Chapter 10

Softgel Formulation of Clarithromycin

10.1 INTRODUCTION

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 levofloxacin softgel for sustained delivery of levofloxacin in stomach.

10.2 MATERIALS

Clarithromycin was received as a gift sample from Gujarat Liqui Pharma Caps Pvt Ltd., (Baroda, India). Hydroxypropylmethylcellulose (HPMC) K100M CR and Polyethylene oxide (Polyox) WSR coagulant were generously gifted by Colorcon Asia Pvt. Ltd. (Goa, India). Sodium alginate (Keltone HVCR) was gift sample from Anshul agencies (Mumbai, India). Xanthan gum was purchased from S.D. Fine Chem (Mumbai, India). Sodium bicarbonate, citric acid and polyethylene glycol 400 (PEG 400)e were purchased from Qualigens Fine Chemicals (Mumbai, India). Gelatin, glycerine, sorbitol solution 70%, methyl paraben and propyl paraben were provided by Gujarat Liqui Pharmacaps Pvt Ltd, Baroda.

10.3 METHODS

10.3.1 Preliminary trials

Role of different polymers for achieving sustained and gastroretentive properties was explored in preliminary trials. PEG 400 was used as a vehicle as it showed satisfactory performance in amoxicillin softgel formulation. Preliminary trials CSF 01 to CSF 03 (Table 10.1) were carried out to determine role of an individual polymer in the formulation. In this case, combination of HPMC K100 M CR and Polyox coagulant was tried as rate controlling polymer. Preliminary formulations were evaluated for floating lag time and total floating duration, matrix integrity, swelling characteristics and drug release pattern.

10.3.2 Experimental design

HPMC K100M CR and Polyox WSR coagulant in 1:1 ratio (X_1) and sodium alginate (X_2) each at three different levels were selected as formulation variables.

Full factorial design (3^2) was implemented for optimizing the formulation and total 9 experiments were designed as shown in Table 10.2

Table 10.1 Composition of preliminary trial batches

Ingredients (mg per Softgel)	CSF 01	CSF 02	CSF 03
Clarithromycin	100	100	100
HPMC K100M CR	70	--	35
Polyox WSR coagulant	--	70	35
Sodium alginate	15	15	15
Xanthan gum	15	15	15
Sodium bicarbonate	60	60	60
Citric acid	20	20	20
PEG 400	300	300	300

Drug release at 1 hour (Y_1), time required for 50% drug release (Y_2), time required for 95% drug release (Y_3) swelling index (Y_4) were selected as response variables. Regression analysis was carried out to get a quantitatively correlate formulation and response variables. The equation can be given as

$$Y_i = b_0 + b_1X_1 + b_2X_2 + b_{11}X_1^2 + b_{22}X_2^2 + b_{12}X_1X_2 \qquad (10.1)$$

where b_0 is arithmetic mean of 9 runs; b_i is an estimated coefficient for factors X_1 and X_2. All experimental results were computed by statistical software DOE v6.0.5 (Stat-Ease Inc., Minneapolis, MN, USA). Response surface plots, showing effect of formulation variables on various response variables, were generated using JMP software v5.1 (SAS Institute Inc., Cary, NC, USA)

10.3.3 Preparation of Softgel capsules

10.3.3.1 Preparation of gelatin for capsule shell

Gelatin for the capsule shell was prepared as described in section 6.3.3.1 (Chapter 6)

10.3.3.2 Blend encapsulation in softgel capsule shell

Encapsulation was carried out by continuous rotary die process as described in section 6.3.3.2

Table 10.2 Formulation designing by 3^2 full factorial design

Formulation No.	Factor Levels	
	X_1	X_2
CSF 04	-1	0
CSF 05	+1	-1
CSF 06	-1	+1
CSF 07	0	0
CSF 08	+1	0
CSF 09	+1	+1
CSF 10	0	-1
CSF 11	-1	-1
CSF 12	0	+1
Coded values	**Actual values***	
	X_1	X_2
-1	20	10
0	40	20
+1	60	30

