Chapter 6

Softgel Formulation of Amoxicillin

6.1 INTRODUCTION

Amoxicillin is reported to have excellent activiy against H. pylori (Walsh and Peterson, 1995). H. pylori lives deep within the gastric mucus layer and prolonged local application of drug is needed for its effective eradication (Emami et al., 2006). Conventional immediate release formulations cannot achieve this objective (Cooreman et al., 1993). Therefore stomach specific drug delivery systems are necessary. Gellan gum based intra-gastric floating in-situ gelling system (Rajinikanth et al., 2007). gliadin nanoparticles (Umamaheshwari et al., 2004), pH-sensitive chitosan / polyvinyl pyrrolidone based controlled drug release system (Risbud et al., 2000) and mucoadhesive microspheres (Liu et al., 2005) have been developed in past. Objective of the present research work was to develop a novel floating softgel formulation of amoxicillin for stomach specific drug delivery.

6.2 MATERIALS

Amoxicillin trihydrate was received as a gift sample from Aristo Pharmaceuticals Ltd. (Mumbai, India). HPMC (Hydroxypropylmethylcellulose) K4MCR, HPMC K100M CR, HPMC K100LV and Polyethylene oxide (Polyox) WSR coagulant were gifted by Colorcon Asia Pvt. Ltd. (Goa, India). Xanthan gum was purchased from S.D.Fine Chemicals (Mumbai, India). Polyethylene Glycol 400 (PEG 400), Light liquid paraffin, sodium bicarbonate, citric acid and hydrochloric acid was 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.

6.3 METHODS

6.3.1 Preliminary Experiments

Preliminary experiments were carried out for selection of key excipients which include rate controlling polymer for sustaining drug delivery, gum component for imparting matrix integrity, gas generating component for achieving floating property and a suitable vehicle capable of producing uniform and free flowing encapsulation blend. Oily vehicle such as light liquid paraffin (LLP) is widely used as a vehicle for preparation of encapsulation blend (suspension or paste) that is filled in the soft gelatin capsule. Also hydrophilic vehicle such as polyethylene

glycols (PEGs) are generally used (Augsburger, 2008). HPMC K4M CR was tried as a rate controlling polymer in the preliminary trials. In trial ASF 01, LLP was used as a vehicle. Amoxicillin and HPMC K4M CR were properly mixed and LLP was added to obtain a smooth suspension. But it was observed that LLP was getting separated from the suspension within 15-20 min after its preparation. Hence hydrophilic vehicle PG was used in trial ASF 02. But it was affecting shell integrity. Therefore PEG 400 was tried as a vehicle in trial ASF 03. Blend prepared using PEG 400 was uniform and there was no separation of the contents as observed for ASF 01. Trial ASF 04 was taken by incorporating sodium bicarbonate and citric acid as a gas generating couple to impart floating feature to the formulation.

Further preliminary experiments ASF 06 to ASF 11 (Table 6.2) were carried out to determine role of an individual polymer in the formulation. Formulations of the preliminary trial were evaluated for buoyancy properties, matrix integrity and drug release pattern.

Table 6.1 Composition of preliminary trial batches

Ingredients (mg per softgel)	ASF 01	ASF 02	ASF 03	ASF 04	ASF 05
Amoxicillin trihydrate	114.77	114.77	114.77	114.77	114.77
HPMC K4M CR	100	100	100	100	100
Xanthan gum	--	--	--	--	50
Sodium bicarbonate	--	--	--	60	60
Citric acid	--	--	--	20	20
Light liquid paraffin	250	--	--	--	--
Propylene glycol	--	250	--	--	--
PEG 400	--	--	250	300	300

*Equivalent to 100 mg of amoxicillin

6.3.2 Experimental design

Design of experiment has been widely used in pharmaceutical field for systematic study of the effect of formulation variables and their interaction on response variables (Lewis et al, 1999; Li et al, 2003; Narendra at al 2006). Aim of the conventional approach in formulation development was not to obtain the best formulation but find a suitable solution under the given set of restrictions. This

Table 6.2 Composition of preliminary trial batches

Ingredients (mg per softgel)	ASF 06	ASF 07	ASF 08	ASF 09	ASF 10	ASF 11
Amoxicillin trihydrate	114.77	114.77	114.77	114.77	114.77	114.77
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 amoxicillin

development approach has several drawbacks which include lengthy development timelines and higher development cost. Also it is unpredictable, not fit to reveal interactions and sometimes even may be unsuccessful. Hence now days, systematic optimization techniques are being widely practiced to alleviate such inconsistencies. These involve experimental designs, mathematical equations and graphic outcomes which are capable of depicting a complete picture of variation of the responses as a function of the formulation variables. The systematic approach give best solution in the presence of competing objectives and simulate product performance using model equation. In full factorial designs an experimental run can be performed at every combination of the factor levels. The sample size is the product of the numbers of levels of the factors.

