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FORMULATION AND OPTIMIZATION OF CONTROLLED RELEASE FLOATING MICROSPHERES OF FUROSEMIDE FROM ETHYLCELLULOSE AND HYDROXYPROPYL METHYLCELLULOSE POLYMER BLENDS

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Keywords:

Furosemide, Ethylcellulose, HPMC, solvent evaporation method, Gastroretentive Floating Microspheres, Optimization, Central Composite Design

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ABSTRACT: Furosemide is a potent and commonly used loop diuretic. It is absorbed largely in the stomach and upper small intestine. This narrow absorption window results in its low (average of 50%) and variable (10-100%) bioavailability from conventional dosage forms. The objective of the present study was to develop an optimized controlled release floating microspheres of furosemide capable of floating on the gastric fluid and delivering the drug over a period of 12 h. The floating microspheres were prepared by solvent evaporation method. Preliminary studies were conducted and, drug loading and EC/HPMC ratio were identified as the most important factors affecting the desired response variables: drug release rate and buoyancy. The effects of drug loading and EC/HPMC ratio were further studied and optimized. Simultaneous optimization of buoyancy and release rate was performed using central composite design and the most desirable optimal point was obtained at release rate of 27 $h^{-1/2}$ and buoyancy of 58.45%, with corresponding levels of 344 mg furosemide and 4.84 EC/HPMC ratio. Evaluation of the optimized formulation showed high yield, good flow property, extended release and buoyancy over a period of 12 h and excellent drug entrapment efficiency. Comparison of the release profiles of the three different batches of the optimized formulation confirmed that there was no statistically significant difference ($p = 0.302$) in the release profiles of the formulations.

INTRODUCTION: Furosemide is absorbed mostly in the stomach and upper small intestine, possibly due to its weak acidic property, pKa 3.8 [1]. This narrow absorption window is responsible for its low bioavailability of about 50%, and variable and erratic absorption [2]. Other reports indicate a poorer and highly variable oral bioavailability of 37–51% [1] and 10–100% [3].

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Attempts have been made to improve the bioavailability of furosemide by enhancing its aqueous solubility. Solid dispersions have been used as an effective method to improve the dissolution properties and bioavailability of poorly water-soluble drugs. Chaulang *et al* prepared furosemide tablets by solid dispersion technique and reported improved *in vitro* release profiles [4].

However, another problem with furosemide oral pharmacotherapy is the dissipation of the natriuretic effect before the next dose of the drug is given, which could not be addressed with solid dispersion formulations. Administration of furosemide as an intravenous infusion has been shown to improve its diuretic and natriuretic

activities in comparison to a bolus injection [5]. The narrow absorption window of furosemide in the upper part of the GI tract, together with its improved effect upon continuous drug input, provides a rationale for developing a gastroretentive dosage form for this drug. Such a dosage form would be retained for prolonged period of time in the stomach and release the drug in a sustained manner, so as to provide the drug continuously to its absorption site in a controlled manner for extended duration of absorption and drug effect [2].

Various approaches have been pursued over the last three decades to increase the retention of oral dosage forms in the stomach. The most common gastroretentive approaches used to increase the gastric residence time of pharmaceutical dosage forms include floating systems, swelling systems, bio/mucoadhesive systems and high density systems. Floating dosage forms are one of the most reliable and commonly used gastroretentive dosage forms and can be classified as single-unit and multiple-unit formulations.

Single-unit floating formulations are associated with problems such as sticking to the stomach wall, which may have a potential danger of producing irritation, and unreliable and irreproducible residence time in the stomach owing to their fortuitous emptying process. On the other hand, multiple-unit floating dosage forms appear to be better suited since they avoid risk of local irritation and the 'all-or-nothing' process. This reduced risk of 'all-or-nothing' effect reduces the intersubject variability in absorption and lower the probability of dose-dumping [6]. These advantages of multiple-unit floating dosage forms gave birth to the development of gastroretentive floating (hollow) microspheres.

An object of the present investigation was to develop an optimized controlled release floating microspheres of furosemide in a polymer blend of ethylcellulose and HPMC, which is capable of floating on the gastric fluid and delivering the therapeutic agent over a period of 12 h.

