Chapter 8

Softgel Formulation of Levofloxacin

8.1 INTRODUCTION

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.

8.2 MATERIALS

Levofloxacin was received as a gift sample from Blue Cross Labs Ltd, (Nashik, India). Hydroxypropylmethylcellulose (HPMC) K100M CR was gifted by Colorcon Asia Pvt. Ltd. (Goa, India) and sodium alginate (Keltone HVCR) was a generously gifted by 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) 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.

8.3 METHODS

8.3.1 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 LSF 01 to LSF 06 (Table 8.1) were carried out to determine role of an individual polymer in the formulation. Formulations of the preliminary trial were evaluated for floating lag time and total floating duration, matrix integrity, swelling characteristics and drug release pattern.

8.3.2 Experimental design

Preliminary trials have shown promising results for HPMC K100M CR and sodium alginate. Full factorial design (3^2) was implemented for optimizing the formulation. HPMC K100M CR (X_1) and sodium alginate (X_2) each at three different levels were selected as formulation variables. Total 9 experiments were designed as

shown in Table 8.2 Formulation variables and their levels were decided from the preliminary studies carried out in the laboratory.

Table 8.1 Composition of preliminary trial batches

Ingredients (mg per Softgel)	LSF 01	LSF 02	LSF 03	LSF 04	LSF 04	LSF 05
Levofloxacin hemihydrate *	102.48	102.48	102.48	102.48	102.48	102.48
HPMC K4M CR	100	--	--	--	--	--
HPMC K100M CR	--	100	--	--	--	--
HPMC K100 LV	--	--	100	--	--	--
Polyox WSR coagulant	--	--	--	100	--	--
Sodium alginate	--	--	--	--	100	
Xanthan gum	--	--	--	--	--	100
Sodium bicarbonate	60	60	60	60	60	60
Citric acid	20	20	20	20	20	20
PEG 400	300	300	300	300	300	300

*Equivalent to 100 mg of levofloxacin

Effect of formulation variables was observed on drug release at 1 hour (Y_1), time required for 50% drug release (Y_2), time required for 95% drug release (Y_3) and swelling index (Y_4) which are called as response variables. These response variables were fitted to quadratic model and regression analysis was carried out to get a quantitative relationship between formulation and response variables. The equation can be given as

$$Y_i = b_0 + b_1X_1 + b_2X_2 + b_{11}X_1^2 + b_{22}X_2^2 + b_{12}X_1X_2 \qquad (8.1)$$

where b_0 is arithmetic mean of 9 runs; b_i is an estimated coefficient for factors X_1 or 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)

8.3.3 Preparation of Softgel capsules

8.3.3.1 Preparation of gelatin for capsule shell

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

Table 8.2 Formulation designing by 3^2 full factorial design

Formulation No.	Factor Levels	
	X_1	X_2
LSF 07	-1	+1
LSF 08	0	-1
LSF 09	-1	-1
LSF 10	0	0
LSF 11	-1	0
LSF 12	+1	-1
LSF 13	+1	+1
LSF 14	0	+1
LSF 15	+1	0
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 8.3 Composition of the factorial design batches

Ingredients (mg per softgel)	LSF 07	LSF 08	LSF 09	LSF 10	LSF 11	LSF 12	LSF 13	LSF 14	LSF 15
Levofloxacin hemihydrate	102.48	102.48	102.48	102.48	102.48	102.5	102.48	102.48	102.48
HPMC K100M CR	20	40	20	40	20	60	60	40	60
Sodium alginate	30	10	10	20	20	10	30	30	20
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	300.52	300.52	300.52	300.52	300.52	300.5	300.52	300.52	300.52
Total Wt	500	500	480	510	490	520	540	520	530

*Equivalent to 100 mg of levofloxacin

8.3.3.2 Blend encapsulation in softgel capsule shell

Encapsulation was carried out by continuous rotary die process as described in chapter 6 (section 6.3.3.2)

8.3.3.3 Drying of softgel capsules

Controlled temperature and humidity conditions were maintained while drying softgel capsules. Temperature was maintained at 26° to 27°C and relative humidity of 20% to 22%.

8.3.4 Evaluation of softgel capsules

8.3.4.1 Weight variation

Twenty softgel capsules were individually weighed and the weight was noted. Contents the capsules were completely removed and empty shell weight was noted. Weight of encapsulated blend was calculated from the difference in weight of intact capsule and its empty shell (IP, 2007).