After determining role of individual polymers in the preliminary trials, it was decided to design the formulations by factorial design. Full factorial design (3^2) was implemented for studying effect combination of the polymers and gas generating couple at different levels. Formulation variables were decided from results of preliminary experiments. HPMC K100M CR (X_1) and gas generating couple [sodium bicarbonate: citric acid (3:1)] (X_2) each at three different levels were selected as formulation variables. The experimental design consists of total 9 experiments as shown in Table 6.3.

Effect of formulation variables was observed on buoyancy lag time (Y_1), drug release at 1 hour (Y_2), time required for 95% drug release (Y_3) and swelling index (Y_4) which are called as response variables.

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. The equation can be given as

$$Y_i = b_0 + b_1X_1 + b_2X_2 + b_{11}X_1^2 + b_{22}X_2^2 + b_{12}A X_1 X_2 \qquad (6.1)$$

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)

Table 6.3 Formulation designing by full factorial design

Formulation	Pattern*	Formulation variables**	
		X_1	X_2
ASF 12	+-	1	-1
ASF 13	-0	-1	0
ASF 14	++	1	1
ASF 15	+0	1	0
ASF 16	0-	0	-1
ASF 17	0+	0	1
ASF 18	00	0	0
ASF 19	--	-1	-1
ASF 20	-+	-1	1

Coded values	Actual values*	
	X_1	X_3
-1	30	32
0	45	40
1	60	48

*Actual values indicate quantity in mg per softgel

Table 6.4 Composition of the factorial design batches

Ingredients (mg per softgel)	ASF 12	ASF 13	ASF 14	ASF 15	ASF 16	ASF 17	ASF 18	ASF 19	ASF 20
Amoxicillin trihydrate*	114.77	114.77	114.77	114.77	114.77	114.77	114.77	114.77	114.77
HPMC K100M CR	60	30	60	60	45	45	45	30	30
Sodium bicarbonate	24	30	36	30	24	36	30	24	36
Citric acid	8	10	12	10	8	12	10	8	12
Xanthan gum	15	15	15	15	15	15	15	15	15
PEG 400	299.23	299.23	299.23	299.23	299.23	299.23	299.23	299.23	299.23
Total Wt	*521*	*499*	*537*	*529*	*506*	*522*	*514*	*491*	*507*

*Equivalent to 100 mg of amoxicillin

6.3.3 Preparation of softgel capsules

6.3.3.1 Preparation of gelatin for capsule shell

Methyl paraben and propyl paraben were dissolved in water at 75° to 80°C in gelatin mass reactor (AV Pharma machinery, Mumbai). Glycerine, sorbitol solution and remaining quantity of water was added and properly mixed. Then gelatin flakes were added and soaked for 45 minutes. This mixture was heated at 65° to 70°C with stirring until completely melted. Vacuum was applied to remove entrapped air. The molten mass was maintained at 40° to 50°C when it was fed to film forming rollers.

Table 6.5 Gelatin Shell composition

Ingredients	% w/w
Gelatin	43.93
Glycerine	6.00
Sorbitol solution (70%)	12.00
Methyl paraben	0.20
Propyl paraben	0.10
Water	37.77

6.3.3.2 Blend encapsulation in softgel capsule shell

Encapsulation was carried out by continuous rotary die process using softgel encapsulation machine (Hitech Pharma Equipments, Bhilad, Gujarat) using 20 mm oblong cavity rollers. The molten gelatin mass was maintained at 40° to 50°C when it was fed to film forming rollers. Film was formed on rollers where

temperature was maintained at 10° to 12°C. The encapsulation process was based on form-fill-seal principle.

6.3.3.3 Drying of softgel capsules

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

6.3.4 Evaluation of softgel capsules

6.3.4.1 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 of intact capsule weight and empty capsule (shell) weight (IP, 2007).

6.3.4.2 *Assay*

Amoxicillin 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 (0.2 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.

6.3.4.3 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 duration of buoyancy was noted.

6.3.4.4 Drug Release Study

Drug release study was carried out using USP type II (paddle type) dissolution test apparatus (VDA 6-DR, Veego Instruments Corporation, Mumbai, India) at 50 rpm. Dissolution medium used was 900 ml of 0.1N HCl at 37±0.5°C. one softgel

was introduced into each dissolution vessel. 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 spectrophotometry for estimating amount of drug release.

