Chapter 9

Minimatrices of Clarithromycin

9.1 INTRODUCTION

Clarithromycin is a semi-synthetic macrolide antibiotic. Clarithromycin exerts its antibacterial action by binding to the 50S ribosomal subunit of susceptible microorganisms resulting in inhibition of protein synthesis. Clarithromycin is active in vitro against a variety of aerobic and anaerobic gram-positive and gram-negative microorganisms. Clarithromycin MIC for different H.Pylori strains vary from 0.03 µg/ml to 4.0 µg/ml (Hasan et al., 1999). Chitosan-based mucoadhesive microspheres (Majithiya and Murthy, 2005) and chitosan and carboxymethylcellulose sodium interpolymer complexes (Gomez-Burgaz et al., 2008) have been reported. Aim of the present investigation was to develop clarithromycin minimatrices for sustained delivery in stomach.

9.2 MATERIALS

Clarithromycin was received as a gift sample from Gujarat Liqui Pharma Caps Pvt Ltd., (Baroda, India). Polyethylene oxide (Polyox) WSR coagulant was gifted by Colorcon Asia Pvt. Ltd. (Goa, India). Chitosan was received from Central Institute of Fisheries & Technology (Kochi, India). Microcrystalline cellulose (Avicel PH102) was obtained from Signet Chemical Corporation (Mumbai, India). Xanthan gum, magnesium stearate and polyvinylpyrrolidone (PVP) K30 were purchased from S.D. Fine Chem (Mumbai, India). Sodium bicarbonate, citric acid, Isopropyl alcohol, sodium dihydrogen orthophosphate and hydrochloric acid were purchased from Qualigens Fine Chemicals (Mumbai, India).

9.3 METHODS

9.3.1 Preliminary studies

Preliminary studies were carried out for selection of suitable excipients and preparation technique. As a simplest technique, drug and excipients can be mixed and compressed. This method of direct compression is convenient and cost effective as it involves minimum processing steps. But its feasibility depends on micromeritic properties of drug and excipients. If the blend is not suitable for direct compression, then granulation is needed for converting the blend in compressible form. Trial C-1 was carried out to ascertain feasibility of direct compression.

As final formulation characteristics were not satisfactory, granulation approach was implemented. To determine role of individual excipients in the formulation, trials C-2

to C-7 (Table 9.1) were carried out. Formulations prepared in the preliminary trials were evaluated for drug release pattern and buoyancy lag time as these are key parameters for sustained release gastroretentive formulation.

Table 9.1 Composition of preliminary formulation trials

Ingredients (mg / minimatrix)	**C-1**	**C-2**	**C-3**	**C-4**	**C-5**	**C-6**	**C-7**
Clarithromycin	12.5	12.5	12.5	12.5	12.5	12.5	12.5
Sodium bicarbonate	2.625	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	0.875
Xanthan gum	0	16.9	0	0	0	0	0
HPMC K100M CR	18.65	0	16.9	0	0	0	0
Polyox WSR coagulant	0	0	0	16.9	0	0	0
Carbopol 974P	0	0	0	0	16.9	0	0
Sodium alginate	0	0	0	0	0	16.9	0
Chitosan	0	0	0	0	0	0	16.9
PVP K30[#]	0	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	0.35
Total wt.	**35**	**35**	**35**	**35**	**35**	**35**	**35**

[#]Used as 5% w/v solution in IPA

Table 9.2 Composition of preliminary formulation trials

Ingredients (mg / minimatrix)	**C-8**	**C-9**
Clarithromycin	12.5	12.5
Sodium bicarbonate	2.625	0
Citric acid	0.875	0
Polyox coagulant	12.25	12.25
Chitosan	3.5	3.5
Avicel PH102	1.15	4.65
PVP K30	1.75	1.75
Magnesium stearate	0.175	0.175
Total wt.	35	35

Formulations C-8 and C-9 were prepared to ascertain necessity of gas generating couple to achieve floating feature.

9.3.2 Drug excipient compatibility study

DSC studies were carried out to study compatibility of clarithromycin with the selected excipients. 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. Clarithromycin alone and alongwith the excipients was subjected for DSC studies. Thermogram was recorded using 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.

9.3.3 Formulation designing by full factorial design

In the present study, formulations were optimized by 3^2 full factorial design which involve study of two factors at three different levels. As shown in Table 9.3, total 9 formulations were designed. Polyox WSR coagulant (X_1) and Chitosan (X_2), each at three different levels, were selected as formulation variables. Levels of formulation variables were decided from the preliminary studies. Effect of the formulation variables was studied on floating lag time (Y_1), drug release at 1 hour (Y_2), time required for 95% drug release, t95 (Y_3), swelling index (Y_4) and bioadhesive strength (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

$$Y_i = b_0 + b_1 X_1 + b_2 X_2 + b_{11} X_1^2 + b_{22} X_2^2 + b_{12} X_1 X_2 \qquad (9.1)$$

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 9.3 Formulation designing by full factorial design

Formulation	Pattern*	Formulation variables	
		X_1	X_2
CMT 01	00	0	0
CMT 02	0-	0	-1
CMT 03	0+	0	1
CMT 04	-0	-1	0
CMT 05	--	-1	-1
CMT 06	-+	-1	1
CMT 07	+0	1	0
CMT 08	+-	1	-1
CMT 09	++	1	1

Coded values	Actual values#	
	A	B
-1	8	3
0	16	6
1	24	9

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

Table 9.4 Composition of the full factorial design batches

Ingredients	CMT 01		CMT 02		CMT 03	
(per minimatrix)	**mg**	**%**	**mg**	**%**	**mg**	**%**
Clarithromycin	12.5	35.71	12.5	35.71	12.5	35.71
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
Xanthan gum	3.85	11.00	3.85	11.00	3.85	11.00
Polyox WSR coagulant	5.6	16.00	5.6	16.00	5.6	16.00
Chitosan	2.1	6.00	1.05	3.00	3.15	9.00
Avicel PH 102	5.35	15.29	6.4	18.29	4.3	12.29
PVP K30#	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	100.00	35	100.00