MATERIALS AND METHODS: Furosemide raw material (China associated Co. Ltd., China) and HPMC 4000 cp (China Associated Co. Ltd., China) were kindly supplied from the Ethiopian Pharmaceutical Manufacturing Sh. Co. (EPHARM). Ethylcellulose (Feicheng Rutai, China) was donated by Cadila Pharmaceuticals PLC. Furosemide reference standard (Greenfield Pharmaceuticals Co. Ltd., China) was obtained from Food, Medicine and Health Care Administration and Control Authority of Ethiopia. Ethanol (Uni. Chem., India), dichloromethane (Research-lab fine Chem. Industries, India), hydrochloric acid (BDH Ltd., England), sodium hydroxide (BDH Ltd., England), and Tween 80 (BDH Ltd., England) were all used as received.

Preparation of microspheres: Various microsphere formulations were prepared using solvent evaporation method. Typically, a fixed weight (1 g), but at varied proportions, of ethylcellulose and HPMC was dissolved in 16 ml of (1:1, v/v) dichloromethane and ethanol at room temperature. Weighed amount of furosemide was added to the polymers solution and mixed.

The resultant slurry was slowly introduced as a thin stream into a 200 ml of water containing 0.01% Tween 80 maintained at different temperatures and stirred at different stirring rates using heating magnetic stirrer (Velp Scientifica, Italy) for 1 h to allow the volatile solvent evaporate completely. The microspheres formed were filtered, repeatedly washed with distilled water and dried overnight in an oven drier (Kotterman 2711, Germany) at 40^{o}C [7].

Percentage yield of Microspheres: The production yield of microspheres of each batch was calculated using the weight of the final product after drying with respect to the initial total weight of the drug and polymers used for preparation of microspheres, and the percentage production yield was calculated as follows [8]:

$$\text{Yield (\%)} = \frac{\text{Practical mass (Microsphere)}}{\text{Theoretical mass (Polymers + Drug)}} \quad \text{...Eq. 1}$$

Particle size distribution: The particle size distribution of the prepared microspheres was determined using sieving method. Weighed microspheres of each formulation were shaken in a set of sieves fixed on a universal drive unit (Erweka, AR 402, Germany).

Microspheres that were retained on each sieve were collected and weighed, and the average particle size was calculated [9].

Density and related properties: For each formulation, sample of 20 g of microspheres was carefully introduced into a 250 ml graduated glass cylinder. The volume of the microspheres was noted. The bulk density of each formulation was then calculated as:

$$D_b = \frac{M}{V_b} \quad \text{.....Eq. 2}$$

Where, D_b is bulk density (g/cm^3), M is weight of sample in grams, and V_b is volume of microspheres in cm^3.

The microsphere sample was tapped 500 times using tapped densitometer (Erweka, SVM 20, Germany). The volume was noted after tapping and the tapped density was obtained using equation:

$$D_t = \frac{M}{V_t} \quad \text{.....Eq. 3}$$

Where D_t is tapped density (g/cm^3) and V_t is final tapped volume of powder in cm^3.

The Carr's index of each formulation was calculated from bulk and tapped densities using the equation:

$$Carr's\ index\ (\%) = \frac{(D_t - D_b)}{D_t} \times 100 \quad \text{.....Eq. 4}$$

Where D_b is bulk density (g/cm^3), and D_t is tapped density (g/cm^3).

Angle of Repose: Angle of repose was measured by using the fixed funnel method. Accordingly, 20 g of microspheres from 10 cm height was allowed to flow through a glass funnel orifice with an inner diameter of 15 mm. The angle of repose (θ, degree) was calculated as:

$$Angle\ of\ repose\ (\theta) = \tan^{-1}\left(\frac{H}{R}\right) \quad \text{.....Eq. 5}$$

Where H is pile height and R is the base radius.