*Actual values indicate quantity in mg per softgel

Table 10.3 Composition of the factorial design batches

Ingredients (mg per softgel)	CSF 04	CSF 05	CSF 06	CSF 07	CSF 08	CSF 09	CSF 10	CSF 11	CSF 12
Clarithromycin	100	100	100	100	100	100	100	100	100
Polyox WSR coagulant	10	30	10	20	30	30	20	10	20
HPMC K100M CR	10	30	10	20	30	30	20	10	20
Sodium alginate	20	10	30	20	20	30	10	10	30
Xanthan gum	15	15	15	15	15	15	15	15	15
Sodium bicarbonate	24	24	24	24	24	24	24	24	24
Citric acid	8	8	8	8	8	8	8	8	8
PEG 400	300	300	300	300	300	300	300	300	300
Total wt	487	517	497	507	527	537	497	477	517

10.3.3.3 Drying of softgel capsules

Prepared softgel capsules were dried at temperature 26° to 27°C and relative humidity 20% to 22% for 48 h.

10.3.4 Evaluation of softgel capsules

10.3.4.1 Weight variation

Contents of twenty softgel capsules were removed and individually weighed. Percent weight variation was calculated with respect to actual fill weight.

10.3.4.2 Assay

Clarithromycin content in the softgel was estimated by HPLC method. Content of twenty softgel capsules were completely removed. Blend equivalent to fill weight of one softgel was accurately weighed and transferred to 100 ml volumetric flask. About 70-80 ml of mobile phase was added and it was sonicated (Modern Industrial Corporation, Mumbai, India) for 20 min. Volume was made upto 100 ml by adding mobile phase. The solution was filtered using Whatman filter paper type I. Suitable portion of the filtrate (0.5 ml) was diluted to 10 ml with mobile phase and 20 µl of the finally diluted solution was injected. Drug content was calculated from the peak area obtained.

10.3.4.3 Buoyancy lag time and total buoyancy time

Buoyancy lag time was determined simultaneously during drug release study. It is the time interval between introduction of softgel in the dissolution vessel to the time when it starts floating towards the surface of dissolution medium. Total time for which the softgel was able to float was also determined.

10.3.4.4 Dissolution Profile

Drug release study was carried out using USP type II dissolution test apparatus (VDA 6-DR, Veego Instruments Corporation, Mumbai, India) at 50 rpm. Nine hundred milliliter of 0.1N HCl was used as dissolution medium. One softgel was introduced in each vessel. Ten milliliter sample was withdrawn at 1,2,4,6,8,10 and 12 h and was replenished with equal volume of dissolution medium. Clarithromycin release was estimated in suitably diluted samples by colorimetric method.

10.3.4.5 Analysis of drug release data

Drug release data was fitted to various kinetic equations such as zero-order (equ 5.8), first-order (equ 5.9), Higuchi's square root of time (equ 5.10) and Ritger and Peppas equation (equ 5.11)

10.3.5 Optimisation by desirability approach

Factorial design approach was used for designing the formulations. Optimum formulation was selected from the designed formulations by desirability approach. Individual desirability was calculated for each response variable. It varies from 0 to 1 according to closeness of the response to its desired value. Individual desirability values were combined to calculate an overall desirability. Formulation with highest overall desirability value was called optimum formulation.

In the context of present optimisation exercise, optimum formulation should release less amount of drug at 1 h, should require high time for 50% drug release and 95% drug release and should have high swelling index. Individual desirability for each response was calculated using the following methods.

As optimum formulation should release less amount of drug at initial hour, this response was minimized while calculating individual desirability value. Desirability value, d_1, of this response was calculated using following equation:

$$d_1 = Y_{max} - Y_i / Y_{max} - Y_{target} \quad \text{for } Y_i > Y_{target} \tag{10.2}$$

$$d_1 = 1 \quad \text{for } Y_i < Y_{target} \tag{10.3}$$

where Y_{max} and Y_{target} indicate maximum and target (minimum) value of experimental result for Y_1 (Table 10.4). Y_i is the experimental result for individual factorial design batch.

