Formulation and optimization of gastric floating drug delivery system using Central composite design and its biopharmaceutical evaluation

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Abstract: The present work investigates the formulation and biopharmaceutical estimation of gastric floating drug delivery system (GFDDS) of propranolol HCl using semi-synthetic polymer carboxymethyl ethyl cellulose (CMEC) and a synthetic polymer polyethylene oxide (PEO). A central composite design was applied for optimization of polymer quantity (CMEC or PEO) and sodium bicarbonate concentration as independent variables. The dependent variables evaluated were: % of drug release at 1 hr (D_{1hr}), % drug release at 3 hr (D_{3hr}) and time taken for 95% of drug release (t_{95}). Numerical optimization and graphical optimization were conducted to optimize the response variables. All observed responses of statistically optimized formulations were in high treaty with predicted values. Accelerated stability studies were conducted on the optimized formulations at 40±2ºC/75% ±5% RH and confirm that formulations were stable. Optimized formulations were evaluated for *in vivo* buoyancy characterization in human volunteers and were found buoyant in gastric fluid. Gastric residence time was enhanced in the fed but not the fasted state. The optimized formulations and marketed formulation were administered to healthy human volunteers and evaluated for pharmacokinetic parameters. Mean residence time (MRT) was prolonged and AUC levels were increased for both optimized floating tablets when compared with marketed product. High relative bioavailability obtained with optimized gastric floating tablets compared to commercial formulation, indicated the improvement of bioavailability.

Keywords: Propranolol HCl, carboxymethyl ethyl cellulose, polyethylene oxide, gastric floating, statistical optimization.

INTRODUCTION

The oral route is considered to be the most common route of drug delivery and especially oral controlled drug delivery offers numerous advantages. Effectiveness of the controlled drug delivery system depends upon the factors like gastrointestinal transit time of the dosage form, gastric residence time, gastric emptying, pH variability in gastrointestinal (GI) tract segments, drug release and the site of absorption of drugs (Hirtz 1985; Shivkumar 2001). These physiological limitations may result improper absorption profiles, partial drug release and shorter gastric residence time of the dosage form in the stomach; these may lead to incomplete absorption of drugs, which are having absorption windows in the upper parts of the small intestine. A gastric floating drug delivery system (GFDDS) may be an alternative for drugs that are absorbed in the upper parts of the small intestine. The GFDDS can prolong the gastric residence time of the dosage form, thereby enhancing the drug bioavailability (Srikanth 2011a).

GFDDS are most regularly used technique for gastric retention of the dosage form. In order to attain floating, an intimate balance required between the mass and volume of the drug delivery system. To obtain floating, the drug delivery system should have an original density of less than 1gm/cc (Moes 1993). One of the major advantages of developing a floating drug delivery system is the capability to attain an increase in GRT without varying the gastric emptying rate (Srikanth 2011a, Moes 1993). GFDDS can be further divided into hydrodynamically balanced systems, low-density systems, hot melt extrusion systems and effervescent systems. Gastric retention is beneficial for the drugs, which are less soluble in alkaline pH (e.g. verapamil, chlordiazepoxide, propranolol HCl and cinnarizine) or those that degrade in alkaline pH (e.g. captopril) (Moes 1993).

Propranolol HCl is a non-selective beta adrenoceptor blocker and mainly used for the treatment of cardiovascular disease. Propranolol HCl is highly lipophilic substance and absorbed completely after oral administration. It is having shorter half-life about 3-4 hrs and undergoes first pass metabolism and only 25% of propranolol reaches the systemic circulation (Indian Pharmacopoeia, 1996). The bioavailability of propranolol HCl is enhanced by the presence of food. P-gp plays a major role in its absorption and it is an acid-soluble basic drug, which make it adequate for GFDDS (Swati 2009).

Polyethylene Oxide (PEO), a non-ionic, high molecular weight, hydrophilic polymer with unique physicochemical properties is used frequently in controlled drug delivery systems as a retarding polymer. CMEC, a novel semi synthetic polymer having excellent water dispersibility, is

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suitable for enteric coating and also used as a matrix forming material in controlled drug delivery (Hideaki 1985). There are no previous reports on the pharmacokinetic evaluation of drug delivery system prepared with CMEC. Preliminary studies were carried out in our laboratory showed CMEC and PEO WSR 301 polymers to be the best vehicle for floating tablets. Srikanth *et al*.(2012) formulated gastro retentive floating tablets of propranolol HCl using a CMEC with 3-factor statistical approach with more number of standard runs. Author characterized *in vivo* buoyancy properties of tablets in human healthy volunteers. The gastric residence time of tablets was enhanced in the fed but not the fasted state (Venkata Srikanth 2012). In the present work, author applied 2-factor central composite design for optimizing the formulations with less number of experimental runs. Optimization of the formulation has been done by different dependent and independent variables compared to previous research work. In addition, pharmacokinetic studies of the optimized formulations from CMEC and PEO polymers were carried out in human volunteers, which were not reported previously.

The conventional optimization process requires a single parameter to be varied while all others are kept constant under a specific set of conditions and it is not possible to alter more than one parameter at a time during the formulation development. This may lead to unreliable data and improper conclusions, in addition to wasting excipients due to the requirement of large number of runs. Statistical optimization is an alternative method to overcome this difficulty; it can be employed to optimize the formulations with suitable experimental design. Statistical optimization permits a deeper understanding of a process or product and has important applications in optimization and in establishing the robustness of the product.

The present study aimed to develop a GFDDS of propranolol HCl using novel semi synthetic polymer carboxymethyl ethyl cellulose (CMEC) and a synthetic polymer PEO (PEO WSR 301). To achieve the objective, response surface methodology (RSM) by central composite design was used with polymer quantity and sodium bicarbonate concentration as independent formulation variables. The dependent variables include % drug release at 1 hr ($D_{1\ hr}$), % drug release at 3 hrs (D_{3hr}) and time to reach 95% of drug release (t_{95}). Statistical analysis was implemented to identify the optimized formulation and model was validated by comparing the experimental results with the theoretical values for the responses. The levels and ranges of the formulation independent variables were selected on the basis of the results got from the preliminary studies conducted in this laboratory.

The hypothesis of the present work is the propranolol HCl gastric floating tablets may retain in the gastric region for more time and thereby it may enhance the bioavailability of the drug.

MATERIALS AND METHODS

Materials

Propranolol HCl was obtained from Dr Reddy's Laboratories, Hyderabad, India. Carboxymethyl ethyl cellulose and PEO WSR 301 were provided by Unichem Laboratories Ltd, Goa, India. Povidone K 30, sodium bicarbonate (NaHCO3) and magnesium stearate were gifts from Hetero drug Ltd, Hyderabad, India. All other chemicals and reagents were of analytical grade.

Experimental design

Experimental design and optimization of formulations were performed by using Design Expert software (Version 7.1.6, Stat Ease Inc., Minneapolis, MN). Two factors, three level, central composite design was applied for the design and optimization. According to the model, 11 experimental runs for each polymer were conducted in total. The design generally involves 2 independent formulation variables such as X_1, polymer quantity (either CMEC or PEO) and X_2, concentration of the sodium bicarbonate. The dependent variables Y_1, Y_2 and Y_3 were D_{1hr}, D_{3hr} and t_{95} respectively. The variables and their ranges studied are summarized in table 1 & 2.