Used as 5%w/v solution in IPA

Table 9.5 Composition of the full factorial design batches

Ingredients (per minimatrix)	CMT 04		CMT 05		CMT 06	
	mg/tab	%	mg/tab	%	mg/tab	%
Clarithromycin	12.5	35.71	12.5	35.71	12.5	35.71
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
Xanthan gum	3.85	11.00	3.85	11.00	3.85	11.00
Polyox WSR coagulant	2.8	8.00	2.8	8.00	2.8	8.00
Chitosan	2.1	6.00	1.05	3.00	3.15	9.00
Avicel PH 102	8.15	23.29	9.2	26.29	7.1	20.29
PVP K30[#]	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	100.00	35	100.00	35	100.00

[#] Used as 5%w/v solution in IPA

Table 9.6 Composition of the full factorial design batches

Ingredients (per minimatrix)	CMT 07		CMT 08		CMT 09	
	mg/tab	%	mg/tab	%	mg/tab	%
Clarithromycin	12.5	35.71	12.5	35.71	12.5	35.71
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
Xanthan gum	3.85	11.00	3.85	11.00	3.85	11.00
Polyox WSR coagulant	8.4	24.00	8.4	24.00	8.4	24.00
Chitosan	2.1	6.00	1.05	3.00	3.15	9.00
Avicel PH 102	2.55	7.29	3.6	10.29	1.5	4.29
PVP K30[#]	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	100.00	35	100.00	35	100.00

[#] Used as 5%w/v solution in IPA

9.3.4 Method of minimatrix preparation

Clarithromycin, xanthan gum, polyox coagulant, chitosan, sodium bicarbonate, citric acid and Avicel PH102 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 isopropyl alcohol. Air drying of

granulated mass was carried out at room temperature for 15-20 min and then it was sifted through sieve no.30. Sifted granules were dried at 40°-42°C for about 20 min in tray dryer (Shree Kailash Industries, Baroda, India). Dried mass was lubricated by adding magnesium stearate (40#) and compressed using 4 mm circular multi-tip punches on rotary tablet compression machine (General Machinery Co., Mumbai, India).

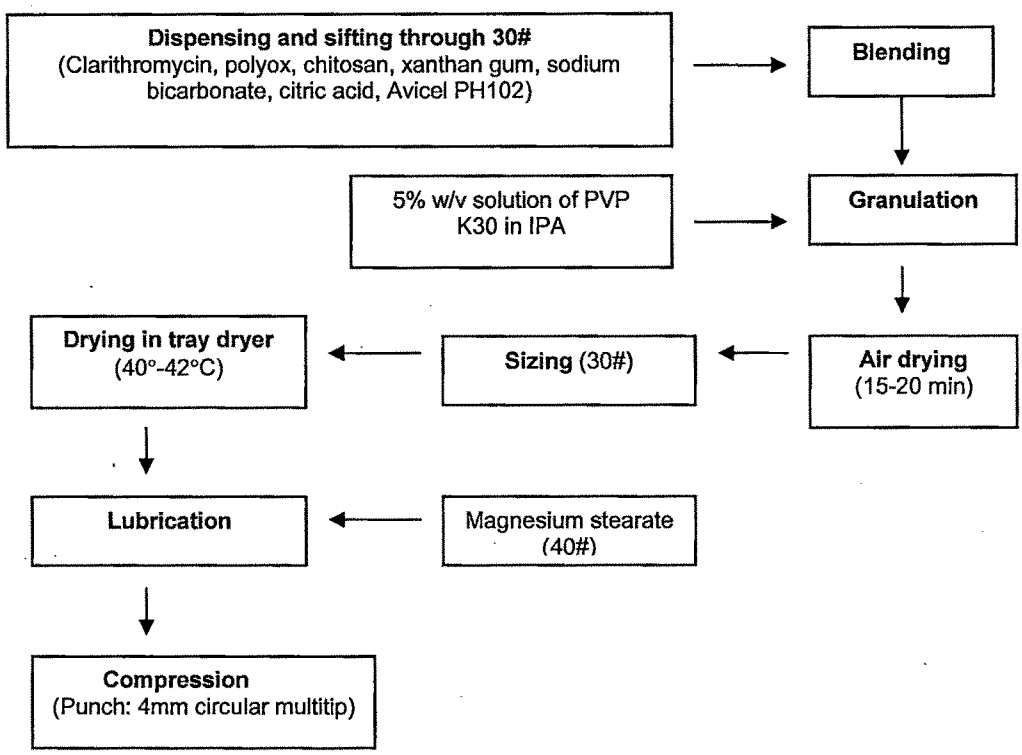

Fig 9.1 Flow diagram for preparation of minimatrices

Bulk density and tapped density of lubricated granules was tested 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.

9.3.5 Evaluation of lubricated granules

9.3.5.1 Bulk Density and Tapped Density

Bulk density and tapped density of the lubricated granules was determined as described in section 5.3.5.1 (Chapter 5)

9.3.5.2 Angle of Repose

Frictional forces in granules can be measured by angle of repose. This is the maximum angle possible between the surface of pile of powder and the horizontal plane. It was determined as per the procedure described in section 5.3.5.2 (Chapter 5)

9.3.6 Characterisation of minimatrices

9.3.6.1 Weight variation

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

9.3.6.2 Thickness and hardness

Thickness of the minimatrices was measured using vernier caliper and hardness by using Monsanto type hardness tester.

9.3.6.3 Friability

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

9.3.6.4 Content of clarithromycin

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 25 min. Final volume was made by adding mobile phase and filtered using type 1 Whatman filter paper. This filtrate was suitably diluted by mobile phase and 20 µl of the finally diluted sample was injected. Content of clarithromycin was estimated from calibration plot.

9.3.6.5 Floating lag time

Floating lag time was determined simultaneously during drug release study. Minimatrices were introduced into dissolution vessel. The time required for the minimatrices to float towards the surface of dissolution medium was noted. This time is called floating lag time.