Values indicating time required for 50% drug release, time required for 95% drug release and swelling index were maximized as optimum formulation should have high values for these response variables. Individual desirability of these response variables were calculated using the following equation:

$$d_2 \text{ or } d_3 \text{ or } d_4 = Y_i - Y_{min} / Y_{target} - Y_{min} \quad \text{for } Y_i < Y_{target} \tag{10.4}$$

$$d_2 \text{ or } d_3 \text{ or } d_4 = 1 \quad \text{for } Y_i > Y_{target} \tag{10.5}$$

where d_2, d_3 and d_4 indicate desirability for Y_2 and Y_3 and Y_4. 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:

$$D = (d_1 d_2 d_3 d_4)^{1/4} \qquad (10.6)$$

10.3.6 In vivo studies by gamma scintigraphy

Optimum formulation was selected from the in vitro studies and it was subjected to in vivo evaluation in healthy human volunteers having age group 25 to 35 years. They were non-alcoholic and there was no history of illness. Gamma scintigraphy technique was used for in vivo studies. Formulation was radiolabelled as described in section 6.3.7. One softgel each was administered to healthy human volunteers after a standard breakfast. Softgel was located in the GIT by capturing images with the help of gamma camera (e-cam signature series, Siemens).

10.3.7 Stability studies

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

10.4 RESULTS AND DISCUSSION

10.4.1 Preliminary Experiments

HPMC K100M CR and Polyox WSR coagulant in 1:1 ratio were found effective in sustaining drug release as compared to the individual polymer. Sodium alginate assist rate controlling polymers in sustaining drug release by forming insoluble matrix in presence of acidic dissolution medium.

10.4.2 Preparation of softgel capsules

Encapsulation blend for different batches were prepared as described in Table 10.3 and encapsulated in the gelatin shell as described in section 6.3.3.2.

10.4.3 Evaluation of softgel

10.4.3.1 Weight variation

Weight variation of softgel contents were 6.2% calculated with respect to actual fill weight.

10.4.3.2 Clarithromycin content

Clarithromycin content was found in the range 97.6 to 101.9% of added amount of clarithromycin per softgel.

10.4.3.3 Floating lag time and total floating time

Designed formulations were found to have same floating lag time of 9-12 min as all of them (CSF 04 to CSF 12) contained same level of gas generating couple. Initial floating property is imparted by the gas generating couple and further floating phenomenon might be achieved as the system become hydrodynamically balanced.

10.4.3.4 Drug release study

Sustaining clarithromycin release form buoyant formulation was prime aim of present development work. Gas generating couple imparted floating feature in the formulation. HPMC K100M CR together with Polyox played crucial role in sustaining clarithromycin release.

HPMC and Polyox, both are hydrophilic polymers which swell in presence of aqueous medium and are widely used as rate-controlling polymers in various modified drug release systems. These are available in different viscosity grades that are capable of providing different sustained release patterns for various drug entities. In the present work, high viscosity grades of HPMC and PEO were used. Combination of both the polymers was found effective in sustaining clarithromycin release. Hence 1:1 ratio at different level was used as one of the formulation variable. Second variable was sodium alginate at three different levels. Sodium alginate gets hydrated in presence of aqueous dissolution medium and forms

insoluble matrix in acidic dissolution medium. As the developed formulation is intended to release clarithromycin in acidic atmosphere of stomach, sodium alginate was considered as one of the potential excipient for sustaining drug release. Results of drug release study proved this assumption. Regression analysis in Table 10.5 clearly shows significant effect of sodium alginate (X_2) in decreasing drug release at 1 h and increasing the time required for 50% drug release as Prob>F values for these terms were less than 0.05