Table 1: Level of formulation variables (Central composite design)

Variables	Range and levels		
	-1	0	+1
CMEC based formulations			
CMEC (mg) X_1	200	240	280
% w/w Sodium bicarbonate concentration (to the total tablet weight) X_2	5	10	15
PEO WSR 301 based formulations			
PEO WSR 301 (mg) X_1	160	200	240
% w/w Sodium bicarbonate concentration (to the total tablet weight) X_2	5	10	15

Preparation of gastric floating tablets (GFT)

All the gastric floating formulations contained an 80 mg of propranolol HCl. Briefly, all the ingredients listed in table 2 were weighed accurately and sifted through # 40 mesh (420 µm). Propranolol HCl and polymer (either CMEC or PEO) were mixed together to form a homogeneous blend. Sodium bicarbonate and povidone K 30 were added and mixed with the above blend wherever necessary. The above blend was lubricated with presifted magnesium stearate (# 60 mesh (250µm)). Final lubricated blends was compressed into tablets on a 16-station rotary tablet punching machine (M/s. Cadmach Machinery Co. Pvt., Ltd., India) using 9 mm plain round punches.

Tablet assay and physical evaluation

The GFT were estimated for drug assay using 0.1 N HCl as the extracting solvent, and the samples were analyzed spectrophotometrically (Shimadzu UV-1601, Shimadzu Corp.) at 289 nm (Srikanth 2011 b). Tablets were also evaluated for weight variation (n=20), hardness (n=5) (Monsanto hardness tester) and friability (n=20) (Roche Friabilator, 100 rpm).

In vitro buoyancy studies

The *in vitro* buoyancy of the GFT was measured by floating lag time (Srikanth *et al* 2011c). The floating tablets were placed in a beaker containing 0.1 N HCl and the time required for the tablet to float on the surface was determined as floating lag time (Srikanth *et al* 2011 c).

In vitro dissolution studies

The *in vitro* dissolution rate of propranolol HCl from GRFT (n=3) was determined. The dissolution test was carried out in USP type- II apparatus (LabIndia, Disso 2000), 900mL of 0.1N HCl and 50 rpm at 37±0.5°C (USP 36-NF31, 2013). 5mL of the dissolution portion was withdrawn at specific time intervals and the quantity was replaced with fresh dissolution medium. All samples were filtered through a 0.45µm membrane filter and followed by suitable dilution with 0.1 N HCl. Absorbance of these samples was measured at 289 nm using a UV-Visible double-beam spectrophotometer (Shimadzu UV-1601,Shimadzu Corp, Kyoto, Japan).

Data analysis and validation

Design Expert was established for the statistical validation of the polynomial equation on the basis of ANOVA provision in the software. A total of 11 runs for each polymer based formulation with replicate of factorial points & axial points and triplicate centre points were produced. The models were estimated in terms of significant coefficients, coefficient of variation (CV), coefficient of determination (R^2) and adjusted coefficient of determination (adj R^2) values. Numerical optimization and graphical optimization were accompanied to obtain the optimized composition. 3-D response surface graphs were plotted by the Design Expert software to check the effect of independent variables on formulation responses. The statistically optimized formulations suggested by software were formulated and evaluated. The experimental values were compared with that of the predicted values.

Fourier transform infrared study

The Fourier transform infrared (FTIR) spectra of pure propranolol HCl, CMEC, PEO WSR 301 and statistically optimized formulations (PCso and PPso) were measured using Shimadzu FTIR 8700 IR spectrophotometer. Samples were prepared by using KBrpellatization method. The samples were mixed with KBr (Spectroscopic grade) and made into disks by means of hydraulic pellet press at a pressure of 8 to 10 tons. The sample pellet was scanned from 3500 to 500 cm^{-1} at ambient temperature (25°C).

Stability studies

Stability studies were carried out on the statistically optimized GFT was carried out as per ICH (ICH 2003) guidelines at 25°C±2°C/60%±5% RH and 40°C±2°C/75% ±5% RH. Physical attributes of the tablets, % assay and *in vitro* drug release profiles were studied over a period of 6 months. Samples were withdrawn at 1, 3 and 6 months for the evaluation.

In vivo studies

In vivo buoyancy characterization

The *in vivo* floating ability studies of statistically optimized formulations (PCso and PPso) were carried out in healthy humans in the fasted and fed state by conducting X-ray evaluation studies.

Four healthy male volunteers of mean age 25±2 yrs, average weight 68±10 kg and an average height of 170±5 cm participated in this study. The volunteers were judged healthy on the basis of their physical examination, past medical history and routine laboratory tests. A full detail of the investigation was explained to the volunteers by verbally and in written form. The entire study was conducted under the guidance of a specialized radiologist. The study was approved from an independent Institutional Ethics Committee of Andhra University, Visakhapatnam (India). The study protocol is as follows:

Group A	Volunteer-1	PCso- Fed state	Wash out (1week)	PCso- Fasted state
	Volunteer-2	PCso- Fasted state		PCso -Fed state
Group B	Volunteer-3	PPso- Fed state		PPso- Fasted state
	Volunteer-4	PPso- Fasted state		PPso- Fed state

1. Fasted state: The subjects were fasted overnight and then administered the gastric floating tablet with 200 ml of water. No food was allowed up to 3 h of dosing. Subjects were not allowed to lie down and received 200 ml water every hour.
2. Fed state: The subjects were fasted overnight and in the morning given a high calorie-high fat breakfast with a total calorie value of approximately 900 Cal. The floating tablet was administered with 200 ml of water 30 min after the breakfast. The subjects were not allowed to eat anything for up to 6 h but were given 200 ml water every hour.

The optimized GFT of propranolol HCl containing barium sulfate (PCsoB and PPsoB) for *in vivo* X-ray evaluation were prepared. The quantity of propranolol HCl was reduced to 40 mg for incorporating the barium sulfate (40 mg) as the radio opaque substance in order to maintain the constant weight of the tablet. The remaining procedure is same as that for the normal gastric floating tablets preparation.

Table 2: Variable levels and formulae of floating tablets by central composite design

Formulation code	Variables		Ingredient (mg/tablet)						Total tablet weight (mg)
	X1	X2	Propranolol HCl	CMEC	PEO WSR 301	Sodium bicarbonate	Povidone	Magnesium stearate	
PC 1	-1	-1	80	200	-	16	16	3	315
PC 2	+1	-1	80	280	-	20	20	4	404
PC 3	-1	+1	80	200	-	53	16	4	353
PC 4	+1	+1	80	280	-	68	22	5	455
PC 5	-1.414	0	80	183.43	-	31.57	16	3	314
PC 6	+1.414	0	80	296.57	-	45.43	22	4	448
PC 7	0	-1.414	80	240	-	10	18	4	352
PC 8	0	+1.414	80	240	-	71	21	4	416
PC 9	0	0	80	240	-	38	19	4	381
PC 10	0	0	80	240	-	38	19	4	381
PC 11	0	0	80	240	-	38	19	4	381
PP 1	-1	-1	80	-	160	13	-	3	256
PP 2	+1	-1	80	-	240	17	-	4	341
PP 3	-1	+1	80	-	160	43	-	3	286
PP 4	+1	+1	80	-	240	57	-	4	381
PP 5	-1.414	0	80	-	143.43	25.57	-	3	252
PP 6	+1.414	0	80	-	256.57	37.43	-	4	378
PP 7	0	-1.414	80	-	200	8.5	-	2.5	291
PP 8	0	+1.414	80	-	200	58.5	-	3.5	342
PP 9	0	0	80	-	200	31.5	-	3.5	315
PP 10	0	0	80	-	200	31.5	-	3.5	315
PP 11	0	0	80	-	200	31.5	-	3.5	315

In vivo study of optimized formulations

The optimized floating tablets containing 80mg of propranolol HCl was compared with a commercial sustained release product Ciplar LA 80, 80mg, (B. No: D80624) Cipla Pharmaceuticals Ltd., India. Six healthy male volunteers of mean age 25±2 yrs, mean weight 68±10Kg and a mean height of 170±5 cm participated in this study with a protocol approved by the Institutional Ethics Committee of Andhra University, Visakhapatnam (India). A randomized, single dose, three-way crossover design (3 groups, each containing 2) with a washout period of 1 week between the two phases was used for the present *in vivo* study.