9.3.6.6 Drug release study

USP type II dissolution test apparatus (VDA 6-DR, Veego Instruments Corporation, Mumbai, India) was used for carrying out drug release study. Dissolution medium was 900 ml of 0.1N HCl maintained at 37±0.5°C and paddle speed was 50 rpm. Eight minimatrices were introduced in each dissolution vessel. Ten milliliter sample was withdrawn at 1,2,3,4,6,8,10 and 12 h and filtered through type I Whatman filter paper. Equal volume of dissolution medium was replenished each time. Five milliliter of the sample was suitably diluted and amount of drug released was estimated by colorimetric method. (UV-1700, Pharmaspec, Shimadzu, Japan) at 760 nm.

9.3.6.7 Scanning Electron Microscopy (SEM) studies

After coming in contact with the dissolution medium, the medium penetrates into the minimatrices slowly over the period of time and dissolves the drug molecules. Drug is released from the matrix network either by diffusion or erosion phenomenon. In the 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 feature, SEM studies were carried out for optimum formulation. Surface features of the minimatrices exposed to the dissolution medium for about 6h were observed by scanning electron microscopy. Minimatrices were introduced in dissolution medium and the test was started with the same test parameters as mentioned in section - Drug release study. Minimatrices were carefully withdrawn at 6h interval 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 minimatrix in dry state were also observed.

9.3.6.8 Kinetic modelling of drug release

Drug may be released from the minimatrices by either diffusion or erosion phenomenon. The type of release pattern followed can be interpreted by fitting the drug release data to various mathematical equations. In the present study, in vitro drug release data was fitted to zero-order release kinetics (equ 5.8), first-order release kinetics (equ 5.9), Higuchi's square root of time equation (equ 5.10) Ritger and Peppas power law (equ 5.11)

9.3.6.9 Swelling index

Swelling Index was determined by a method described in section 5.3.6.10. The study was carried upto 12 h. Swelling index was calculated by an equation 5.12.

9.3.6.10 Bioadhesion strength

Bioadhesive strength of the minimatrices was evaluated by Instron tensiometer (Instron 1121, UK). 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 minimatrix 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 minimatrix was completely detached from the tissue. Detachment force in "dynes/cm^{2}" was measured.

9.3.7 Optimisation of responses by desirability approach

Desirability is a process or formulation optimisation tool using multiple response data from a statistically planned experiment. This approach is used to achieve an optimum process or formula based on desirability values of different response variables. Overall desirability can be obtained by combining individual desirability values.

Goal of the present optimisation exercise is to obtain a formulation which should have low floating lag time, low % drug release at 1 h, high time t95, high swelling index and high bioadhesive strength. The individual desirability for each response was calculated using the following methods.

Floating lag time and % drug release at 1h values were minimized in the optimization procedure, as suitable formulation should have low lag time and less amount of drug should be released at initial hour. Desirability of these responses were calculated using following equation:

$$d_1 \text{ or } d_2 = Y_{max} - Y_i / Y_{max} - Y_{target} \quad \text{for } Y_i > Y_{target} \qquad (9.2)$$

$$d_1 \text{ or } d_2 = 1 \quad \text{for } Y_i < Y_{target} \qquad (9.3)$$

where d_1 is individual desirability for floating lag time and d_2 for % drug release at 1h.

Time required for 95% drug release, swelling index and bioadhesion strength values were maximized in the optimization procedure, as suitable formulation should have high values for these response variables. Desirability function of these responses were calculated using the following equation:

$$d_3 \text{ or } d_4 \text{ or } d_5 = Y_i - Y_{min} / Y_{target} - Y_{min} \text{ for } Y_i < Y_{target} \quad (9.4)$$

$$d_3 \text{ or } d_4 \text{ or } d_5 = 1 \text{ for } Y_i > Y_{target} \quad (9.5)$$

where d_3 is desirability for t95, d_4 for swelling index and d_5 for bioadhesive strength. Y_i is the experimental result. The overall desirability values were calculated from the individual values by using the following equation:

$$D = (d_1 d_2 d_3 d_4 d_5)^{1/5} \quad (9.6)$$

9.3.8 In vivo study by gamma scintigraphy

Optimum formulation was subjected for in vivo studies. These studies were carried out in healthy human volunteers having age group of 25 to 35 years. There was no history of previous illness in near past. Technetium (Tc99m) coupled with DTPA was used as radioactive material. Minimatrices were radiolabelled as described in section 5.3.8 (chapter 5.3.8). Small hole was made on one side of the minimatrix. Radioactive material having 6 milicurie of radioactivity was introduced using a fine needle. The hole was sealed using non-aqueous solution of ethyl cellulose to avoid leakage of the radioactive material. Radiolabelled formulation was introduced into hard gelatin capsule. Volunteers were fasted overnight and the radiolabelled formulation was administered after a standard breakfast. In vivo transit behaviour of the formulation was studied by capturing the images by Gamma camera (Siemens signature series). Images were taken at every hour.

9.3.9 Stability studies

Stability study of an optimum formulation was carried out as per ICH guidelines. Samples were packed in aluminium pouches using 0.04mm aluminium foil and subjected for stability at storage condition of 40°C/75%RH and 30°C/65%RH. Samples were withdrawn at 1, 2, 3 and 6 months time interval and analysed for physical appearance, floating lag time, clarithromycin content, bioadhesive strength and in vitro drug release pattern.

9.4 RESULTS AND DISCUSSION

9.4.1 Preliminary Experiments

Direct compression (Trial C-1) was tried as first attempt to prepare a formulation due to its minimum processing steps. Final blend was free flowing and compressible. It was observed during preparation of amoxicillin and levofloxacin minimatrices that direct compression blend did not yield free flowing formulation due to fluffy nature of

drug. This difficulty was not observed for clarithromycin as it is granular material having good flow properties. The final blend was compressible but the prepared minimatrices were having low hardness. Floating lag time of the formulation was 8-10 min. During dissolution testing the minimatrices disintegrated within 2 h. Low hardness and early disintegration of the minimatrices indicated that the formulation blend was not having sufficient cohesive strength which could keep the matrix in intact position for longer duration during dissolution test. For imparting sufficient cohesiveness in the blend, granulation technique was implemented. Non-aqueous solution of PVP K30 (5% w/v solution in IPA) was used as granulating agent. Resultant granular blend could produce minimatrices with higher hardness and good matrix integrity.