8.3.4.2 Levofloxacin content

Levofloxacin content in the softgel was estimated by HPLC method. Contents of twenty softgel capsules were completely removed 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 (0.1 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.

8.3.4.3 Buoyancy lag time and total buoyancy time

Buoyancy lag time was determined by introducing softgel in dissolution vessel containing 900 ml of 0.1 N HCl. Paddle rotation was started at 50 rpm. The time interval between introduction of softgel in the dissolution vessel to the time when it starts floating towards the surface of dissolution medium was noted. The total duration for which softgel was capable to float, called total buoyancy time, was also noted.

8.3.4.4 Dissolution Profile

Dissolution profile was carried out using USP type II (paddle type) 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. Five

milliliter sample was withdrawn at 1,2,4,6,8,10 and 12 h and was replenished with equal volume of dissolution medium. Suitably diluted sample was analysed by UV spectrophotometric method for determining amount of drug release.

8.3.4.5 Analysis of drug release data

Dissolution profile data was analysed by fitting it to various kinetic equations such as zero-order (equation 5.8), first-order (equation 5.9), Higuchi's square root of time (equation 5.10) and Ritger and Peppas (equation 5.11)

8.3.5 Optimisation by desirability function

Formulations were designed by 3^2 full factorial design. Optimum formulation was selected from the designed formulations by desirability approach. In this approach, individual desirability was calculated for each response variable that varies from 0 to 1 according to closeness of the response to its target 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 values of this response was calculated using following equation:

$$d_1 = Y_{max} - Y_i / Y_{max} - Y_{target} \quad \text{for } Y_i > Y_{target} \tag{8.2}$$

$$d_1 = 1 \quad \text{for } Y_i < Y_{target} \tag{8.3}$$

where d_1 individual desirability for % drug release at 1 h respectively. The Y_{max} and Y_{target} indicate maximum and target (minimum) value of this response variables in factorial design batches (Table 8.4). Y_i is the experimental result of 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:

$$d_2 \text{ or } d_3 \text{ or } d_4 = Y_i - Y_{min} / Y_{target} - Y_{min} \text{ for } Y_i < Y_{target} \quad (8.4)$$

$$d_2 \text{ or } d_3 \text{ or } d_4 = 1 \text{ for } Y_i > Y_{target} \quad (8.5)$$

where d_2, d_3 and d_4 indicate desirability for Y_2, Y_3 and Y_4 respectively. Y_i is the experimental result. The Y_{min} and Y_{target} indicate minimum and target (maximum) value of experimental result for respective response variables in factorial design batches. Y_i is the experimental result of the response variable for respective factorial design batch.

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

$$D = (d_1 d_2 d_3 d_4)^{1/4} \quad (8.6)$$

8.3.6 In vivo gastric residence time by gamma scintigraphy

In vivo studies were carried out in three healthy human volunteers having age 25 to 35 years. They were non-alcoholic and there was no history of illness. Softgel was radiolabelled as described in section 6.3.7 One radiolabelled softgel was administered to each healthy human volunteer after a standard breakfast and a glass of water. Images were captured by gamma camera (e-cam signature series, Siemens) to ascertain gastric residence time of softgel.

8.3.7 Stability studies

Optimum formulation was subjected to stability studies 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 months time interval and analysed for physical appearance, floating lag time, drug content and drug release pattern.

8.4 RESULTS AND DISCUSSION

8.4.1 Preliminary Experiments

Preliminary trials indicated that HPMC K100M CR and sodium alginate showed promising role in sustaining drug release. Gas generating couple was essential for obtaining floating phenomenon. PEG 400 was found to be satisfactory vehicle for preparation of fill material suspension.

8.4.2 Preparation of softgel capsules

Encapsulation blends were prepared for the factorial design batches as shown in table 8.3 and it was encapsulated by continuous rotary die process.

8.4.3 Evaluation of softgel

8.4.3.1 Weight variation

Weight variation of softgel contents was 3.9% calculated with respect to theoretical fill weight. It passes the test as per pharmacopoeial specification (IP, 2007) as the variation is less than 7.5% which is allowable limit for the soft gelatin capsules having fill weight more than 300 mg.

8.4.3.2 Content uniformity

Levofloxacin content was found in the range 97.9% to 101.3% of added amount of levofloxacin per softgel. These results shown satisfactory content uniformity.