The subjects were fasted overnight at least 12 hours prior to dosing. Six volunteers were randomly divided into 3 groups of 2 each. A standardized high fat breakfast approximately 900 Cal was given in the morning. 30 min after breakfast, a zero hour blood sample (blank) was collected, followed by administration of optimized floating tablets or reference product with 200 ml of water and the subjects were 200 ml water every 2 hours. Blood samples were collected by cannulation method using pre labelled 5 ml K3 EDTA tubes. Samples were withdrawn at 0, 0.5, 1, 2, 3, 4, 6, 8, 10, 12 and 24h. Centrifugation was carried at 7500 rpm for 15 minutes to separate the plasma from the blood samples and separated plasma samples were stored in properly labelled tubes at -20°C prior to analysis. The *in vivo* evaluation of the selected formulations was carried out under the strict supervision of the physician.

The plasma propranolol concentration was determined using a rapid, sensitive, accurate and validated high performance liquid chromatographic (HPLC) method (Srikanth *et al* 2012a). Human plasma samples were exposed to protein precipitation by methanol. And final samples were directly injected into HPLC C18 column. Mobile phase comprising of a mixture of acetonitrile: pH 4.5 phosphate buffer (35:65) was delivered at a 1 ml/min of flow rate. Column temperature was maintained at 35±2°C. Ultraviolet absorption was measured at the wavelength of 214 nm using PDA detector. A good separation was achieved for propranolol HCl and diltiazem HCl with retention times of 6.6 min and 9.9 min respectively.

Pharmacokinetic parameters

Pharmacokinetic parameters such as elimination rate constant, half-life, AUC and MRT were estimated by using KINETICA 4.4.1 Software (Thermo Electron Corp., UK). The time to reach maximum concentration (T_{max}) and maximum concentration (C_{max}) were directly measured from the plasma concentration Vs time profile. The elimination rate constant (K_{el}) was estimated from the terminal portion of the log plasma concentration Vs time profile. The absorption rate constant (Ka) was measured by the method of residuals. The elimination half-life ($t_{1/2}$) was calculated by$0.693/K_{el}$. The area under the concentration time curve (AUC) and area under first moment curve (AUMC) was determined by the trapezoidal method. The mean residence time (MRT) was determined by AUMC divided by AUC.

Table 3: Tableting characteristics and observed responses of propranolol HCl floating tablets

Formulation	Tableting characteristics		Observed responses (n=3)		
	Assay[y] (%)	FLT* (S)	D_{1hr} (%)	D_{3hr} (%)	t_{95} (h)
PC 1	99.57±0.42	404	32.12	60.98	9
PC 2	99.84±0.23	259	23.45	49.98	11
PC 3	99.01±0.04	199	22.12	45.09	12
PC 4	99.98±0.99	97	13.84	37.98	14
PC 5	99.45±0.45	341	27.23	48.98	10
PC 6	99.63±0.23	148	18.12	41.12	12.5
PC 7	99.24±0.74	396	28.15	49.43	10
PC 8	100.01±0.05	104	18.99	40.05	13
PC 9	100.56±0.04	241	17.67	42.23	11.5
PC 10	99.86±0.26	227	17.9	41.15	11.8
PC 11	101.01±0.09	219	16.01	40.62	11.5
PP 1	100.08±0.07	395	25.77	53.86	5
PP 2	99.97±0.3	247	17.89	36.32	12
PP 3	99.68±0.45	193	20.52	40.12	9
PP 4	99.45±0.56	73	13.24	27.12	14
PP 5	98.95±0.17	314	23.98	45.05	8
PP 6	101.04±0.04	124	16.31	40.73	12
PP 7	100.06±0.09	349	22.95	44.81	7
PP 8	99.78±0.25	91	17.03	38.72	12
PP 9	99.56±0.54	210	17.49	39.12	11.5
PP 10	99.18±0.97	224	17.01	37.12	11.2
PP 11	100.04±0.45	217	18.43	40.72	13

STATISTICAL ANALYSIS

The pharmacokinetic parameters such as C_{max}, T_{max}, Ka, AUC_{0-24}, $t_{1/2}$ and MRT_{0-24} values of the tested formulations were statistically analyzed using paired sample's t-test using Origin Pro 8.5 software. A value of $P<0.05$ was considered to be significant.

RESULTS

Physical evaluation

Drug assay in the formulations was estimated spectrophotometrically at 289 nm. The assayed content of drug in all formulations were varied between 98.95% and 101.04% (table 3). All the prepared floating formulations complied with the compendial standards for uniformity of weight. Tablet hardness of the formulations was found to be between 4-6 kg/cm^2. Friability of all the formulations was found to be less than 0.5%.

In vitro buoyancy studies

Tablets of all formulations had desired floating lag times in the range of (<7 min) (table 3) i.e. all the formulations were floated in the 0.1 N HCl by less than 7 min indicating that all are having good buoyancy properties.

In vitro dissolution studies

The *in vitro* dissolution profiles of propranolol HCl floating tablets prepared by CMEC and PEO are shown in fig. 1. In the preliminary studies, CMEC based formulations exhibited higher/faster release at initial stages (1-2 hrs) which failed to meet USP specifications (USP 36-NF31) i.e. not more than 20% of the drug should release in 1 h. Hence to reduce the initial drug release, 5%w/w of povidone K30 was added to all CMEC based formulations. The dissolution parameters (D_{1hr}, D_{3hr} and t_{95}) of all the floating tablets are shown in table 3. From dissolution profile and parameters data, out of all CMEC based formulations, the formulations PC 4, 8,9,10 and 11 met the USP specifications. (Notgreater than 20% of the drug in 1 h and 20-45% of the drug should be release in 3 h). Among the PEO based formulations, only the PP 2, 4, 6, 8, 9, 10 and 11 met USP specifications.