Drug entrapment efficiency: Drug entrapment efficiency (DEE) was determined taking a sample of 50 mg drug loaded microspheres of each formulation. The weighed microspheres were dissolved in 10 ml dichloromethane in a separating funnel and the drug was repeatedly extracted with aliquots of 0.1 N NaOH. The extract was transferred to a 100 ml volumetric flask and the volume was made up using 0.1 N NaOH. The solution was filtered and the absorbance was measured at 271 nm against 0.1 N NaOH as blank. The amount of drug entrapped in the microspheres was calculated by the following formula [10]:

$$DEE\ (\%) = \frac{\text{Amount of drug actually present in the sample}}{\text{Theoretical drug content in the sample}} \times 100 \quad \text{.....Eq. 6}$$

***In vitro* buoyancy:** A sample of drug loaded microspheres weighing 300 mg were spread over the surface of USP Type II (paddle) dissolution apparatus (Erweka, DT 600, Germany) filled with 900 ml of 0.1 N HCl containing 0.02% of Tween 80. The medium was maintained at 37^{o}C and agitated with a paddle rotating at 100 rpm for 12 h. At the end of this period, the layer of buoyant particles on the surface of the medium was collected and the sinking particulates were separated by filtration. Both particle types were dried overnight in an oven drier (Kotterman 2711, Germany) at 40^{o}C. Dried weights were measured, and buoyancy was determined by the weight ratio of the floating particles to the sum of floating and sinking particles [11].

$$Buoyancy\ (\%) = \frac{\text{Dry weight of floated Microspheres}}{\text{Total dry weight of Floated and Settled Microspheres}} \quad \text{..... Eq. 7}$$

***In vitro* drug release:** A USP type II (paddle) dissolution apparatus (Erweka, DT 600, Germany) was used to study the *in vitro* drug release of the microspheres. Accordingly, an amount of the microspheres equivalent to 10 mg of furosemide filled in a hard gelatin capsule (size 0) was placed in the dissolution medium containing 900 ml of 0.1 N HCl and 0.02% of Tween 80 maintained at 37±0.5°C with paddle rotating at 100 rpm.

Samples of 10 ml were withdrawn at 0.5, 1, 2, 4, 6, 8, 10 and 12 h and filtered. Equal volume (10 ml) of the dissolution medium was replaced in the vessel after each withdrawal to maintain sink condition. Then, each of the sample solutions was analyzed spectrophotometrically for the drug content at 274 nm and the percentage of drug release was calculated and plotted as a function of time [12].

Release profiles comparison: Dissolution efficiency (DE) is one of the model independent approaches used for comparing drug release profiles. In this study, DE was determined from the *in vitro* dissolution data of the various formulations to compare their release profiles using the equation:

$$DE(\%) = \frac{\int_{t_1}^{t_2} y.dt}{y_{100}(t_2 - t_1)} \times 100 \quad \text{.....Eq. 8}$$

Where *y* is the percentage of dissolved product at any time *t*, y_{100} denotes 100% dissolution, and the integral represents the area under dissolution curve between time points t_1 and t_2.[13] DE could be defined for every sampling time. In this study, DE was calculated for the first 12 h release, setting t_1 at zero and t_2 at 12 h. One way ANOVA was applied to determine whether the existing differences were significant or not.

Kinetics and mechanism of drug release: In order to describe the drug release kinetics from the different microsphere formulations, the drug release data were fitted to the following release kinetic models:

a. Zero order release model:

$$Q = Q_o - Kt \quad \text{.....Eq. 9}$$

Where Q is the amount of drug remaining in the dosage form at time t, Q_o is the quantity of drug present initially in the dosage form and K is the zero order release constant.

b. First order release model:

$$\ln Q = \ln Q_o - Kt \quad \text{.....Eq. 10}$$

Where Q is the amount of drug remaining in the dosage form at time t, Q_o is the quantity of drug present initially in the dosage form, and K is the first order release constant.

c. Higuchi square root model:

$$\frac{M_t}{M_\infty} = Kt^{1/2} \quad \text{.....Eq. 11}$$

Where M_t/M_∞ is the fraction release of drug at time t, and K is rate constant.

d. Hixson-Crowell cube root model:

$$Q^{1/3} = Q_0^{1/3} - Kt \quad \text{.....Eq. 12}$$

Where Q is the amount of drug remaining in the dosage form at time t, Q_o is the quantity of drug present initially in the dosage form and K is the rate constant for Hixson-Crowell rate equation.