6.3.4.5 Analysis of drug release data

Dissolution profile data was analysed by fitting it 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)

6.3.4.6 Swelling Index

One softgel was placed in a basket of dissolution test apparatus and the basket was immersed in a petri-dish having 100 ml of 0.1N HCl. At every hour baskets were removed, excess of the 0.1N HCl was soaked by tissue paper and weight was measured. This study was carried upto 12 h. Swelling index calculated by equation

$$\text{Swelling Index} = [(W_2-W_1) / W_1] \times 100 \qquad (6.2)$$

Where W_1 = Initial weight of softgel and W_2= Weight of wet softgel at 12 h

6.3.5 Regression analysis

Regression analysis was carried out by using equation 6.1, for determining significance of formulation variables on the measured response variables. All experimental results were computed by statistical software DOE v6.0.5 (Stat-Ease Inc., Minneapolis, MN, USA).

6.3.6 Selection of optimum formulation by desirability function

After carrying out preliminary trials, formulations were systematically designed by 3^2 full factorial design to study effect of formulation variables on key response variables. Amongst the designed formulations, best or optimum formulation was selected by desirability approach. In this approach, individual desirability is calculated for each response variable which varies from 0 to 1 according to closeness of the response to its target value. Individual desirability values are combined to calculate an overall desirability. Formulation with highest overall desirability value is called optimum formulation (Mashru et al., 2005).

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

As optimum formulation should have low buoyancy lag time and it should release less amount of drug at initial hour, these responses were minimized while calculating individual desirability values. Desirability values of these responses were calculated using following equation:

$$d_1 \text{ or } d_2 = Y_{max} - Y_i / Y_{max} - Y_{target} \quad \text{for } Y_i > Y_{target} \qquad (6.3)$$

$$d_1 \text{ or } d_2 = 1 \quad \text{for } Y_i < Y_{target} \qquad (6.4)$$

where d_1 and d_2 is individual desirability for buoyancy lag time and % drug release at 1 h respectively. The Y_{max} and Y_{target} indicate maximum and target (minimum) value of experimental result for respective response variables for factorial design batches (Table 6.6). Y_i is the experimental result of the response variable for individual factorial design batch.

Time required for 95% drug release and swelling index values 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:

$$d_3 \text{ or } d_4 = Y_i - Y_{min} / Y_{target} - Y_{min} \quad \text{for } Y_i < Y_{target} \qquad (6.5)$$

$$d_3 \text{ or } d_4 = 1 \text{ for } Y_i > Y_{target} \qquad (6.6)$$

where d_3 and d_4 indicate desirability for Y_3 and Y_4. 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.

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

$$D = (d_1 d_2 d_3 d_4)^{1/4} \qquad (6.7)$$

6.3.7 In vivo study by gamma scintigraphy

Transit of a optimum softgel formulation was non-invasively imaged in vivo by gamma scintigraphy. Softgel formulation was radiolabelled by using Technetium Tc 99m pentetate as a radiolabelling agent. It emits very low intensity, non hazardous gamma rays which can be detected by using gamma camera. For radiolabelling the formulation, softgel content was removed by small puncture. Radioactive material (Tc 99m pentetate having 6 milicurie activity) was mixed with the contents and radiolabelled blend was refilled in softgel using disposable syringe. One softgel was administered to each volunteer after a standard breakfast and a glass of water. Location of radiolabelled softgel was monitored in GIT by capturing Images by gamma camera (e-cam signature series, Siemens). Images were captured at one hour interval until the formulation was observed in stomach.

6.3.8 Stability studies

Stability study is an integral part of the formulation development process. Stability indicates capability of the developed formulation to retain the desired characteristics under influence of temperature and humidity during its storage. 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 in stability chambers (Newtronic, Mumbai). Samples were withdrawn at 1, 2, 3 and 6 month time interval and analysed for physical appearance, buoyancy lag time, drug release pattern and amoxicillin content.