8.4.3.3 Floating lag time and total floating time

Floating lag time for all the designed formulations (LSF 07 to LSF 15) was in the range 8-10 min. it did not differ significantly amongst the designed formulations which indicated that this parameter totally depends on the level of gas generating couple. The formulations that were capable to maintain matrix integrity upto 12h were also capable to float till that time. Initial floating property is imparted by the gas generating couple and further floating phenomenon might be achieved as the system becomes hydrodynamically balanced.

8.4.3.4 Dissolution profile

Aim of the present investigation was to obtain buoyant and sustained release formulation. Buoyancy was achieved by incorporating sodium bicarbonate and citric acid as a gas generating couple. For obtaining a second feature, HPMC K100M CR and sodium alginate were used as sustained release polymers. These two polymers, each at three different levels were selected as formulation variables and experiments were designed by factorial design technique. Dissolution profile of the designed formulations is shown in Fig 8.1.

HPMC K100M CR, a hydrophilic polymer, swells in presence of aqueous medium. It is widely used as a rate-controlling polymer in various novel drug

delivery systems. Sodium alginate gets hydrated in presence of aqueous medium. In presence of acidic dissolution medium (which simulates gastric fluid),

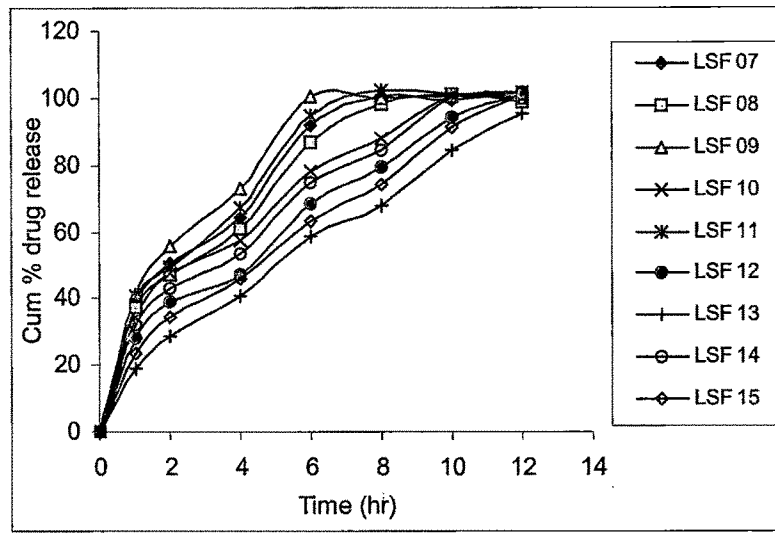

Fig 8.1 Dissolution Profile

the hydrated sodium alginate is converted into a porous, insoluble alginic acid skin that suppresses drug release (ISP technical bulletin). As the designed formulation is intended for drug delivery in stomach, sodium alginate might prove as an efficient formulation component due to its acid insoluble nature. It shows synergistic action regarding sustained release phenomenon along with HPMC K100M CR. As HPMC K100M CR level increased from lower to higher, levofloxacin release was significantly decreased (Fig.8.4). At specific level of HPMC K100 CR, increase in sodium alginate level decreased levofloxacin release.

Xanthan gum is one of the formulation component. This component mainly helped in retarding drug release by creating viscous network in the polymeric drug diffusion channels and helped in maintaining matrix integrity. Ultimately levofloxacin release was sustained due to intact matrix. Preliminary trials were conducted without xanthan gum but the matrix integrity was not appropriate. Drug release study was carried out for 12h. From the drug release profile, drug release at 1 h, time required for 50% drug release and time required for 95% drug release was estimated. Effect of the formulation variables on these response variables was observed from response surface plots (Fig 8.2) and prediction profiler (Fig 8.4)