Preliminary trials revealed that HPMC K100M CR (formulation C-3) and polyox coagulant (formulation C-4) were excellent polymers for sustaining drug release. Polyox containing formulation trial C-4 was found to have minimum lag time. Carbopol based matrices (formulation C-5) could not maintain integrity due to its highly hydrophilic nature. Similarly chitosan based matrices (formulation C-7) disintegrated rapidly due to solubility of chitosan in acidic atmosphere. Xanthan gum (formulation C-2) was capable of maintaining matrix integrity due to its gelling property but it was unable to sustain drug release.

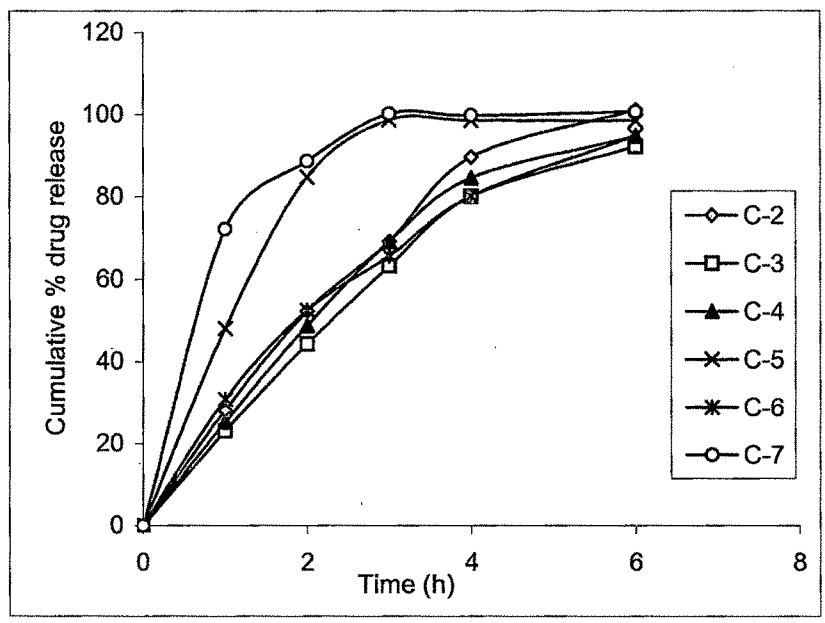

Fig 9.2 Dissolution profile of preliminary trial batches

Floating lag time of formulation C-8 and C-9 was evaluated. Inability of formulation C-9 to float indicated that presence of gas generating couple is essential for imparting floating feature to the formulation.

Dissolution profile of formulation C-8 was carried out. About 89% drug was released in 6 hours. During dissolution study of the preliminary studies, it was observed that for maintaining matrix integrity and thus sustaining drug release for longer duration, xanthan gum should be added to the formulation. It was decided to optimize the formulation systematically by 3^2 full factorial design approach. Polyox WSR coagulant was selected as one of the formulation variable as it shows good floating property and is also capable of sustaining drug release. Chitosan was selected as second variable due to its good bioadhesive property.

9.4.2 Drug excipient interaction study

Clarithromycin thermogram showed endothermic peak at 227.12°C (Fig 9.3). The blend of clarithromycin and other formulation excipients also showed an endothermic peak at the same temperature as shown by an individual clarithromycin. It clearly indicated that there is no interaction between drug and the excipients.

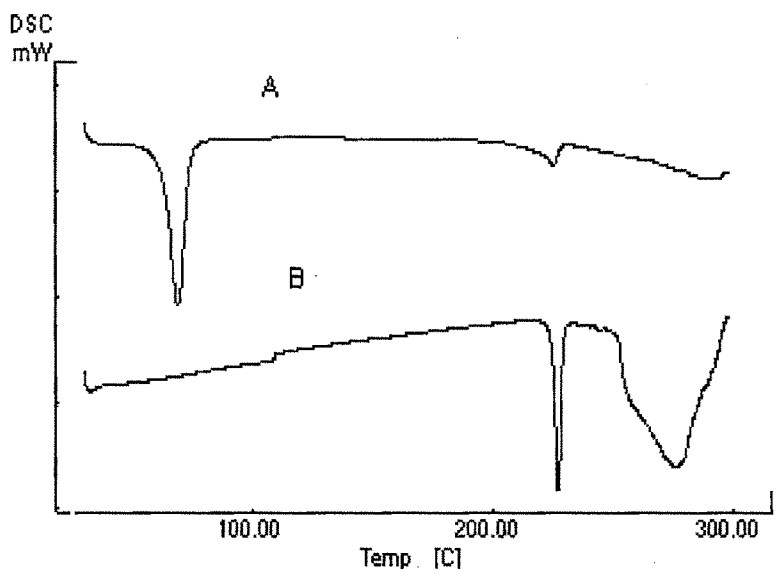

Fig 9.3 DSC thermograms (A) Blend of Clarithromycin with excipients (B) Pure Clarithromycin

9.4.3 Evaluation of granule properties

Bulk density and angle of repose of the lubricated blend was evaluated. Bulk density is an important aspect as it affects die fill and compression. It was found between 0.52 and 0.61 gm/cm^3 for the designed formulations. Flow properties of the granules were predicted from angle of repose. Uniform flow is important for avoiding weight variation during compression of the minimatrices. Angle of repose values were between 32° and 40° which indicates good flow properties.

9.4.4 Characterisation of minimatrices

9.4.4.1 Weight variation

Weight variation was 6.9% calculated with respect to theoretical weight of the minimatrix. It was found satisfactory as it is less than 10% which is allowable limit for the uncoated tablets having unit weight less than 80mg.