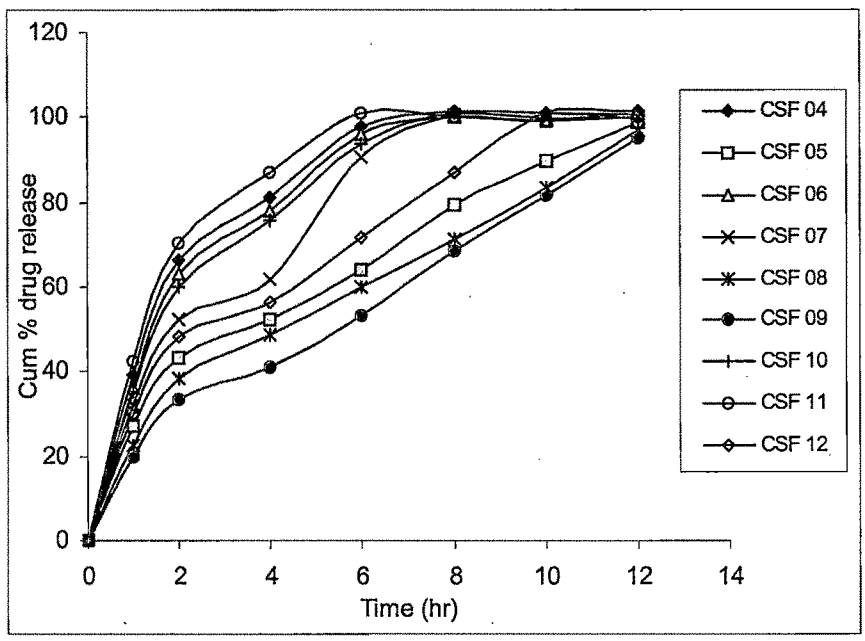

Fig 10.1 Dissolution profile of factorial design batches

Table 10.4 Experimental Results

Formulation no.	Y_1 (%)	Y_2 (h)	Y_3 (h)	Y_4
CSF 04	39.2	1.3	5.5	345.2
CSF 05	27.1	3.4	11.1	455.7
CSF 06	36.3	1.4	5.8	360.3
CSF 07	32.1	1.8	6.6	400.3
CSF 08	22.4	4.2	11.7	496.3
CSF 09	19.8	5.5	11.9	525.9
CSF 10	35.2	1.5	6.2	382.5
CSF 11	42.3	1.2	5	340.3
CSF 12	29.9	2.3	9	426.4

Xanthan gum retards drug release by creating viscous network in the polymeric drug diffusion channels. Also preliminary trials indicated that xanthan gum played crucial role in maintaining matrix integrity. This phenomenon ultimately helped in sustaining drug release. Hence xanthan gum was included as one of the formulation component. Drug release study was carried out upto 12h. Drug release at 1 h, time required for 50% drug release and time required for 95% drug release was estimated from dissolution study. Effect of the formulation variables on these response variables was interpreted from prediction profiler in Fig 10.4 and response surface plots in Fig 10.2

10.4.3.5 Kinetic modelling of drug release

Dissolution profile data was fitted to zero order, first order, Higuchi and Ritger and Peppas equation. This curve fitting exercise provided release rate constant and coefficient of determination (Table 10.6). Dissolution profile data of optimum formulation CSF 09 best fitted to Ritger and Peppas equation. Value of release exponent "n" was 0.524 (Table 10.6) indicating anomalous type of drug release.

10.4.3.6 Regression analysis

Regression analysis was carried out to correlate formulation and response variables. Results of regression analysis indicated that formulation variables X_1 and X_2 significantly reduced initial hour drug release (Table 10.5) Time required for 50% drug release was extended due to X_1 and X_2 but magnitude of X_1 in decreasing the drug release was higher as compared to X_2 as can be seen from the regression analysis in Table 10.5. It may be due to effective sustained release properties of HPMC K100M CR and Polyox. Time required for 95% drug release was significantly affected due to only X_1 and not X_2 (Table 10.5)