Data analysis and validation

The observed responses for the CMEC and PEO based floating tablets are shown in table 3. An appropriate polynomial equation was selected based upon the several statistical parameters estimation like standard deviation, R^2, adj R^2, predicted R^2 values and the predicted residual sum of squares (PRESS) provided by the Design-Expert software. By the model summary static studies, the linear model was nominated as a suitable statistical model for all responses in both (CMEC and PEO based) formulations except D_{1hr} in CMEC based formulations, which followed quadratic model. PRESS value indicates the fitness of the model. Smaller PRESS is the better the model fits to the data points. The comparative values of R^2, adjusted R^2,

Table 4: Model summary statistics for responses $D_{1hr,}$ D_{3hr} and t_{95}

Source	Standard deviation	R^2	Adjusted R^2	Predicted R^2	PRESS	
CMEC based formulations						
D_{1hr}						
Linear	3.25	0.7430	0.6788	0.5938	133.29	
2FI	3.47	0.7432	0.6331	0.4554	178.68	
Quadratic	1.40	0.9700	0.9400	0.8181	59.68	Suggested
D_{3hr}						
Linear	3.09	0.7828	0.7285	0.5914	144.33	Suggested
2FI	3.31	0.7828	0.6898	0.2326	271.09	
Quadratic	3.03	0.8698	0.7397	0.1735	291.96	
t_{95}						
Linear	0.28	0.9700	0.9625	0.9364	1.33	Suggested
2FI	0.30	0.9700	0.9572	0.8997	2.09	
Quadratic	0.32	0.9758	0.9516	0.8419	3.29	
Regression equations[a]						
D_{1hr} = +17.19 -3.73 *A- 4.07 *B +0.097 *A*B+2.68 *A^2+3.13*B^2						
D_{3hr} = +40.38 -3.04 *A- 5.03*B						
t_{95} = +11.60 +0.94*A +1.28*B +0.000*A*B- 0.14*A^2-0.019*B^2						
PEO based formulations						
D_{1hr}						
Linear	1.33	0.8994	0.8743	0.8294	23.95	Suggested
2FI	1.42	0.9001	0.8572	0.7921	29.19	
Quadratic	0.97	0.9667	0.9334	0.7993	28.18	
D_{3hr}						
Linear	4.16	0.6787	0.5984	0.2908	305.46	Suggested
2FI	4.36	0.6907	0.5582	-0.1420	491.87	
Quadratic	4.99	0.7111	0.4221	-0.9812	853.31	
t_{95}						
Linear	1.47	0.7766	0.7207	0.6033	30.81	Suggested
2FI	1.53	0.7895	0.6992	0.4761	40.70	
Quadratic	1.20	0.9079	0.8157	0.4612	41.86	
Regression equations[a]						
D_{1hr} = +19.15 -3.25*A- 2.28*B						
D_{3hr}= +40.34- 4.58*A- 3.94*B						
t_{95} = +10.43 +2.21*A+1.63*B						

predicted R^2, standard deviation (SD) and PRESS are given in table 4 including regression equation generated for each response. A positive sign favors the optimization process, whereas a negative sign designates opposite relationship between the response and factor (Vijaykumar 2009). It is confirms that both polymers (CMEC and PEO) and sodium bicarbonate have negative effect on the responses D_{1hr} and D_{3hr} and have positive effect on t_{95}.

Using Design Expert software, the model was evaluated for lack of fit (LOF) and found that adequate. A summary of ANOVA results is shown in table 5. If the 'p-value' > F value is very small (<0.05) then it indicates LOF is significant. If LOF is significant, this indicates that the model that is considered for fitting the response does not adequately explain the variation in response. It may need a higher order model or perhaps a transformation. In some instance, it may indicate that a polynomial may be inadequate to describe the system (Liberman 1988). The LOF was not significant for all of the responses of both CMEC and PEO based formulations.

The model significance depends upon the p-value and it should be <0.05. If a term is non-significant, it can be removed from the model unless it is needed to satisfy hierarchy (Yang 2009). In the present investigation, P-value for all the responses were found to be <0.05 indicating that the models were significant.

Figs. 2 & 3 showed the bi-dimensional contour plots and tri-dimensional response surface respectively. These plots have shown the effect of two factors on the response at any specified time.

A numerical optimization technique and a graphical optimization technique were used to produce the optimum

Table 5: Summary of ANOVA results in analyzing lack of fit and pure error.

Source	Sum of squares	df	Mean square	F value	p-value prob>F	
CMEC based formulations						
D_{1hr}						
Model	318.28	5	63.66	32.32	0.0008	Significant
Residual	9.85	5	1.97			
Lack of fit	7.72	3	2.57	2.42	0.3058	Not significant
Pure error	2.13	2	1.06			
Core total	328.12	10				
D_{3hr}						
Model	276.56	2	138.28	14.42	0.0022	Significant
Residual	76.71	8	9.59			
Lack of fit	69.51	6	11.59	3.22	0.2559	not significant
Pure error	7.20	2	3.60			
Core total	353.28	10				
t_{95}						
Model	20.33	5	4.07	40.32	0.0005	Significant
Residual	0.50	5	0.10			
Lack of fit	0.44	3	0.15	4.94	0.1731	Not significant
Pure error	0.060	2	0.030			
Core total	20.84	10				
PEO based formulations						
D_{1hr}						
Model	126.28	2	63.14	35.77	0.0001	Significant
Residual	14.12	8	1.76			
Lack of fit	13.08	6	2.18	4.18	0.2057	Not significant
Pure error	1.04	2	0.52			
Core total	140.40	10				
D_{3hr}						
Model	292.34	2	146.17	8.45	0.0107	Significant
Residual	138.37	8	17.30			
Lack of fit	131.86	6	21.98	6.76	0.1345	Not significant
Pure error	6.51	2	3.25			
Core total	430.71	10				
t_{95}						
Model	60.33	2	30.16	13.90	0.0025	Significant
Residual	17.35	8	2.17			
Lack of fit	15.49	6	2.58	2.78	0.2883	Not significant
Pure error	1.86	2	0.93			
Core total	77.68	10				

quantities of independent variables (X_1 and X_2). The process was optimized for response variables D_{1hr}, D_{3hr} and t_{95}. The optimized formulation was selected based on constrains on D_{1hr} (<20% drug release), D_{3hr} ($20 \leq Y_2 \geq 45$) which are fixed by USP dissolution conditions (USP 36-NF31) and t_{95} (11.5 h). The acceptance criteria of propranolol HCl extended release dosage form according to the USP specifications are as follows; not more than 20%, between 20-45%, between 45-80% and not less than 80% of the drug should be dissolved at 1, 3, 6 and 12h respectively. Most of the experimental formulations obeyed the dissolution acceptance criteria at the 6^{th}& 12^{th} h, but not at the 1^{st}& 3^{rd} h. From the dissolution results, it was observed that the drug dissolution at 1^{st} and 3^{rd} h time intervals are crucial and hence, in the optimization process D_{1hr} and D_{3hr} were included along with t_{95}. These constrains are common for all the formulations.

The extensive grid and feasibility search provided the optimum formulations. The desired function response plot i.e. desirability plot and overlay plot are as shown in fig. 4, where one solution was found with a highest desirability. Upon swapping of all variables and evaluation of grid & feasibility searches, Design Expert software suggested 247.16 mg of CMEC and 8.97% of sodium bicarbonate for CMEC based formulations and 210.39 mg of PEO WSR 301 and 11.53% of sodium bicarbonate for PEO based formulations.