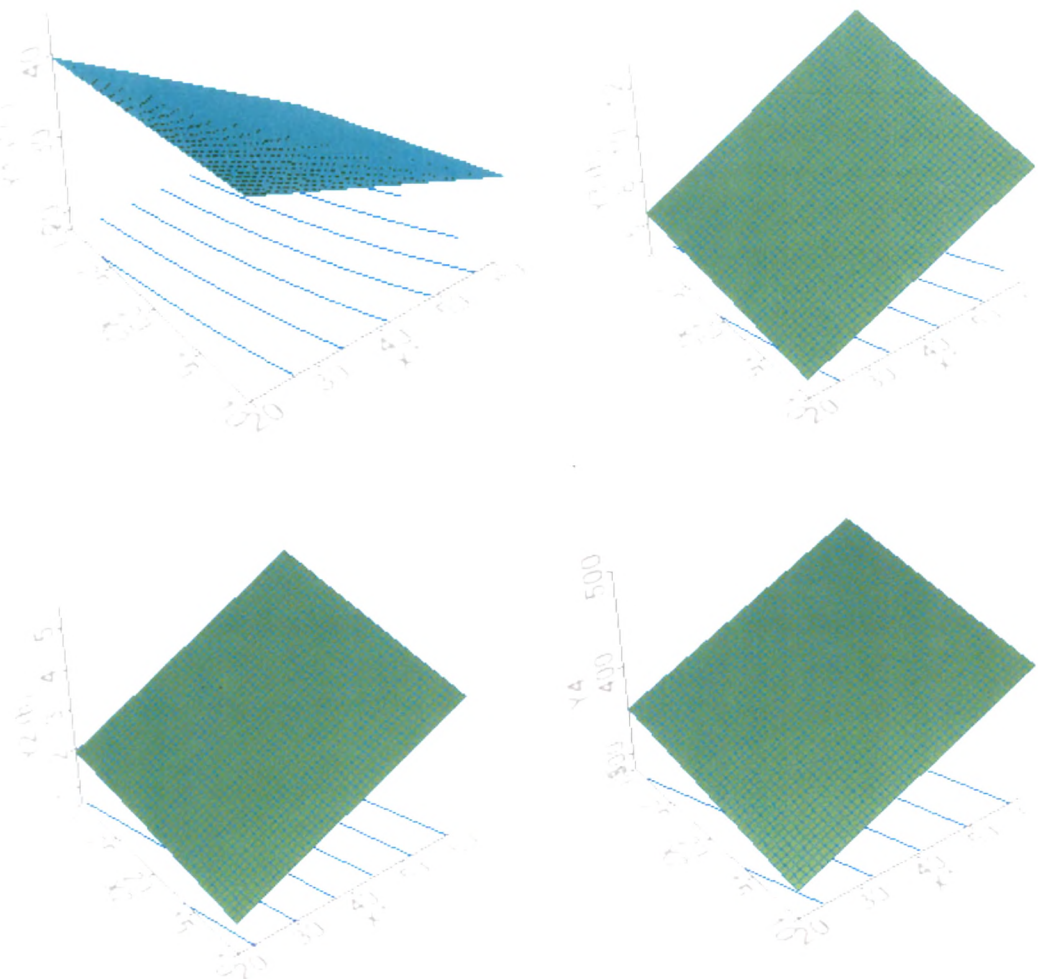

Fig 8.2 Response surface plots showing effect of formulation variables on various response variables

8.4.3.5 Kinetic modelling of drug release

Drug release 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 8.5). Optimum formulation LSF 13 best fitted to Ritger and Peppas equation. In this equation, values of release exponent "n" indicated the type of drug release pattern followed by the respective formulation.

Table 8.4 Experimental Results

Formulation no.	Y_1 (%)	Y_2 (h)	Y_3 (h)	Y_4
LSF 07	38.2	1.9	6.3	352.3
LSF 08	37.1	2.4	7.2	361.6
LSF 09	41.2	1.4	5.4	312.8
LSF 10	34.5	2.4	9	387.9
LSF 11	40.8	2	6	341.4
LSF 12	28.2	4.3	10	432.8
LSF 13	18.7	5	11.9	469.7
LSF 14	31.9	3.4	9.2	402.1
LSF 15	23.7	4.5	10.6	455.2

This equation predicts two independent mechanisms of drug release from a swelling polymer, Fickian diffusion and a case-II transport describes. When value of "n" is between 0.45 and 0.89, it indicates anomalous transport. Optimum formulation LSF 13 was having release exponent 0.614 which indicate anomalous type of drug release.