9.4.4.2 Thickness and hardness

Thickness of the minimatrices was between 2.7 and 2.9 mm and hardness about 2 – 3 Kg/cm^2

9.4.4.3 Friability

Friability of the minimatrices was nil indicating that the minimatrices completely withstand the stress during friability test.

9.4.4.4 Clarithromycin content

Clarithromycin content was estimated by HPLC method. The developed minimatrices were found to contain 98.8 to 101.7% of added amount of clarithromycin per minimatrix. Estimation procedure is described in detail in method section.

9.4.4.5 Floating lag time

Floating lag time is an important criterion for gastroretentive formulation. 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. The designed formulations were found to have floating lag time 8 to 11 min. Sodium bicarbonate and citric acid react in presence of acidic dissolution medium to generate carbon dioxide which gets entrapped as bubbles in polymer network and ultimately

decreases minimatrix density. Citric acid ensures gas bubble generation even in raised gastric pH. Viscous network formed by xanthan gum minimizes escaping of the bubbles from the polymer network. As the time passes the minimatrix system becomes hydrodynamically balanced and retains its floating property. Once started floating the formulations did not sink. Results of regression analysis indicate that formulation variables don't have significant effect on floating time. Buoyancy was achieved due to presence of gas generating couple in the formulation.

9.4.4.6 Drug release study

Sustained release feature ensures drug delivery over longer period of time. Gastroretentive feature retains the formulation in stomach and thus facilitates drug delivery at this site. This was desired goal of the present investigation as H. pylori resides in stomach mucosa necessitating longer exposure of therapeutic moiety for its complete eradication. Considering this goal, sustained release feature was imparted in the formulation. Minimatrices of preliminary trial C-8 could not maintain matrix integrity beyond 6h. This formulation was without xanthan gum. Hence it was necessary to include xanthan gum as one of the formulation components because it forms very viscous network after getting hydrated. This viscous network creates obstacles in drug diffusion channels formed by polymeric network. Ultimately it might have helped in retarding drug release at initial hour. Amongst the factorial design batches, drug release at 1h varied from 21.6% to 39.3%. Polyox and chitosan together played crucial role in decreasing drug release at initial hour (Fig 9.7). It was observed that even at high level of polyox, as chitosan level increased from low to high (CMT 08, CMT 07 and CMT 09), drug release at initial hour was increased from 21.6 % to 25.6%. It may be due to solubility of chitosan in acidic atmosphere. Minimum drug release of 21.6 % was observed for CMT 08 which contains high level of polyox and low level of chitosan.

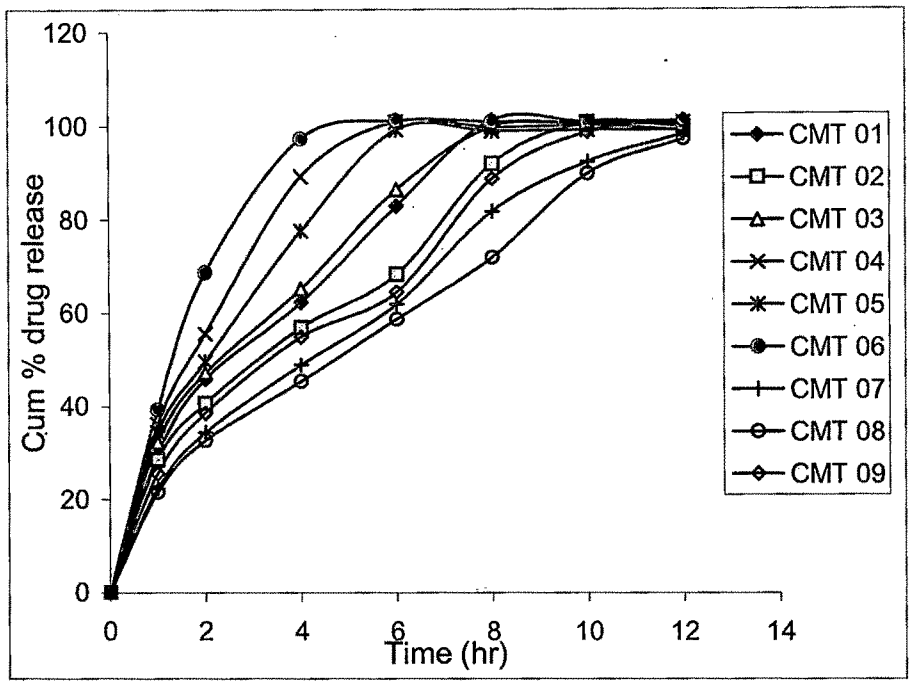

Fig 9.4 Dissolution profile of factorial design batches (CMT 01 to CMT 09)

Table 9.7 Experimental result of the response variables

Formulation	Pattern*	Y_1 (min)	Y_2 (%)	Y_3	Y_4	Y_5 (x10^{3}dynes/cm)
CMT 01	00	10	29.7	7.2	370.4	6.22
CMT 02	0-	8	28.6	8.4	390.8	5.69
CMT 03	0+	9	32.3	7	345.1	8.02
CMT 04	-0	8	36.2	4.6	310.6	6.13
CMT 05	--	11	34.4	5.4	324.1	5.34
CMT 06	-+	6	39.3	3.7	302.7	7.82
CMT 07	+0	6	22.4	10.6	460.9	6.57
CMT 08	+-	8	21.6	10.8	492.1	5.97
CMT 09	++	8	25.6	8.8	418.9	9.51

Table 9.8 Regression coefficients for different response variables

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_o	6.99	--	48.67	--	2.17	--	269.13	--	16.34	--
X_1	2.12	0.2075	-3.06	0.0012	1.24	0.0442	4.99	0.1145	0.40	0.2348
X_2	0.79	0.9507	12.42	0.0119	-2.50	0.5017	-40.97	0.1342	9.53	0.0286
X_1^2	-0.22	0.3917	-0.05	0.2958	-0.04	0.5531	3.08	0.0040	0.05	0.3766
X_2^2	0.93	0.7940	2.41	0.0307	-0.32	0.7437	-4.68	0.4605	2.09	0.0511
X_1X_2	0.91	0.2276	-0.16	0.2511	-0.05	0.7659	-9.44	0.0027	0.19	0.2138
R^2	0.6198	--	0.9990	--	0.9874	--	0.9993	--	0.9769	--

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

9.4.4.7 Scanning Electron Microscopy (SEM) studies

SEM image of minimatrix before exposure to dissolution medium shows intact surface (Fig 9.5a). Minimatrices were exposed to dissolution medium for 6h and surface images were captured at 25x, 50x and 100x (9.5b,c,d). These images show prominent pores which are openings of the drug release channels on minimatrix surface.