Table 6: Comparison of observed and predicted responses

Dependent variables	Observed	Predicted	Relative error (%)
PCso (statistical optimized CMEC based formulation)			
D_{1hr}	16.87	17.576	4.01
D_{3hr}	42.87	40.865	-4.90
t_{95}	11.49	11.50	0.08
PPso (statistical optimized PEO based formulation)			
D_{1hr}	17.98	17.605	-2.13
D_{3hr}	39.58	37.94	-4.32
t_{95}	11.52	11.499	-0.18

Table 7: Accelerated stability data of optimized floating tablets

PTSso	Weight[x] (mg)	Assay[y](%)	FLT[z] (S)	*D_{1hr}	*D_{3hr}	*t_{95}
PCso						
initial	386±1.21	99.12±0.87	238	16.12	42.87	11.49
1st month	387±0.87	99.98±0.83	229	16.78	42.56	11.56
3rd month	389±1.43	99.67±1.87	245	17.28	40.09	11.42
6th month	388±1.67	100.01±0.54	234	18.31	41.98	11.50
PPso						
Initial	333±1.02	99.93±0.74	197	17.98	39.58	11.52
1st month	331±0.58	99.78±0.38	201	18.12	36.48	11.49
3rd month	334±1.04	99.03±1.84	184	16.94	37.23	11.34
6th month	334±0.82	100.08±0.73	210	17.02	39.56	11.59

x: mean ± s.d. (n=20) ; y: mean ± s.d. (n=10); z: mean (n=5); FLT-floating lag time; *n=3

Table 8: *In vivo* gastric residence time of the optimized GRFT of propranolol HCl containing barium sulfate

Time (h)	Position of the tablet in GIT			
	Fed state		Fasted state	
	PCsoB	PPsoB	PCsoB	PPsoB
0.5	Stomach	Stomach	Stomach	Stomach
3	Stomach	Stomach	Small intestine	Small intestine
6	Stomach	Stomach	Disappeared from gastric region	Disappeared from gastric region
9	Disappeared from gastric region	Disappeared from gastric region		

Table 9: Pharmacokinetic parameters (mean ± s.d.) (N=6)

Pharmacokinetic parameter	PCso	PPso	Ciplar LA 80
C_{max}(ng/mL)	70.78±2.14	72.13±2.34	65.49±1.61
T_{max} (hrs)	6.87±0.85	6.59±0.88	4.94±0.91
K_{el} (hrs^{-1})	0.131±0.022	0.126±0.015	0.137±0.015
K_a(hrs^{-1})	0.534±0.013	0.517±0.101	0.755±0.021
$t_{1/2}$ (hrs)	5.79±0.988	5.52±0.691	5.11±0.565
AUC_{0-24} (ng.hrs/mL)	854.4±22.29	841.3±33.18	654.6±21.24
$AUC_{0-\infty}$ (ng .hrs/mL)	914.8±37.92	894.4±38.3	686.6±28.46
MRT_{0-24} (hrs)	12.75±0.204	11.68±0.231	9.71±0.268
$MRT_{0-\infty}$ (hrs)	13.53±0.69	12.94±0.57	10.79±0.52

Fourier transform infrared spectroscopy studies

The FTIR spectrum of propranolol HCl and optimized formulations was shown in the fig. 5. Propranolol HCl exhibited a peaks at 3275 cm^{-1}due to the presence of secondary amine -NH group, 2959 cm^{-1}due to C-H group, 1585 cm^{-1}due to presence of aryl C=C stretch and another stretch at 791 cm^{-1}due to the presence of alpha-substituted naphathalene (Venkata 2012; Srikanth 2012 b) (fig. 5).

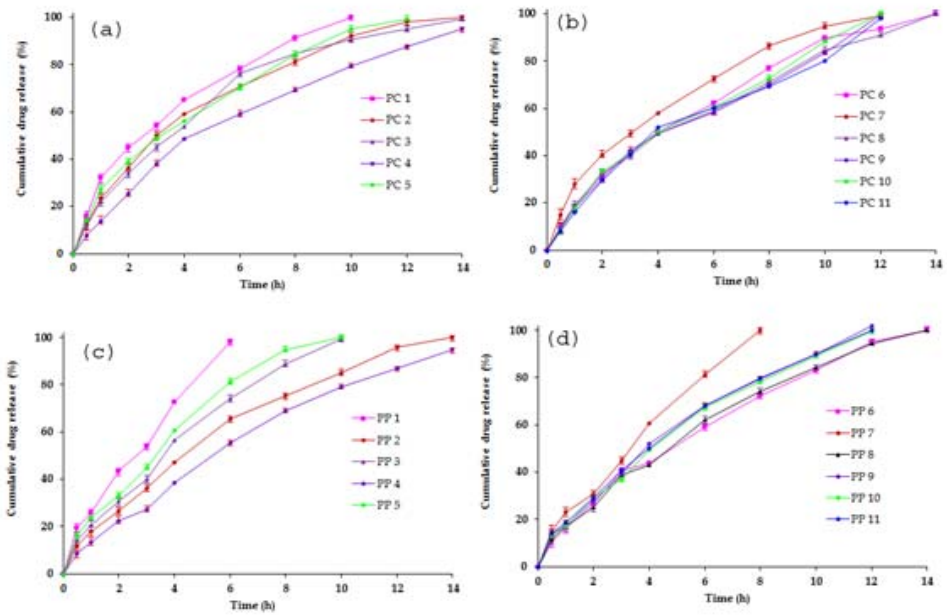

Fig. 1: Dissolution profile of propranolol HCl floating tablets: (a-b): CMEC based floating tablets, (c-d): PEO based floating tablets

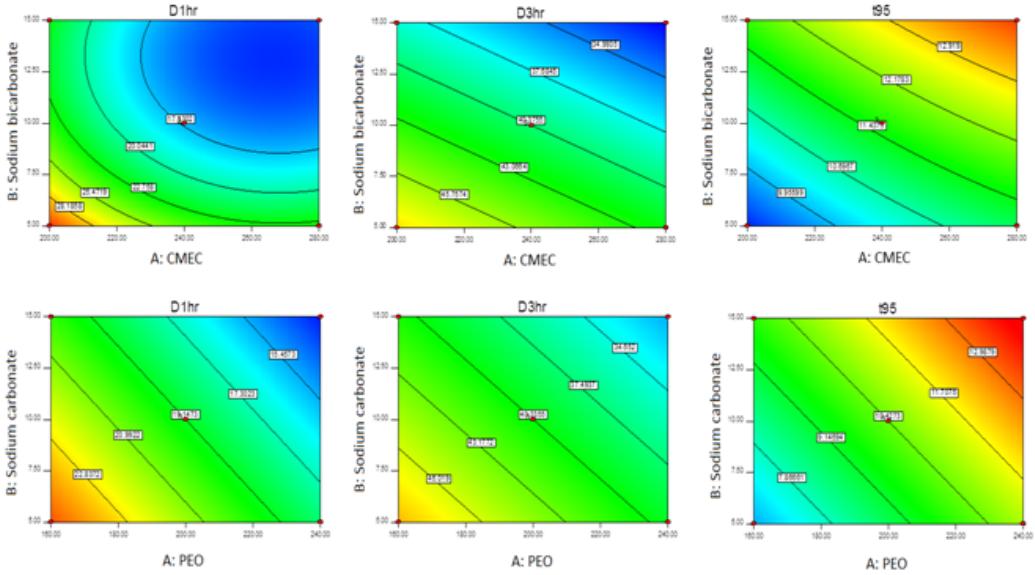

Fig. 2: Contour plot for the effect of independent variables on observed responses.

The FTIR spectrum of CMEC showed the characteristic peak at 3467 cm^{-1}indicating alcoholic -OH stretch, C-H stretch at 2968 cm^{-1}, -C=O stretch at 1755 cm^{-1} and another peak at 1369 cm^{-1} indicating -C-O-C asymmetric stretch. The FTIR spectrum of PEO WSR 301 showed the characteristic alcoholic -OH stretch at 3448 cm^{-1}, -C-O-C asymmetric stretch at 1238 cm^{-1} and -C-O-C symmetric stretch at 1072 cm^{-1}.