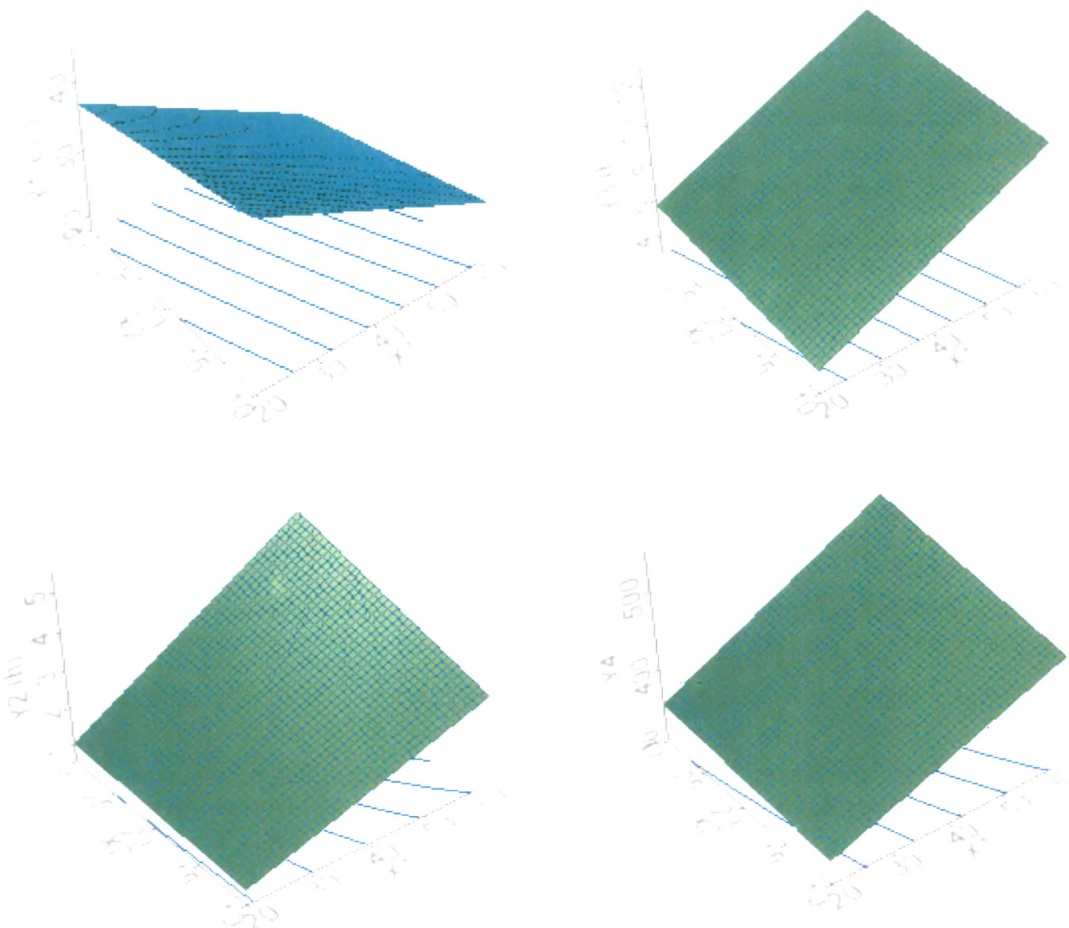

Fig 10.2 Response surface plots

Table 10.5 Results of regression analysis

Term	Y_1		Y_2		Y_3		Y_4	
	EC	Prob > F	EC	Prob > F	EC	Prob > F	EC	Prob > F
b_o	32.044		1.789		7.111		402.233	
X_1	-8.083	< 0.0001	1.533	0.0001	3.067	0.0026	72.017	< 0.0001
X_2	-3.100	0.0008	0.517	0.0031	0.733	0.1107	22.350	0.0008
X_1^2	-1.217	0.0527	0.967	0.0025	1.233	0.1176	17.550	0.0080
X_2^2	0.533	0.2655	0.117	0.3341	0.233	0.7080	1.250	0.6834
X_1X_2	-0.325	0.3242	0.475	0.0070	0.000	1.0000	12.550	0.0078
R^2	0.998	-	0.997	-	0.970		0.999	-