9.4.4.8 Kinetic modelling of drug release

Dissolution profile data was fitted to zero-order, first-order, Higuchi and Ritger and Peppas equations. Formulation having highest desirability i.e. CMT07 best fitted to Ritger and Peppas equations as maximum R^2 value of 0.995 was observed for this equation (Table 9.9). Value of release exponent "n" was 0.561 indicating anomalous type of drug release.

9.4.4.9 Fluid uptake study

Polyox has great tendency to absorb water and get rapidly hydrated. Overall hydration phenomenon depends upon the matrix composition. The minimatrix composition includes polyox and chitosan alongwith xanthan gum. Fluid uptake study was carried out in presence of 0.1N HCl as it simulates acidic conditions of stomach. Chitosan gets readily soluble in acidic conditions while polyox forms hydrated network. Fluid uptake was expressed in terms of swelling index. Highest value of 485.7 was observed for CMT 08 having high level of polyox and low level of chitosan. Swelling index indicates overall capability of the minimatrix to absorb the fluid and

ultimately hold it for longer duration. Hence it depends on overall matrix integrity of the formulation. As chitosan is soluble in acidic atmosphere, formulations containing high level of this variable were found to have less swelling index which may be due to improper matrix integrity over the period of time.

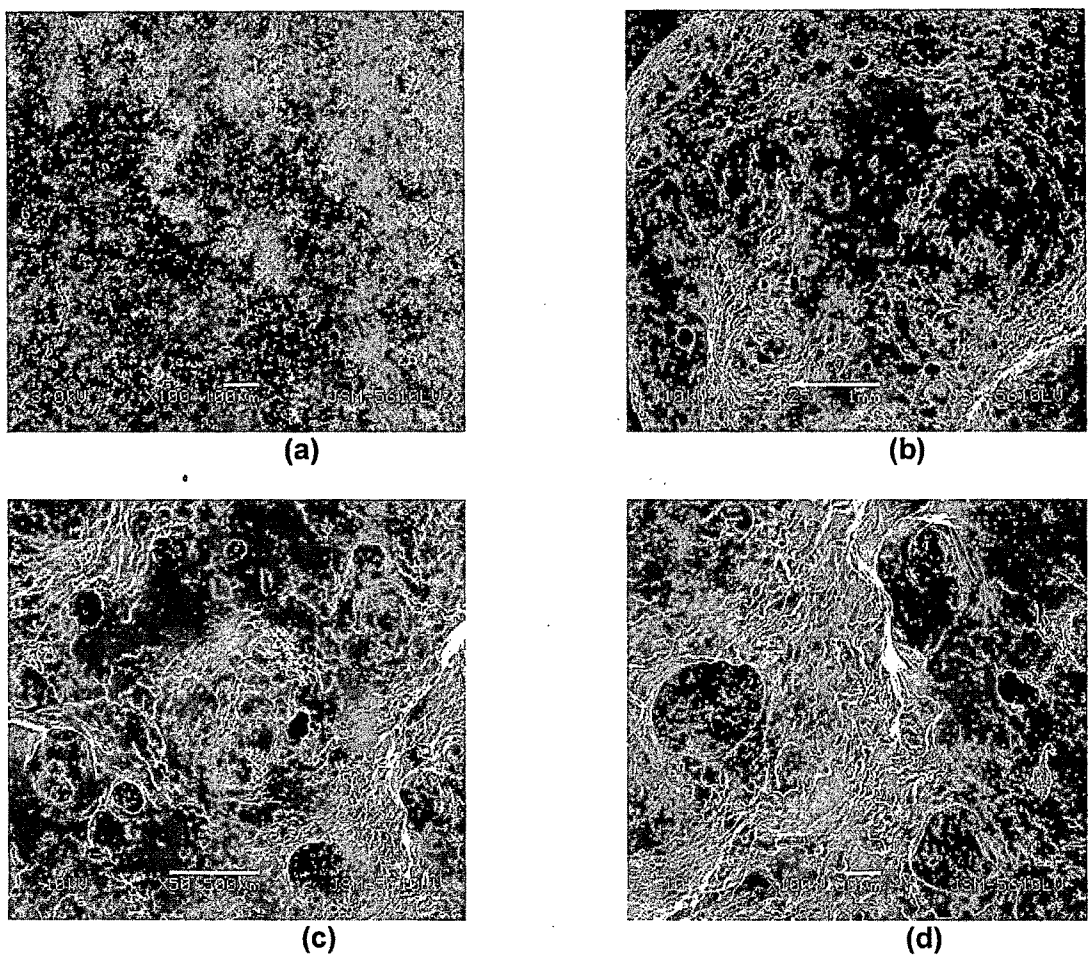

Fig 9.5 Scanning Electron Micrographs of formulation CMT07 (a) Initial dry surface of minimatrix at 50x; (b) (c) and (d) minimatrix surface after 6h exposure in dissolution medium at 25x, 50x and 100x magnification