Stability studies

The stability studies were performed on the statistically optimized formulations (PCso and PPso). The formulations were kept at 40±2°C/75±5% RH aboutsix months to evaluate their stability at accelerated conditions. The study protocol conformed to ICH guidelines for stability testing. After a time interval of 1, 3 and 6 months, samples were withdrawn and retested for average weight, assay, buoyancy lag time and *in vitro* dissolution studies as described in Sec. 2.4-2.6 (table 7). Dissolution parameters such as D_{1hr}, D_{3hr} and t_{95} showed no significant difference between the initial and accelerated product at regular intervals. The results indicated stability of both the statistically optimized formulations (PCso and PPso) for a period of six months in accelerated conditions.

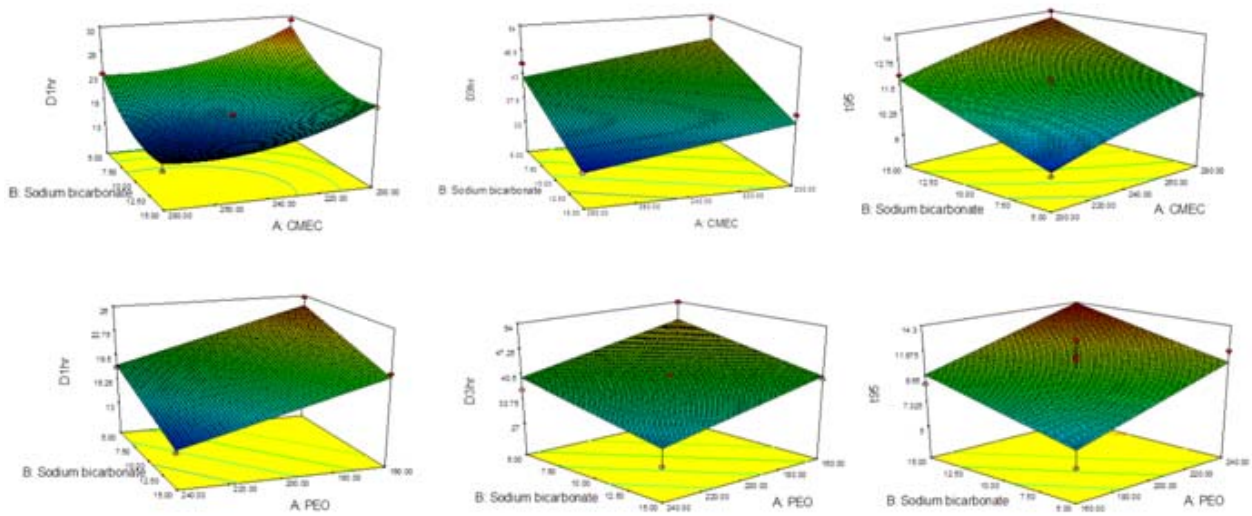

Fig 3: Response surface plot for the effect of independent variables on observed responses.

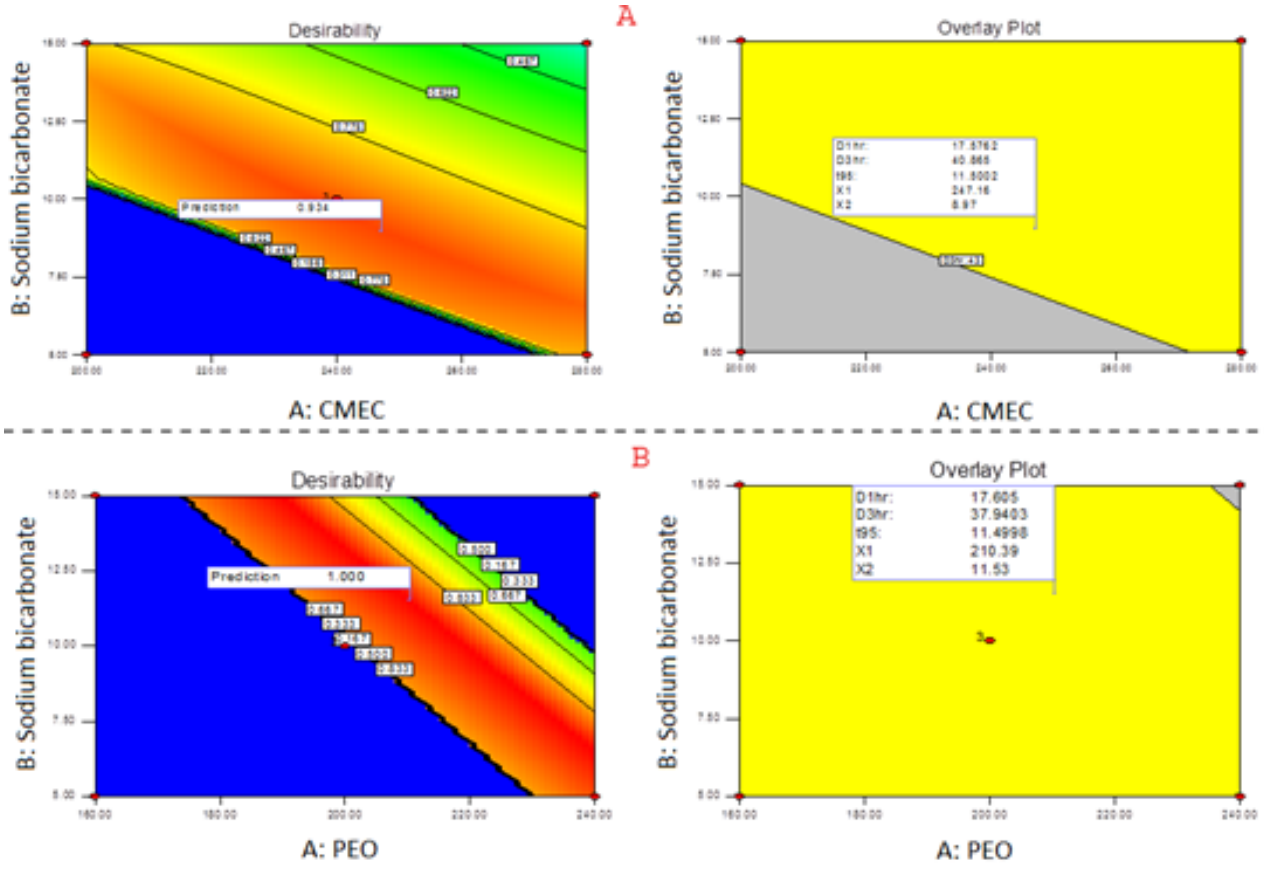

Fig 4: Desirability and overlay plot for optimization of floating tablets: A) CMEC based formulations; B) PEO based formulations

In vivo evaluation

In vivo buoyancy studies

Gastric floating tablets of propranolol HCl were designed to be retained in the gastric region for longer durations, assuring a control delivery of the drug above its absorption window, thus providing increased and more reproducible bioavailability. Their actual efficacy in the biological system was evaluated using X-ray studies in human volunteers to examine the intragastric floating behavior of the statistically optimized GFT of propranolol HCl both in the fasted and fed states.