6.4 RESULTS AND DISCUSSION

6.4.1 Preliminary Experiments

In the preliminary experiments LLP was used as a vehicle for preparation of encapsulation blend. But LLP got separated in the prepared blend which may be due to its hydrophobic nature. Hence it was decided to use hydrophilic vehicle such as PEG. Literature review indicate that PEGs are also widely used for preparation of encapsulation material blend (Augsburger, 2008). Blend prepared in trial ASF 03 using PEG was found uniform and there was no separation. Hydrophilic nature of PEG might have helped in obtaining the consistent blend (Rowe et al., 2006) One of the most important parameter considered while

preparing encapsulation blend is its flowability. It is of great significance because the encapsulation blend has to flow properly so that weight variation can be avoided in continuous operation. This factor was kept in mind while selecting amount of vehicle in the formulation

Aim of the present investigation was to prepare floating formulation with sustained release feature. In order to obtain floating phenomenon, the matrix should either have inherently low density or it should be imparted by using specific excipients such as gas generating agents. For obtaining low density matrix (i.e. encapsulation blend) LLP was used as a vehicle as it has low density than water i.e. <1 gm/ml ((Rowe et al., 2006). Encapsulation blend was prepared by using LLP in trial ASF 01. But phase separation was observed. Also LLP based formulation would not have allowed matrix swelling in presence of aqueous gastric contents. For obtaining sustained release feature, the matrix formulation should be able to get hydrated and swell. This will be possible in case of softgel matrix if the vehicle used is hydrophilic in nature. Hence it was decided to use hydrophilic vehicle with low density. Propylene glycol (PG) fits this criteria. Uniform blend was obtained in trial ASF 02 by using PG, but capsule shell brittleness was observed within 2-3 days of storage. Further (ASF 03) was taken by using PEG 400, a widely used vehicle in softgel formulation. Uniform blend was obtained and softgel shell was also not affected. But the formulation could not float as such. Gas generating components such as sodium bicarbonate and citric acid can nicely impart buoyant properties in the formulation as it was observed during development of the minimatrices. Hence trial ASF 04 was taken by incorporating sodium bicarbonate and citric acid and the formulation obtained was found to have good floating properties. Sodium bicarbonate and citric acid were taken in 3:1 proportion as this ratio imparted best floating phenomenon in amoxicillin minimatrix formulation (Chapter 5). Gas generating couple produces gas bubbles in the matrix. These bubbles get entrapped in the matrix which are responsible for floating of the formulation. But the drawback of this phenomenon is that it hampers matrix integrity. To overcome this difficulty, it was necessary to introduce a viscosity increasing component such as xanthan gum. it helps in keeping the matrix intact by creating viscous network in the polymer network. Experiment ASF 05 was carried out by incorporating xanthan gum as an additional component. Significant difference was observed in ASF 04 (without

xanthan gum) and ASF 05 (with xanthan gum) with respect to matrix integrity during dissolution study.

Further preliminary experiments (ASF 06 to ASF 11) were conducted to select best polymer for introducing sustained release feature. HPMC K4M CR, HPMC K100M CR, HPMC K100 LV, sodium alginate, polyox WSR coagulant and xanthan gum were explored individually. Minimum floating lag time was observed for HPMC K4M CR based (ASF 06) and Polyox based formulation (ASF 09). But the matrix disintegrated within 3 h. HPMC K100 M CR based formulation (ASF 07) had somewhat higher lag time as compared to ASF 06 and ASF 09, but matrix integrity was observed for 5 h. Formulation ASF10 containing sodium alginate and ASF 11 containing xanthan gum could not float.

6.4.2 Experimental design

Role of individual polymers was determined in preliminary experiments. HPMC K100M CR based formulation was having satisfactory floating properties and sustained drug release for limited period. It was necessary to reduce floating lag time and further retard drug release. Hence systematic optimisation exercise was undertaken by using full factorial design. HPMC K100M CR was selected due to its rate controlling property (X_1) and gas generating couple (X_2) was incorporated for imparting floating feature. Aim of the optimisation exercise was to select the most appropriate level of these components in order to achieve a formulation with minimum floating lag time and having capability to sustain drug release upto 12 h.

6.4.3 Preparation of softgel capsules

Encapsulation blends were prepared for the factorial design batches as shown in Table 6.4 and it was encapsulated as per the procedure described in section 6.3.3.2

6.4.4 Evaluation of softgel

6.4.4.1 Weight variation

Weight variation of softgel contents was 4.7% 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.

6.4.4.2 Assay

Amoxicillin content was found in the range 98.2 to 102% of added amount of amoxicillin per softgel as determined by HPLC method.