Table 9.9 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
CMT 01	0.872	8.089	0.825	0.105	0.976	31.795	0.979	0.307	0.534
CMT 02	0.921	7.993	0.898	0.112	0.985	30.697	0.988	0.282	0.496
CMT 03	0.845	7.811	0.810	0.097	0.971	31.092	0.975	0.339	0.507
CMT 04	0.710	7.370	0.641	0.079	0.904	30.873	0.895	0.409	0.619
CMT 05	0.763	7.559	0.710	0.088	0.932	31.002	0.933	0.375	0.528
CMT 06	0.628	6.878	0.540	0.064	0.854	29.774	0.822	0.478	0.806
CMT 07	0.960	7.843	0.914	0.128	0.986	29.510	**0.995**	**0.225**	**0.561**
CMT 08	0.972	7.600	0.937	0.130	0.980	28.339	0.992	0.216	0.547
CMT 09	0.941	8.085	0.904	0.120	0.986	30.715	0.990	0.252	0.519

9.4.4.10 Ex vivo bioadhesion study

Orally administered formulation can be retained in stomach by making it capable to float on gastric contents and further it can be ensured by imparting gastric mucoadhesive properties in the formulation. Chitosan is a cationic polyamine with a high charge density at pH <6.5. It is a linear polyelectrolyte with reactive hydroxyl and amino groups. It reacts with the negatively charged sialic acid residues of mucin glycoproteins and hence it shows excellent mucoadhesive property. Due to this feature chitosan was selected as one of the formulation variables. Formulation CMT 03, CMT 06 and CMT 09 containing high amount of chitosan was found to have higher bioadhesive strength (Table 9.7). But it was also observed that bioadhesion strength also depends on the amount of polyox present in the respective formulation. CMT 09 was having high amount of chitosan and polyox. Hence its bioadhesive strength was 9.51×10^3 dynes/cm^2 that was highest amongst the designed formulations.

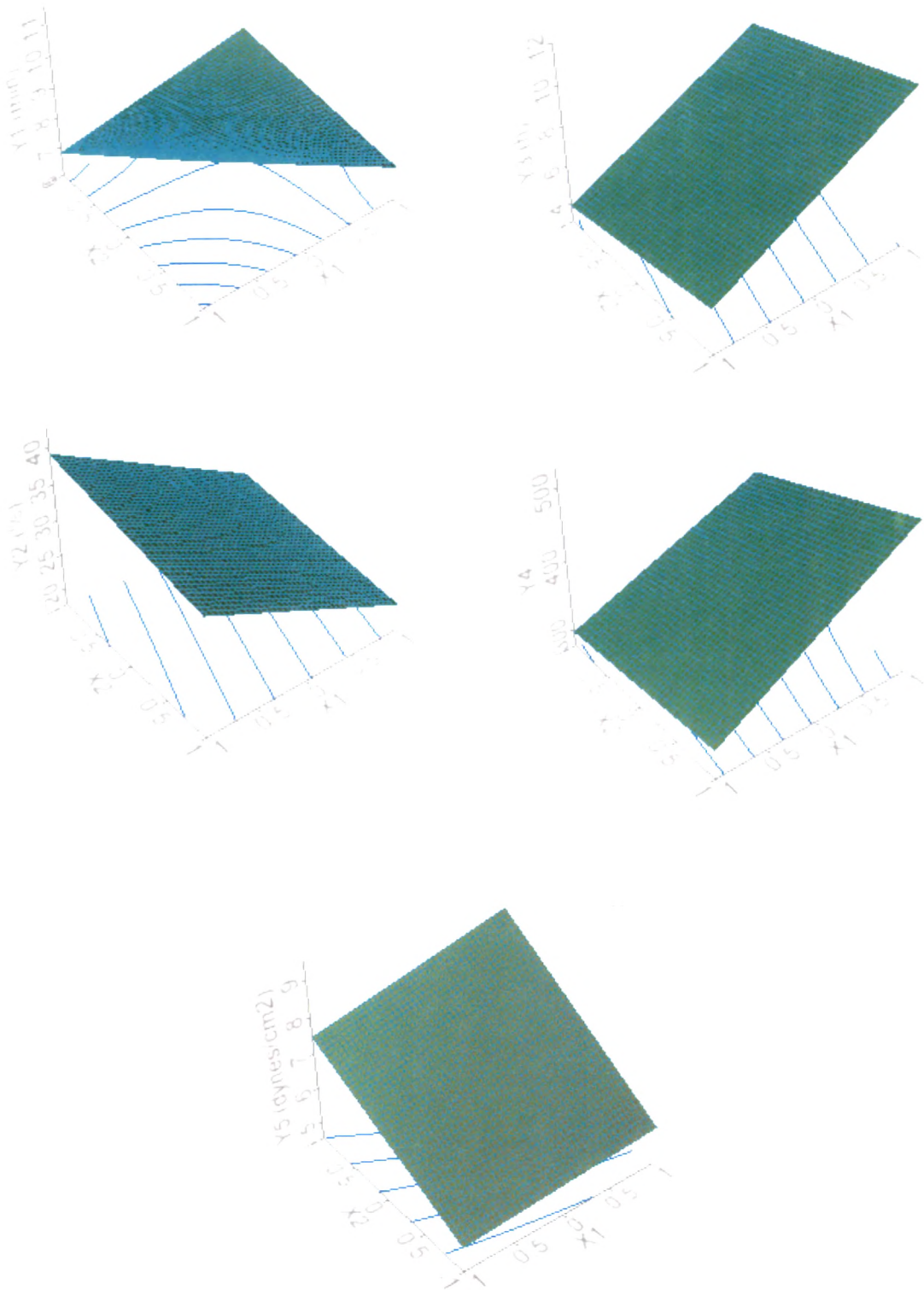

Fig. 9.6 Response surface plots

9.4.5 Optimisation of responses using desirability function

Optimum formulation was selected by using desirability function. Individual desirability was calculated for each response variable (equation 9.2 to 9.5) and then overall desirability was calculated form individual desirability values by equation 9.6. Overall desirability values are shown in Table 9.10. Formulation CMT 07 was having maximum desirability of 0.75 and hence it was selected as an optimum formulation.