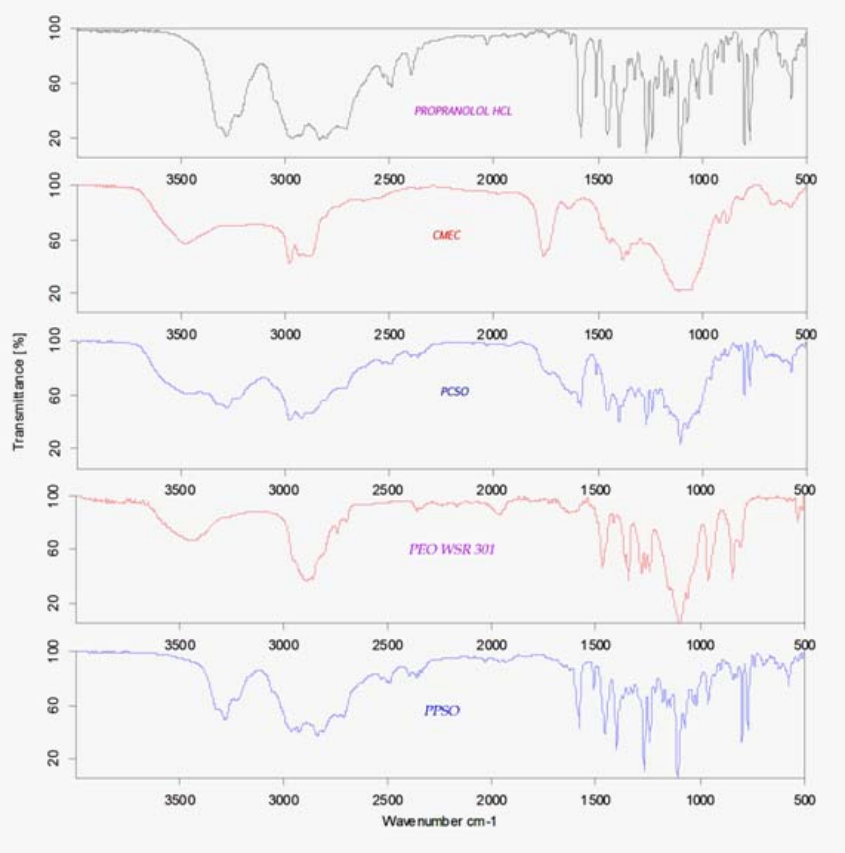

Fig. 5: FTIR spectra of propranolol HCl, polymers and optimized formulations

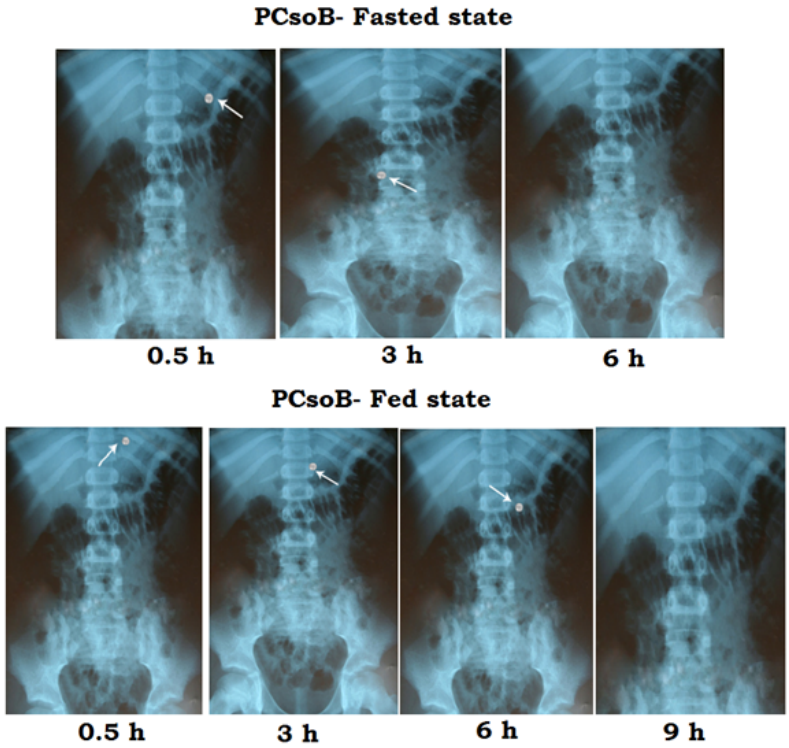

Fig. 6: X-ray photographs of gastric floating tablets of PCsoB containing propranolol HCl under fasted and fed state

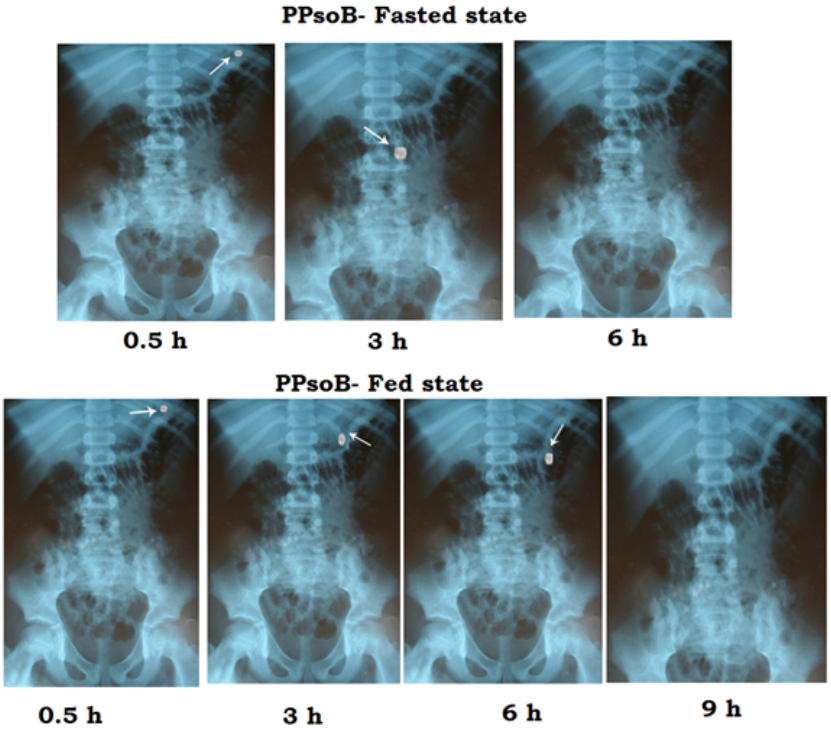

Fig. 7: X-ray photographs of gastric floating tablets of PPsoB containing propranolol HCl under fasted and fed state

The GFT remained buoyant on the gastric content under both fasted and fed states. However, a difference in floating and gastric retention time was found according to the feeding conditions (table 8).

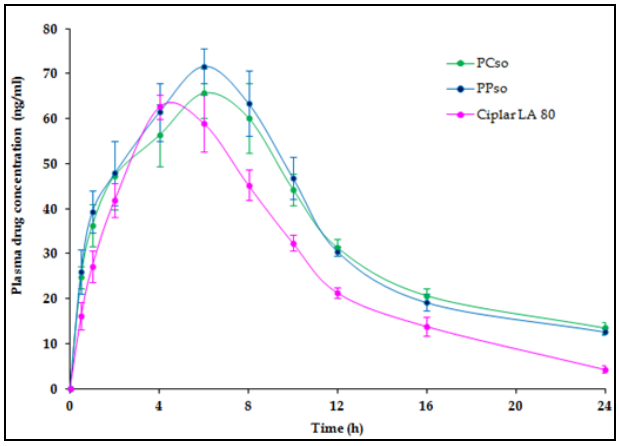

Fig. 8: Profile of mean plasma concentration Vs time of propranolol HCl in healthy human volunteers (n=6).