6.4.4.3 Buoyancy lag time and total buoyancy time

For imparting buoyant feature in the formulation, gas generating couple was incorporated in the formulation. Sodium bicarbonate and citric acid react in presence of acidic gastric atmosphere to produce carbon dioxide gas bubbles. After introducing softgel formulation into the dissolution medium, gas bubbles were generated in the softgel matrix and got entrapped in the matrix that imparted floating feature. Preliminary trials indicated that this component was essential and the formulation without this component could not float (ASF 03). Accordingly it was selected as one of the formulation variables in factorial design batches and its effect was studied on buoyancy lag time. Increasing amount of gas generating couple significantly decreased lag time. This can be clearly observed from the prediction profiler (Fig 6.4) and results of regression analysis (Table 6.8). Increasing amount of sodium bicarbonate and citric acid generates more bubbles inside the softgel matrix. The generated bubbles get entrapped inside the matrix and ultimately creates floating phenomenon. Increasing HPMC concentration also helped in lag time reduction (Fig 6.4). It may be due to the reason that gas bubbles generated inside the matrix can only impart buoyancy if the polymer network does not allow them to escape. It depends on matrix viscosity. Increase in HPMC concentration leads to more hydration and increased matrix viscosity. It might have helped in trapping the bubbles inside the matrix and decreased floating lag time.

Due to long contact with the dissolution medium, the formulation swells and becomes hydrodynamically balanced. This phenomenon continuously kept the softgel in buoyant condition and did not allow it to settle.

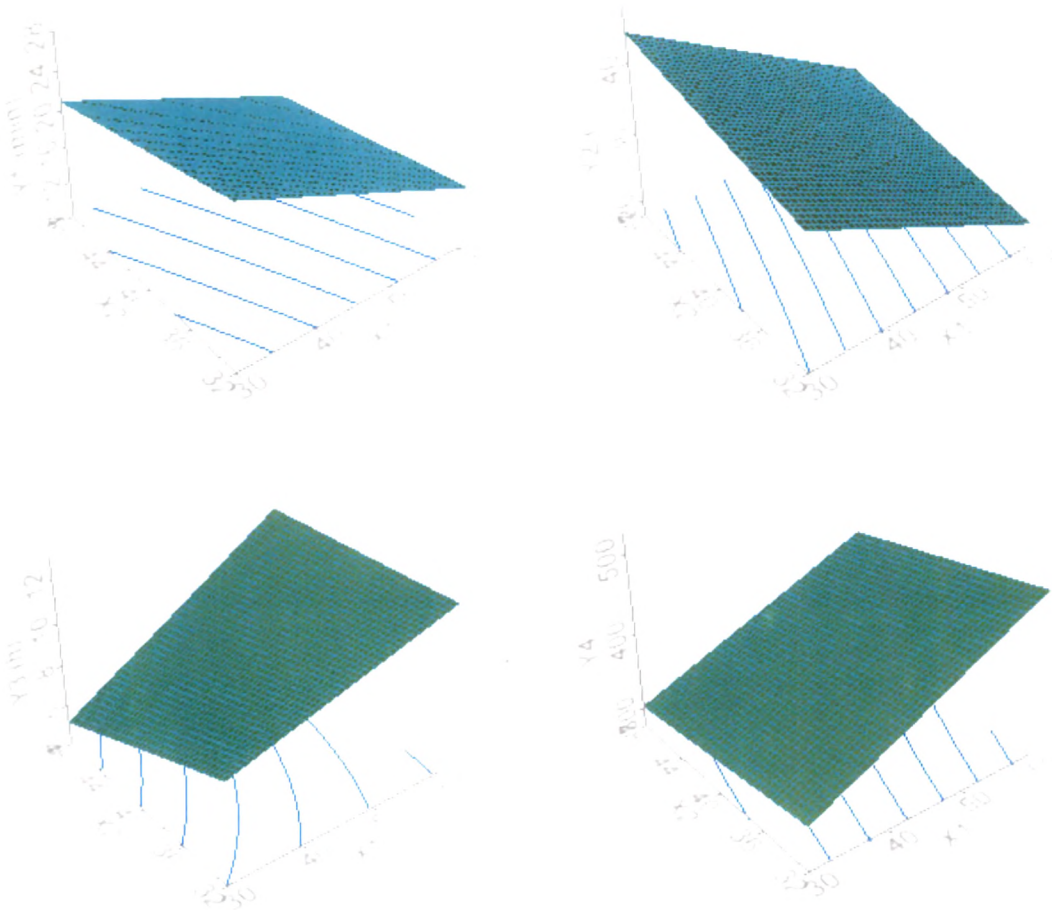

Fig 6.1 Response surface plots showing effect of X_1 and X_2 on response variables;

6.4.4.4 Drug release study

HPMC K100M CR was selected as one of the formulation variable in order to sustain the drug release for longer duration in stomach, which is pre-requisite criteria for effective eradication of H. pylori. Xanthan gum was included as one of the formulation components as it plays crucial role along with HPMC in sustaining drug release by forming viscous network in the hydrated matrix.