Table 9.10 Overall desirability values

Formulation	Desirability
CMT 01	0.33
CMT 02	0.39
CMT 03	0.41
CMT 04	0.00
CMT 05	0.00
CMT 06	0.00
CMT 07	**0.75**
CMT 08	0.62
CMT 09	0.73

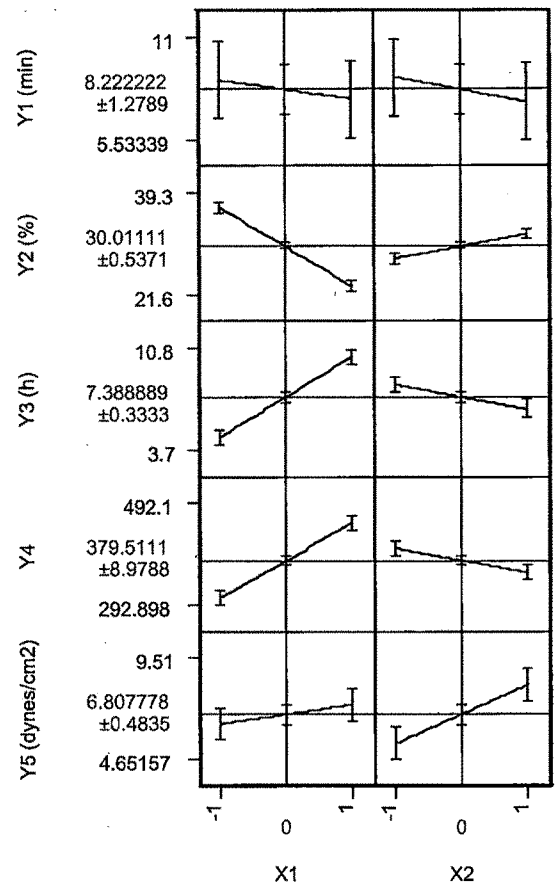

Fig 9.7 Prediction profiler showing effect of different levels of Formulation variables on response variables

9.4.6 In vivo study by gamma scintigraphy

Minimatrices were radiolabelled as described in section 5.3.8. Hard gelatin capsule containing four radiolabelled minimatrices was administered to volunteers and immediately first image was captured which shows intact capsule (Fig 9.8). In vitro studies have shown that the capsule gets disintegrated within 5 min and releases the minimatrices. Though exact disintegration time of the capsule was not determined in vivo, the image at 2 h shows four individual minimatrices indicating capsule disintegration. Images were captured every hour to ascertain location of the minimatrices. The images captured at 0, 2, 6, 8 and 9 h are shown here. Three minimatrices were observed in stomach till 8 h. One minimatrix might have escaped into small intestine or disintegrated. Image taken at 9 h did not show any minimatrix in stomach. It shows gastric residence time of 8 h for the developed minimatrices.

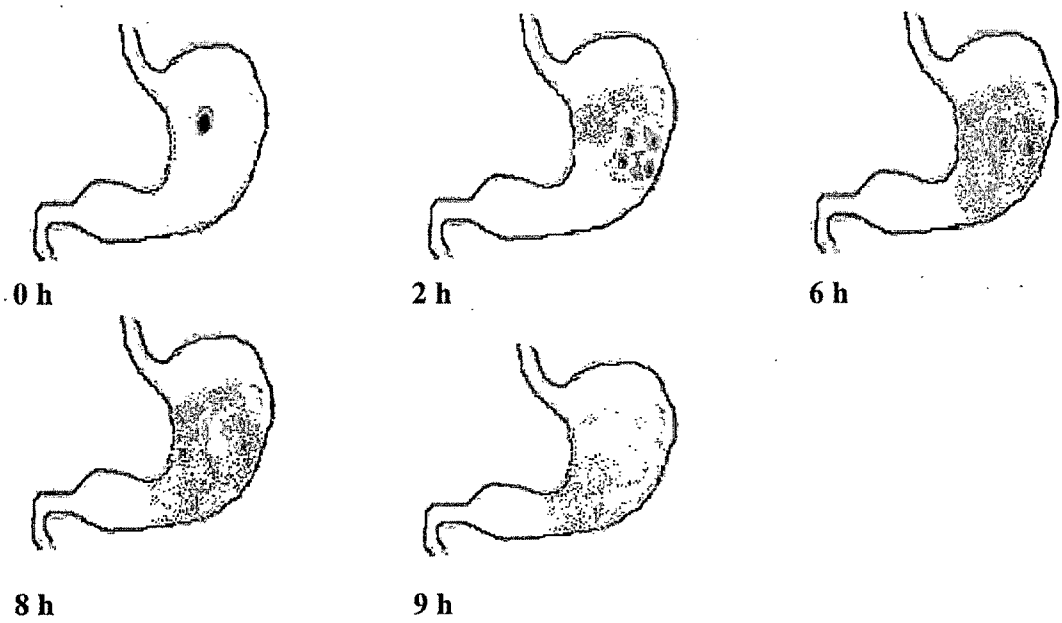

Fig 9.8 Gamma scintigraphy images showing gastric residence time of minimatrices

9.4.7 Stability studies

Stability samples of CMT 07 were analysed for the individual parameters as per the procedure described in method section. There was no significant change in buoyancy lag time, bioadhesive strength and dissolution profile which are critical parameters for gastroretention and sustained release phenomenon. Dissolution profile of stability samples were compared with initial sample profile by using similarity factor and it ranged from 78 to 84 which indicated their similarity.

Table 9.11 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 (%)	100.6	99.2	98.9	98.3	98.1

9.5 CONCLUSION

Clarithromycin minimatrices were designed by full factorial design approach. Optimum formulation was selected by desirability function approach. Regression analysis was carried to determine significance of the formulation variables on the

selected response variables. Formulation CMT 07 was optimum formulation. Minimatrices having very less buoyancy lag time and sustained drug release phenomenon has been successfully developed. In vivo studies show that the formulation has capability to remain in stomach upto 8 h. Thus the developed minimatrices can be utilized for sustained delivery of clarithromycin for longer duration in stomach which is very desirable phenomenon for effective treatment of *H.pylori* infection.