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

Table 10.6 Kinetic modelling of drug release data

Formulation	Zero order		First order		Higuchi		Ritger & Peppas		
	R^2	K_0	R^2	K_1	R^2	K_H	R^2	K_R	n
CSF 04	0.713	7.137	0.664	0.070	0.915	30.016	0.905	0.451	0.758
CSF 05	0.932	7.337	0.911	0.106	0.993	28.118	0.984	0.282	0.474
CSF 06	0.740	7.258	0.680	0.076	0.929	30.188	0.914	0.417	0.800
CSF 07	0.831	7.800	0.789	0.095	0.961	31.147	0.955	0.344	0.470
CSF 08	0.951	7.160	0.905	0.117	0.989	27.100	0.986	0.241	0.529
CSF 09	0.974	7.226	0.937	0.130	0.972	26.802	**0.981**	**0.208**	**0.524**
CSF 10	0.762	7.396	0.705	0.081	0.940	30.492	0.930	0.405	0.774
CSF 11	0.652	6.767	0.601	0.061	0.877	29.127	0.865	0.496	0.733
CSF 12	0.912	7.797	0.891	0.112	0.989	30.146	0.980	0.305	0.457

10.4.4 Optimisation of responses using desirability function

Individual desirability values were calculated for the response variables and then overall desirability value was calculated from the individual values (equ 10.6). Highest desirability value of 1.00 was observed for formulation CSF 09 (Table 10.7) which indicates that it is an optimum formulation. Overall desirability value is an indicator of optimum formulation as it is calculated from the individual values which are actually based on the desirable response.

Table 10.6 Overall desirability values

Formulation	Desirability
CSF 04	0.05
CSF 05	0.66
CSF 06	0.11
CSF 07	0.26
CSF 08	0.84
CSF 09	**1.00**
CSF 10	0.17
CSF 11	0.00
CSF 12	0.44

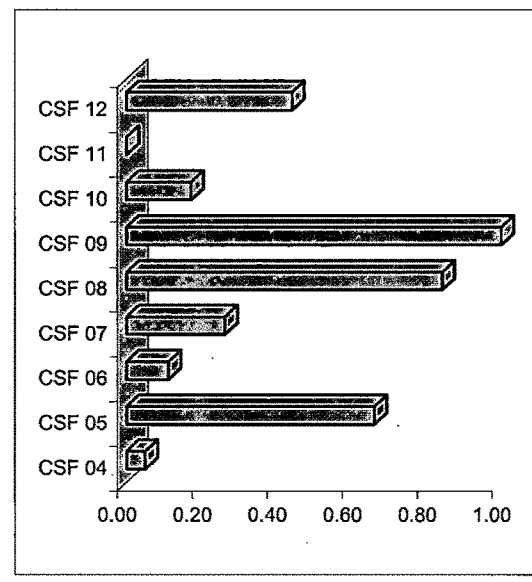

Fig 10.3 Overall Desirability

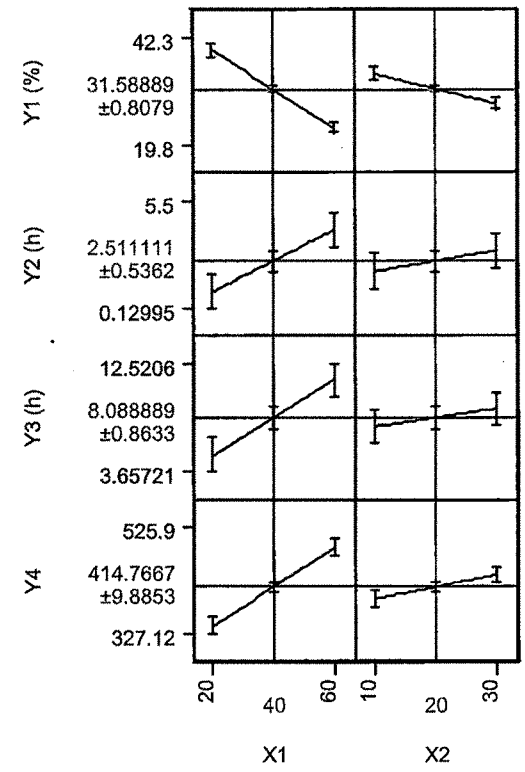

Fig 10.4 Prediction profiler showing effect of increasing level of formulation variable on response variables