Sustained drug release phenomenon was measured by estimating drug release at 1 h and time required for 95% drug release. Experimental results for these responses are shown in Table 6.6. Effect of the formulation variables on these response variables was interpreted from prediction profiler (Fig 6.4) and response surface plots (Fig 6.1).

Results of regression analysis (Table 6.8) shown significant effect of HPMC on drug release parameters (Y_2 and Y_3) and swelling index Y_4. Drug release at 1 h was decreased while time required for 95% drug release and swelling index were increased with increasing HPMC level from –1 to +1. HPMC is a hydrophilic polymer. The polymer molecular chains of HPMC hydrate in presence of aqueous medium and form a gel matrix. During hydration process, channels are formed in the matrix network which are responsible for drug diffusion. An increase in polymer concentration causes an increase in the viscosity of the gel layer while swelling increases diffusional path length. It ultimately decreases drug release (Miranda et al., 2006).

Gas generating couple (X_2) shown negative effect on drug release. Increasing level of X_2 increased drug release at 1 h while it decreased the time required for 95% drug release as well as decreased swelling index. Formulation ASF 20 containing highest level of gas generating couple and lowest level of HPMC K100M CR, released the drug fast as compared to other formulations (Fig 6.2). At high level of gas generating couple, more bubbles were generated in short span of time. While trying to escape from the matrix the bubbles might have created drug diffusion channels as well as it hampered matrix integrity. Both of these might be responsible for faster drug release as compared to the formulations having lower levels of X_2. In such cases matrix integrity depended on level of HPMC K100M CR.

6.4.4.5 Kinetic modelling of drug release

Dissolution profile data was fitted to zero order, first order, Higuchi and Ritger and Peppas equation. This exercise provided release rate constant and coefficient of determination (R^2). Formulation ASF 12 was an optimum formulation as decided from desirability approach (Table 6.9). This formulation followed first order release kinetics as interpreted from the highest value of coefficient of determination (Table 6.7)

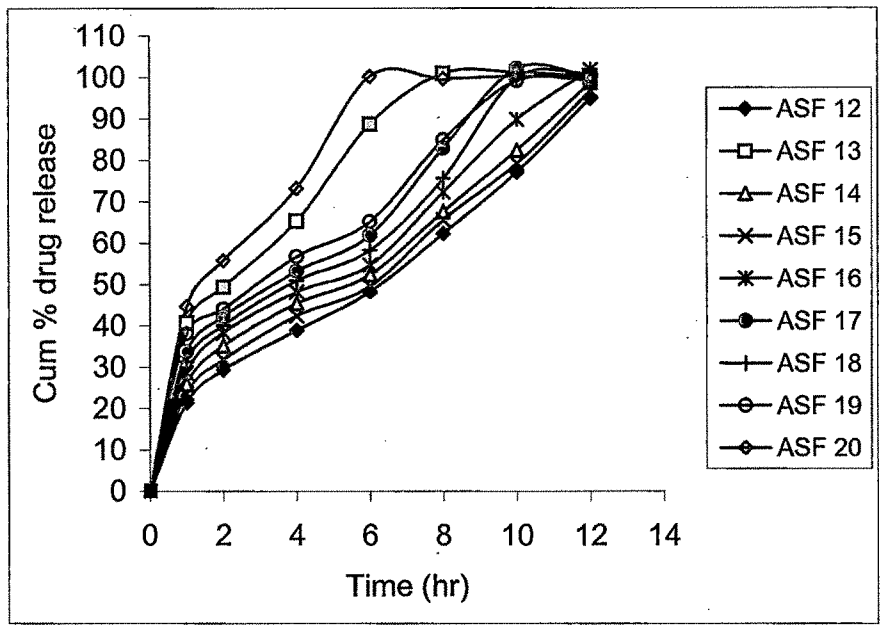

Fig 6.2 Dissolution profile of factorial design batches

Table 6.6 Experimental Results

Formulation no.	Y_1 (min)	Y_2 (%)	Y_3 (h)	Y_4
ASF 12	15	21.4	12	489.8
ASF 13	23	40.8	6.7	324.2
ASF 14	11	25.9	11.4	420.8
ASF 15	13	23.7	11.8	465.5
ASF 16	20	28.7	10.8	402.3
ASF 17	16	33.8	9	361.7
ASF 18	18	31.4	9.4	381.3
ASF 19	26	38.2	9.1	340.2
ASF 20	21	44.7	5.1	302.8