Table 8.5 Kinetic modelling of drug release data

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
LSF 07	0.825	7.664	0.829	0.087	0.964	30.753	0.967	0.376	0.377
LSF 08	0.848	7.690	0.855	0.092	0.971	30.540	0.968	0.364	0.358
LSF 09	0.755	7.336	0.753	0.076	0.933	30.285	0.946	0.430	0.415
LSF 10	0.890	7.584	0.903	0.095	0.988	29.675	0.938	0.341	0.365
LSF 11	0.810	7.691	0.819	0.085	0.956	31.026	0.957	0.391	0.362
LSF 12	0.949	7.733	0.944	0.114	0.987	29.278	0.977	0.265	0.372
LSF 13	0.981	7.476	0.934	0.140	0.974	27.657	**0.993**	**0.189**	**0.614**
LSF 14	0.923	7.802	0.928	0.104	0.991	30.011	0.983	0.301	0.370
LSF 15	0.970	7.797	0.945	0.126	0.982	29.131	0.988	0.225	0.474

8.4.4 Regression analysis

Regression analysis was carried out to determine significance of formulation variables on various response variables. Results of regression analysis (Table 8.6) indicated that formulation variables X_1 and X_2 significantly affected drug release at 1 h (Y_1), time required for 95% drug release (Y_3) and swelling index (Y_4) while only X_2 had significantly increased time required for 50% drug release (Y_2)

Table 8.6 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	34.800	--	2.667	--	8.600	--	388.056	--
X_1	-8.267	< 0.0001	1.417	0.0016	2.467	0.0008	58.533	< 0.0001
X_2	-2.950	0.0008	0.367	0.0648	0.800	0.0202	19.483	0.0001
X_1^2	-2.700	0.0052	0.450	0.1362	-0.100	0.7656	10.167	0.0042
X_2^2	-0.450	0.3083	0.100	0.6834	-0.200	0.5605	-6.283	0.0162
X_1X_2	-1.625	0.0083	0.050	0.7713	0.250	0.3321	-0.650	0.5244
R^2	0.998	--	0.978	--	0.986	--	1.000	--

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

8.4.5 Optimisation of responses using desirability function

Individual desirability values were calculated for the respective response variables and then overall desirability (equ 8.6) value was calculated from the individual values. Formulation LSF 13 was having highest desirability value of 1.00 amongst the designed formulations. Hence it was said to be an optimum formulation. Overall desirability value is an indicator of optimum formulation as it is calculated from the individual values which in turn and the same are calculated based on the desirable target response.

Table 8.7 Overall desirability values

Formulation	Desirability
LSF 07	0.16
LFS 08	0.26
LSF 09	0.00
LSF 10	0.38
LSF 11	0.08
LSF 12	0.71
LSF 13	**1.00**
LSF 14	0.53
LSF 15	0.84

