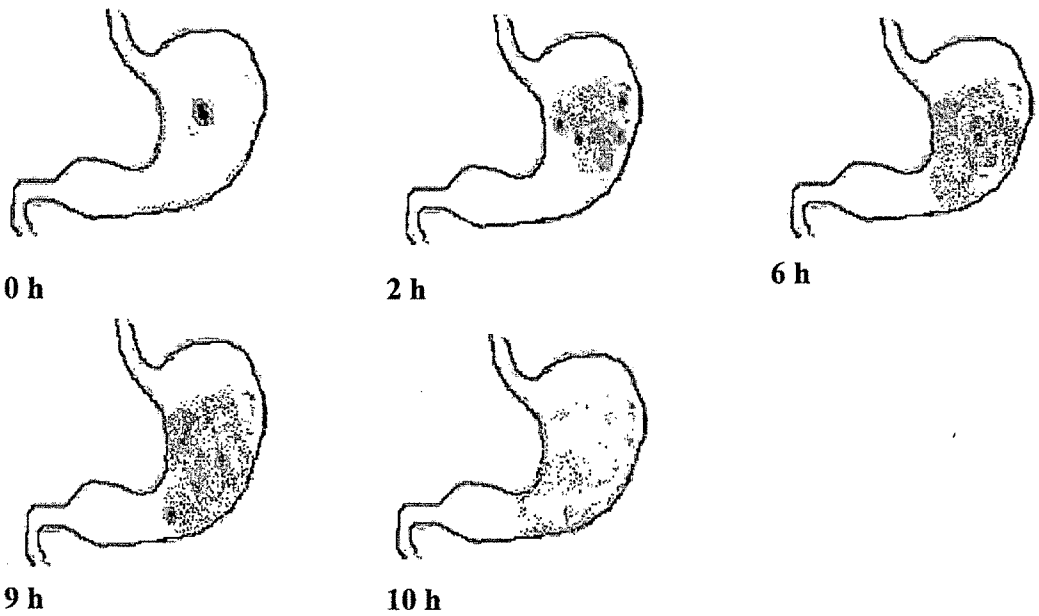

Fig 7.9 Gamma scintigraphy images showing gastric residence time of dosage form

7.4.7 Stability studies

Stability samples were analysed for various the individual parameters as per the procedure described in method section. There was no significant change in floating lag time, bioadhesive strength and dissolution profile which are critical parameters for gastroretention and sustained release phenomenon.

Stability samples of the optimum formulation (LMT 03) were analysed for various parameters as per the procedure described in method section. There was no significant change in levofloxacin content, floating lag time and dissolution profile The parameters like floating lag time and dissolution profile were tested as these are critical gastroretention and sustained release phenomenon. Dissolution profile of stability samples were compared with initial sample profile by using similarity factor and it ranged from 85 to 91 which indicated their similarity.

Table7.10 Stability data

Parameter	Initial	Storage condition and duration			
		30°C/65%RH		40°C/75%RH	
		3 Months	6 Months	3 Months	6 Months
Buoyancy lag time (min)	6	8	10	10	11
Levofloxacin content (%)	100.2	99.7	98.9	98.1	97.5

7.5 CONCLUSION

Gastroretentive formulation in the form of minimatrices have been developed in the present investigation by using HPMC K100M CR, polyox WSR coagulant, sodium alginate. Chitosan was included as one of the formulation component due to its mucoadhesive properties. Xanthan gum was used as viscolysing agent for assisting rate controlling polymers in further retarding drug release. Effect of different levels of sodium alginate and HPMC K100M CR : polyox coagulant (1:1) was studied systematically by 3^2 full factorial design. Optimum formulation (LMT 03) was decided by desirability approach. In vivo gastric residence time of the optimum formulation was evaluated by gamma scintigraphy technique by using Technetium Tc 99m pentetate as a radioactive material. The optimum formulation was found to have gastric residence time of about 9 h. Simple and cost effective technique has been implemented in preparation of the minimatrices. Using the conventional tablet manufacturing facility the formulation scale up can be done. Thus levofloxacin delivery through developed minimatrices may effectively eradicate H.pylori as compared to conventional formulations.