In the fasted state, both the CMEC and PEO based floating tablets (PCsoB and PPsoB respectively) were observed to be buoyant on the gastric fluid at 0.5 h, in the small intestine after 3 h and disappeared from the gastric region at 6 h, as shown in figs. 6 and 7. Therefore, in such conditions, the floating property did not enhance gastric retention time (GRT). The rapid gastric emptying is occurred under fasting conditions for every 1 to 2 h due to strong contractile activity, and which commendably sweep the undigested material from the stomach. Thus, if any dosage forms taken/administered under fasting conditions, it can be emptied from the stomach within 1-2 h depends upon the contractile activity (Minami 1984). Hence the observation that the floating tablets were present in the small intestine after 3 h may be because of this phase III activity, capable of ejecting even the large floating dosage form from the stomach.

In the fed state, after the high calorie, high fat breakfast, the formulations PCsoB and PPsoB were observed to be buoyant on the gastric contents up to 6 h after administration and disappeared at the ninth hour (figs. 6 and 7)**.**

Therefore, in the fed state, the floating system showed a GRT prolonged by about 4 to 5h over the fasted state. The enhanced GRT of the floating tablets is attributed to their position in the upper part of the stomach, which might have protected the dosage form from the strong, antral peristalsis. This favorable floating condition depends on the presence of food. The results were also in agreement with those of Iannuccelli *et al (*1998) and Timmermans *et al*. (1994).

This study has confirmed that in the fasted state, there was no enhancement of GRT of gastric floating matrix tablet, whereas there was a prolonged GRT of approximately 6 h in fed state.

Pharmacokinetic evaluation

The time - mean plasma drug concentration profiles following oral administration of the statistically optimized formulations (Pcso & Ppso) and marketed formulation (Ciplar LA 80) are shown in fig. 8. The mean pharmacokinetic parameters are shown in table 9.

The peak plasma concentrations (C_{max}) of propranolol HCl from Pcso, Ppso and Ciplar LA 80 were found to be 70.78±2.14, 72.13±2.34 and 65.49±1.61ng/mL respectively. The mean T_{max} values of optimized formulations were higher compared to marketed formulation, indicating a relatively slower absorption rate of Pcso and Ppso. Ciplar LA 80 tablets could not withstand GI movements and released the propranolol HCl quickly within 4.94±0.91 h, resulting in faster absorption, thus producing high peak concentration Cmax with earlier Tmax, when compared to Pcso and Ppso. The order of absorption rate among the selected polymers is PEO WSR 301 >CMEC >Ciplar LA 80.The K_a values obtained by application of Wagner-Nelson method to the plasma concentration data were found to be 0.534±0.013, 0.517±0.101 and 0.755±0.021 h^{-1} for Pcso, Ppso and Ciplar LA 80 respectively. The values of AUC_{0-24} and MRT_{0-24} for Pcso were 854.4±22.29ng.h.mL^{-1} and 12.75±0.204 h, for Ppso were 841.3±33.18 ng.h.mL^{-1} and 11.68±0.231 h and for Ciplar LA 80 654.6±21.24 ng.h.mL^{-1} and 9.71±0.268 h respectively.

The *in vivo* data demonstrated that the experimental formulations (Pcso and Ppso) exhibited larger AUC and high MRT values than the marketed formulation, which indicated that experimental formulations exhibited a more sustained drug release pattern compared to that of the marketed formulation.

There was no significant difference exists in K_a and $t_{1/2}$ and a significant difference in other pharmacokinetic parameters like $C_{max,}$ T_{max}, AUC_{0-24} and MRT_{0-24} values between the Pcso-Ciplar LA 80 and Ppso-Ciplar LA 80. No significant differences were observed between the experimental formulations Pcso and Ppso ($p<0.05$).

A good *in vitro in vivo* correlation exhibited with the prepared floating tablets. The relative bioavailability obtained with optimized gastric floating tablets Pcso and Ppso are 130.58 and 128.52% respectively compared to commercial formulation, indicated the improvement in bioavailability with these formulations. This indicated that the developed GRFT (Pcso and Ppso) are more effective in improving the oral bioavailability of propranolol HCl. This may be due to the developed GRFT releasing propranolol HCl constantly to the absorption sites and slower absorption over longer periods of time. Increased AUC and MRT values of prepared GRFT indicated that effective plasma concentrations were maintained for longer times compared to marketed formulation.

DISCUSSION

All the physical parameters of the compressed tablets were found to be in acceptable range.

The buoyancy properties depend upon the quantity of polymer and sodium bicarbonate concentration. As the quantity of polymer (CMEC or PEO) increased, the floating lag time was found to be decreased due to the increasing hydrophilic nature of the polymer, allowing penetration of liquid through pores formed on the surface of the tablet. Sodium bicarbonate generates carbon dioxide on reaction with acidic aqueous media; this helps the tablet to become buoyant and remain entrapped in the gel layer (Narendra 2008).

As the concentration of polymer increased along with concentration of sodium bicarbonate, the drug release rate was retarded. This may be due to increased intensity of air pockets surrounding the jellified surface of the tablet. Increases in the concentration of sodium bicarbonate at constant polymer concentration also retarded the drug release due to high intensity of the carbon dioxide gas pockets (Meka 2012).

Statistical optimized formulations (PCso and PPso for CMEC and PEO respectively) were prepared according to the software suggested quantities and tablets were evaluated for all physicochemical properties. The values for D_{1hr}, D_{3hr} and t_{95} of the floating tablets were comparable to those predicted by the model and are summarized in table 6. The relative error between predicted and observed values of all responses was found to be less than 5%. The results are in good agreement for product properties with theoretical predictions, justifying the predictability and validity of model used in our experimental design.

Statistically optimized formulations (Pcso & PPso) showed all the characteristic peaks of propranolol HCl with minor shifts in its FTIR spectrum. The FTIR data indicated the absence of chemical interaction between propranolol HCl and studied polymers CMEC & PEO WSR 301.

The in vivo buoyancy study has confirmed that in the fasted state, there was no enhancement of GRT of gastric floating matrix tablet, whereas there was a prolonged GRT of approximately 6 h in fed state.

A good *in vitro in vivo* correlation exhibited with the prepared floating tablets. The relative bioavailability obtained with optimized gastric floating tablets Pcso and Ppso are 130.58 and 128.52% respectively compared to commercial formulation, indicated the improvement in bioavailability with these formulations. This indicated that the developed GRFT (Pcso and Ppso) are more

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CONCLUSIONS

The statistical optimization is a suitable tool, particularly when several variables are to be evaluated simultaneously. The article discussed the application of a statistical optimization by central composite design for the development of GFDDS, in which formulations were prepared and optimized. The responses of the optimized formulations were in close agreement with the predicted values. There was a significant increase in the bioavailability of the propranolol HCl from the developed GRFT, which was evident from increased AUC levels and larger MRT values than the marketed product, Ciplar LA 80. This study revealed that the dosing after a standard high calorie high breakfast can prolong the GRT of the gastric floating tablet and will definitely increase the oral bioavailability of drugs. From the present investigation it may be concluded that the novel semi synthetic polymer, CMEC and synthetic polymer PEO is an effective carriers for the design of GFDDS of higher soluble drugs like propranolol HCl.

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