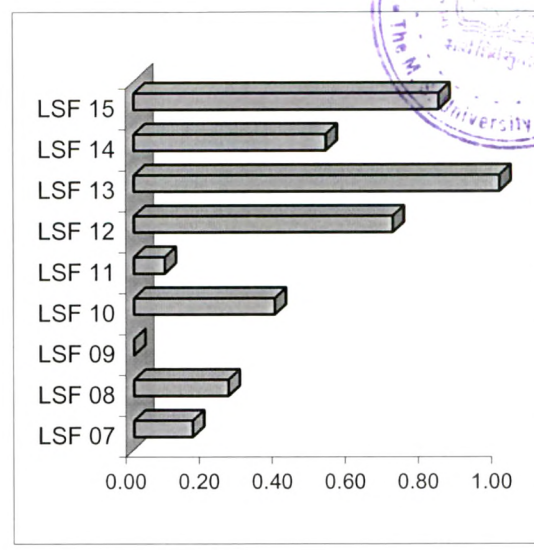

Fig 8.3 Overall Desirability

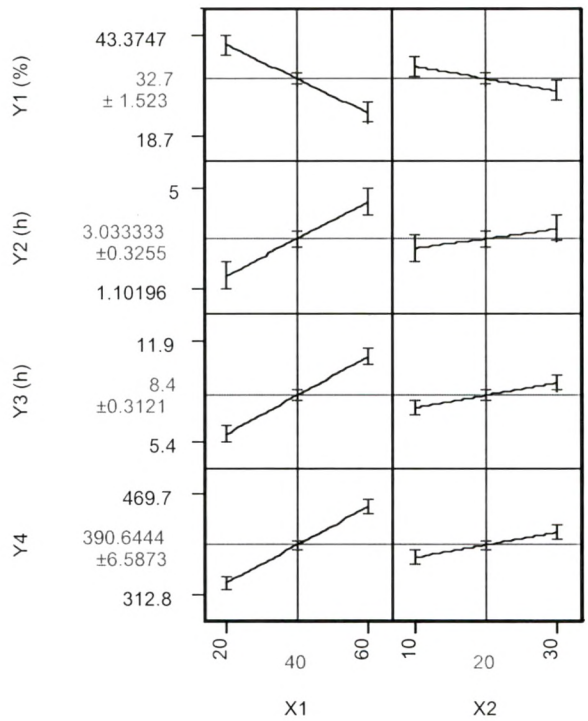

Fig 8.4 Prediction profiler showing effect of different levels of formulation variables on response variables

8.4.6 In vivo study by gamma scintigraphy

Radiolabelled softgel was administered to human volunteers and first image was taken immediately at 0 hour. Afterwards images were taken every hour to ascertain location of the formulation in stomach and the study was continued until softgel disappeared from stomach. Images captured at 0, 2, 6, 10 and 11 h have been shown in Fig 8.5. Imaging exercise shown presence of softgel till 10 h in stomach. It indicated gastric residence time of 10 h.

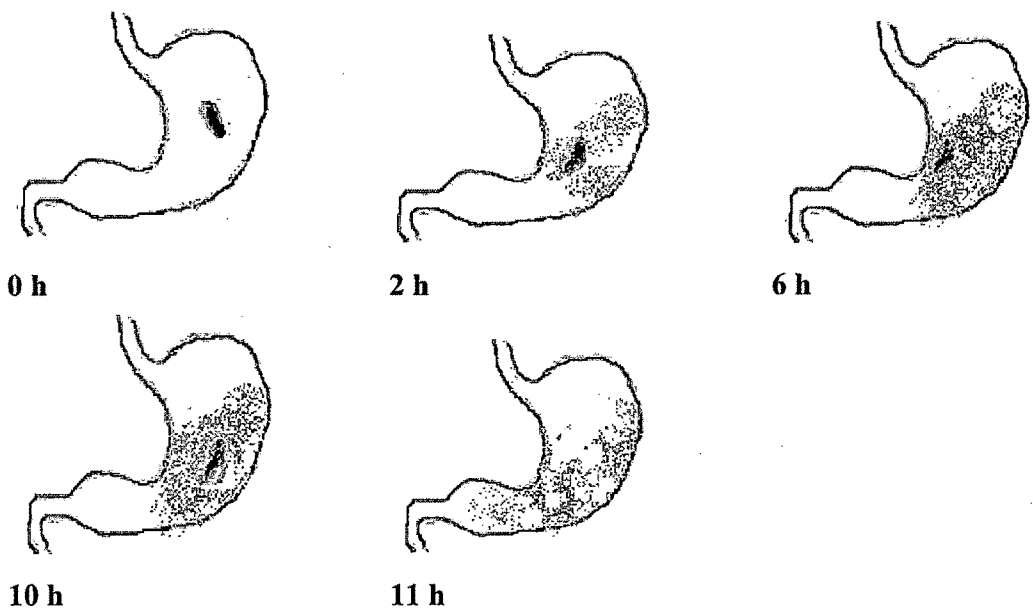

Fig 8.5 Gamma scintigraphy images showing gastric residence time of softgel

8.4.7 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 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 80 to 90 which indicated their similarity.

Table 8.8 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 (%)	98.7	97.9	97.5	97.4	97.1

8.5 CONCLUSION

Softgel formulation of levofloxacin having gastroretentive feature has been developed in the present investigation. Formulations were designed by full factorial design. HPMC K100M CR and sodium alginate were formulation variables. The softgel formulation was optimized with respect to in vitro drug release parameters. Optimum formulation was selected by using desirability approach. Formulation LSF 13 was found to be an optimum formulation as it was having highest desirability value of 1.00. Drug release data for this formulation best fitted Ritger and Peppas equation which indicated anomalous type of drug release. In vivo studies carried out in human volunteers shown that the formulation was capable to remain in stomach for 10 h. Stability studies indicated that there was no significant change in the levofloxacin content and drug release pattern at accelerated condition. Gastroretentive and sustained release properties of the developed softgel have tremendous potential for eradication of H.pylori. The formulation can be scaled up on large scale using softgel manufacturing facility.