10.4.5 In vivo study by gamma scintigraphy

Gastric residence time of the optimum formulation CSF 09 was determined by gamma scintigraphy technique. The radioactive moiety introduced in the formulation emits non hazardous and low intensity gamma rays which can be detected by gamma camera. Ultimately the emitted radiation indicated location of the softgel in gastrointestinal tract. First image was captured immediately after administration (0 h) and then imaging was done at every hour. Images captured at 0, 2, 6, 8 and 9 h have been shown in Fig 10.5. Imaging was continued until the softgel disappeared from stomach. This exercise shown presence of softgel till 8 h in stomach. With increasing time, softgel matrix was found eroding.

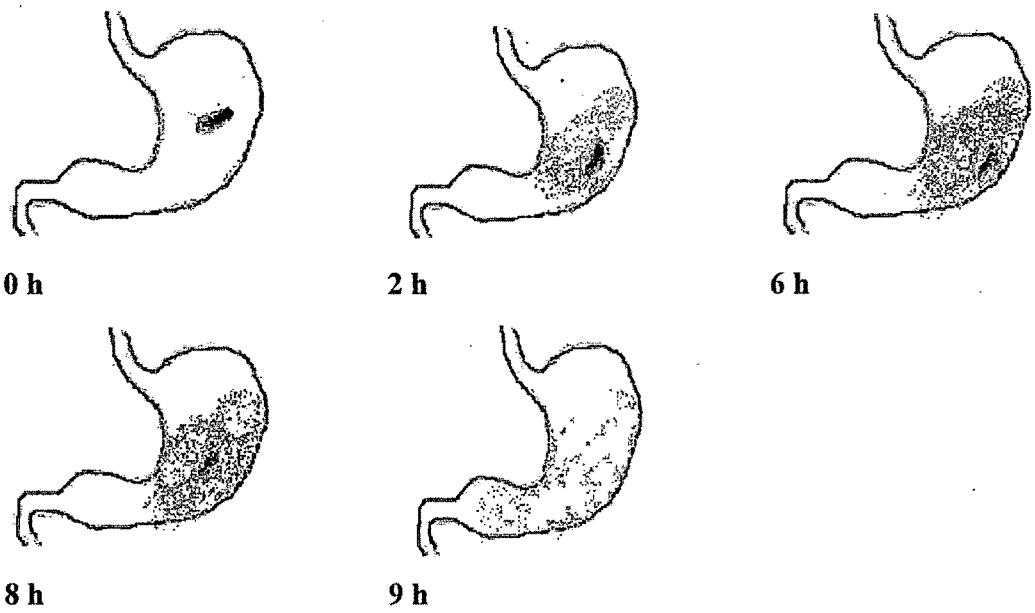

Fig 10.5 Gamma scintigraphy images showing gastric residence time of softgel formulation

10.4.6 Stability studies

Stability samples were analysed for various parameters as per the procedure described in method section. There was no significant change in assay, floating lag time and dissolution profile. It indicates that the formulation is stable. Dissolution profile of stability samples were compared with initial sample profile by using similarity factor and it ranged from 83 to 94 which indicated their similarity.

Table 10.8 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)	10	8	12	11	12
Levofloxacin content (%)	101.6	99.5	98.6	98.4	98.3

10.5 CONCLUSION

Softgel formulation having gastroretentive feature has been developed for clarithromycin in the present investigation. Full factorial design (3^2) was used as an optimisation tool. Formulation was optimized with respect to various in vitro parameters. Desirability approach was proved as useful tool for selection of optimum formulation. Formulation CSF 09 was optimum formulation. Gamma scintigraphy technique shown that optimum formulation was having gastric residence time of 8 h. Hence the developed formulation is an effective tool for clarithromycin delivery in stomach for longer duration which is important therapeutic strategy for H.Pylori eradication. Also the scale up on large scale is feasible by using softgel manufacturing facility.