Table 6.7 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
ASF 12	0.974	6.967	**0.980**	**2.258**	0.952	25.576	0.963	0.207	0.490
ASF 13	0.826	7.603	0.848	1.952	0.965	30.518	0.966	0.393	0.339
ASF 14	0.957	7.101	0.976	2.247	0.965	26.467	0.960	0.246	0.433
ASF 15	0.966	7.025	0.979	2.255	0.959	25.984	0.961	0.226	0.461
ASF 16	0.951	7.412	0.976	2.249	0.966	27.738	0.951	0.263	0.358
ASF 17	0.923	7.683	0.959	2.207	0.972	29.272	0.951	0.311	0.336
ASF 18	0.936	7.633	0.969	2.231	0.965	28.766	0.946	0.287	0.345
ASF 19	0.902	7.368	0.961	2.214	0.979	28.505	0.953	0.351	0.304
ASF 20	0.747	7.222	0.770	1.774	0.930	29.910	0.945	0.452	0.320

Table 6.8 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	17.889	--	31.200	--	9.556	--	384.478	--
X_1	-5.167	< 0.0001	-8.783	< 0.0001	2.383	0.0002	68.150	< 0.0001
X_2	-2.167	0.0002	2.683	0.0003	-1.067	0.0024	-24.500	0.0017
X_1^2	0.167	0.4228	1.150	0.0147	-0.383	0.1398	8.783	0.1136
X_2^2	0.167	0.4228	0.150	0.5544	0.267	0.2590	-4.067	0.3807
X_1X_2	0.250	0.1443	-0.500	0.0522	0.850	0.0082	-7.900	0.0669
R^2	0.999	--	0.999	--	0.995	--	0.997	--

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

6.4.4.6 Swelling Index

HPMC K100M CR has the property to absorb large amount of water and get hydrated. Xanthan gum also has hydration tendency though it is less as compared to HPMC. But it assists HPMC in holding the absorbed water. Swelling index, as calculated by equ 6.2, increased with increase in HPMC level. It might be due to the fact that HPMC matrix hydration is dependent on its concentration (Miranda et al., 2006). In the present context, swelling index also depended on the amount of gas generating couple present in the formulation as it affected matrix integrity. Swelling index increases with time due to increase in matrix

hydration phenomenon. It is possible only when matrix is capable to hold the absorbed water by maintaining its integrity. Formulations with high level of gas generating couple and low level of HPMC were having low swelling index as the matrix was unable to hold absorbed fluid for longer duration due to distorted integrity.

6.4.5 Optimisation of responses using desirability function

Individual desirability values were calculated for the respective response variables and then overall desirability (equ 6.7) value was calculated from the individual values. The Y_{max} and Y_{target} values were 26 and 11 for floating lag time (Y_1) and 44.7 and 21.4 for %drug release at 1 h (Y_2). Respective Y_{min} and Y_{target} values were 5.1 and 12 for Y_3 and 302.8 and 489.8 for Y_4 (Table 6.6). Overall desirability value decides an optimum formulation as it is calculated from the individual values and the same are calculated based on the desirable target response. Amongst the factorial design batches, formulation ASF 12 was having highest desirability value of 0.93 and hence it was said to be optimum. This formulation contains highest level of HPMC K100M CR and lowest level of gas generating couple.

Table 6.9 Overall desirability values

Formulation	Desirability
ASF 12	**0.93**
ASF 13	0.17
ASF 14	0.83
ASF 15	0.90
ASF 16	0.59
ASF 17	0.49
ASF 18	0.53
ASF 19	0.00
ASF 20	0.00

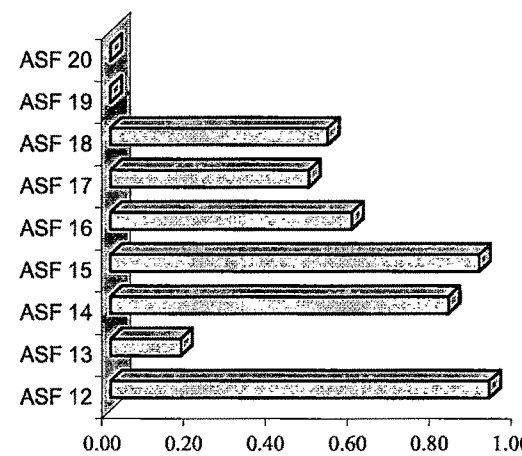

Fig 6.3 Overall Desirability

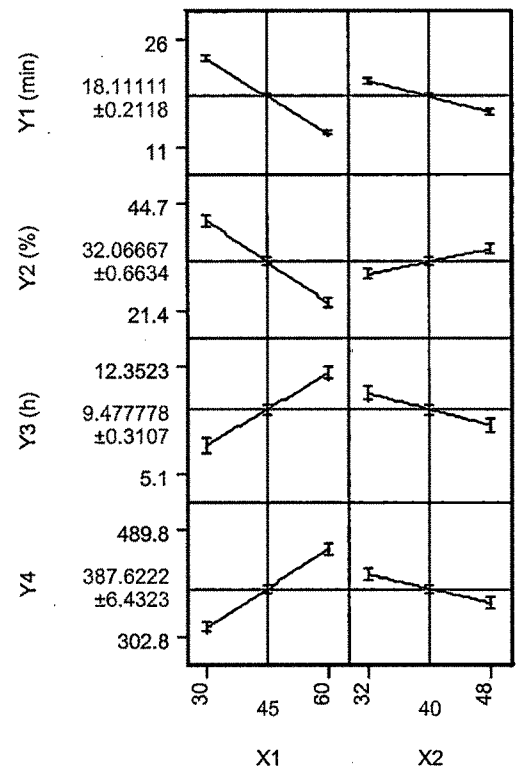

Fig 6.4 Prediction profiler showing effect of formulation variable levels on response variables

6.4.6 In vivo study by gamma scintigraphy

After administering the radiolabelled softgel to human volunteers, its GI transit was monitored by capturing the images with gamma camera. First image was captured immediately after administration (0 h) and then imaging was done at every hour. Images captured at 0, 2, 6, 9 and 10 h have been shown in Fig 6.5. Imaging was continued until the softgel disappeared from stomach. This exercise shown presence of softgel till 9 h in stomach. As the time elapsed, softgel was found eroding.

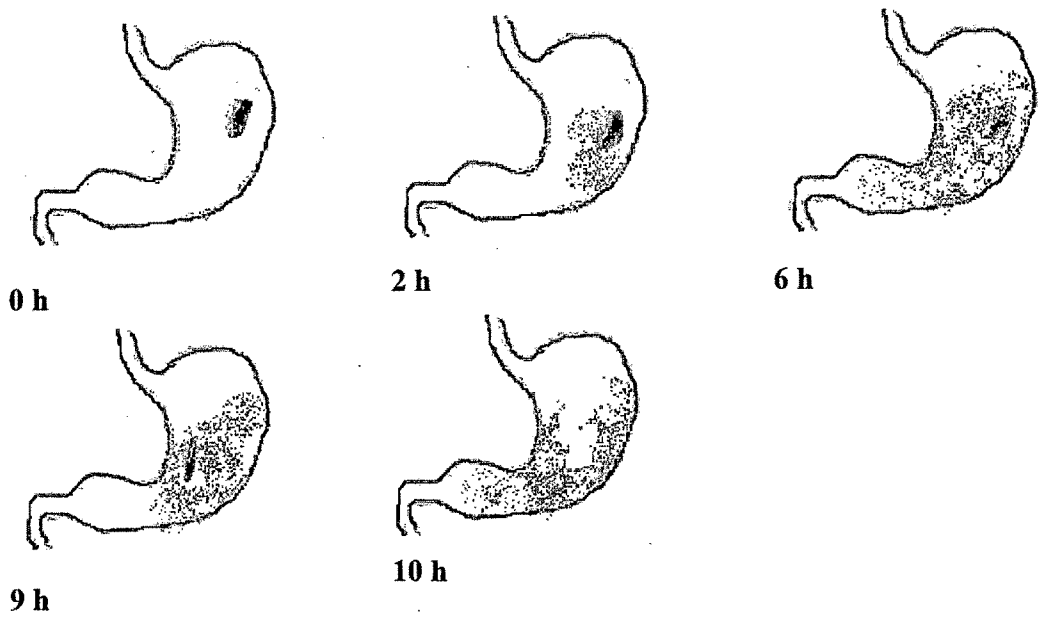

Fig 6.5 Gamma scintigraphy images showing gastric residence time of softgel

6.4.7 Stability studies

Stability samples of the optimum formulation (ASF 12) were analysed for the individual 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 79 to 87 which indicated their similarity.

Table 6.10 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)	15	18	20	17	18
Assay (%)	99.2	98.6	98.4	98.2	97.9

6.5 CONCLUSION

Preliminary trials were carried out for selection of suitable excipients and final optimisation exercise was carried out by full factorial design. Novel gastroretentive softgel formulation with minimum floating lag time and capable to

sustain in vitro drug release upto 12 h has been successfully developed. In vivo study in human volunteers shows that it can remain in stomach for 9 h. The technique and concept of preparing a gastroretentive formulation in the form of softgel is a novel concept. The formulation can be scaled up on large scale using softgel manufacturing facility. Hence the developed formulation has therapeutic as well as manufacturing advantages.