In order to find out the mechanism of drug release from the polymeric microspheres, drug release data were fitted to the Korsemeyer-Peppas model:

$$\frac{M_t}{M_\infty} = Kt^n \quad \text{.....Eq. 13}$$

Where M_t/M_∞ is fraction of drug released at time t, K is the rate constant and 'n' is the release exponent used to characterize different release mechanisms [14, 15].

Experimental design: On the basis of one-at-a-time preliminary experiments, the critical factors, furosemide amount (X_1) and EC/HPMC ratio (X_2), and their levels were chosen for the optimization procedure. These critical factors were further investigated to achieve optimized responses of release rate and buoyancy. For this, central composite design (CCD) was employed to determine the optimal conditions for the critical factors. According to the CCD matrix for two independent variables (n = 2), the total number of experiments (N) was determined as: $N = (2^n + 2n + n_c) = 2^2 + (2\times2) + 5 = 13$. The 13 experimental runs of the CCD matrix were performed and their observations were analyzed using Design-Expert 8.0.4 software.

Experiments were performed in random order to minimize the effects of uncontrolled variables that may introduce bias into the measurements. The selected formulation variables with their limits, units and notations are given in **Table 1**.

TABLE 1: FACTORS AND THEIR LIMITS USED IN THE CCD EXPERIMENTAL DESIGN

Factor	Limits				
	-α	-1	0	+1	+α
Furosemide X_1 (mg)	20.6	120	360	600	699.0
EC/HPMC X_2 (w/w)	1.1	2.8	6.9	11	12.7

N.B.: α = 1.414

RESULTS AND DISCUSSION:

Optimization study: The results of the preliminary experiments on the furosemide floating microsphere formulations indicated that the most important factors were the proportion of EC/HPMC and furosemide amount. Hence, these factors were considered as the independent variables and their effects on the characteristics of controlled release floating microspheres were further studied and optimized using response surface methodology (RSM). CCD of RSM that considers each of the design variables at five distinct levels was used to calculate quadratic regression model coefficients more efficiently. The desired response variables selected for optimization were drug release rate and buoyancy. Prolonged drug release and longer duration of buoyancy are considered critical for increased bioavailability of drugs with narrow absorption window.

On the basis of the preliminary experiments, the factor space of this design was expanded within the ranges 2.8:1 to 11:1 (w/w) for EC/HPMC and 120 to 600 mg for furosemide amount, and thus they were used as low and high levels, respectively, in the design matrix.

***In vitro* drug release:** The drug release behaviors of the different formulations in **Figure 1** indicate that the EC/HPMC ratio and amount of furosemide appeared to influence the drug release pattern remarkably. For instance, formulations with lower EC/HPMC ratio (f2, f4 and f8) exhibited a massive initial burst release of 44.45 ± 1.73%, 71.63 ± 1.79% and 54.97± 2.36%, respectively, in the first 1 h. This finding is analogous to that reported by Nighute and Bhise where drug release from EC/HPMC microspheres exhibited initial burst effect depending on the polymers proportion [16]. The formulation with the lowest drug amount (f6) also showed large initial burst release of 47.57±0.77% in the first 1 h. This could be attributed to the decrease in particle size of microspheres as a result of decreased amount of drug. It has been reported that the release rate depends on the overall viscosity of the system.[8] In the present study, formulation f6 had the lowest overall viscosity as it contains the lowest amount of drug of 20.6 mg in the one gram polymers mixture during its preparation. This reduction in viscosity resulted in formation of smaller droplets and hence smaller microspheres with larger surface area exposed to the dissolution medium that give rise to faster drug release.

All other formulations (f1, f3, f5, f7, f9, f10, f11, f12 and f13) exhibited better extended drug release with lower burst effects (Figure 1). The reason for this extended drug release may be due to the increased proportion of the hydrophobic polymer ethylcellulose and formation of larger microspheres upon increasing the amount of the drug. Hydrophobic polymers prevent the penetration of dissolution medium into the microspheres leading to slower dissolution and diffusion of drug molecules [17].

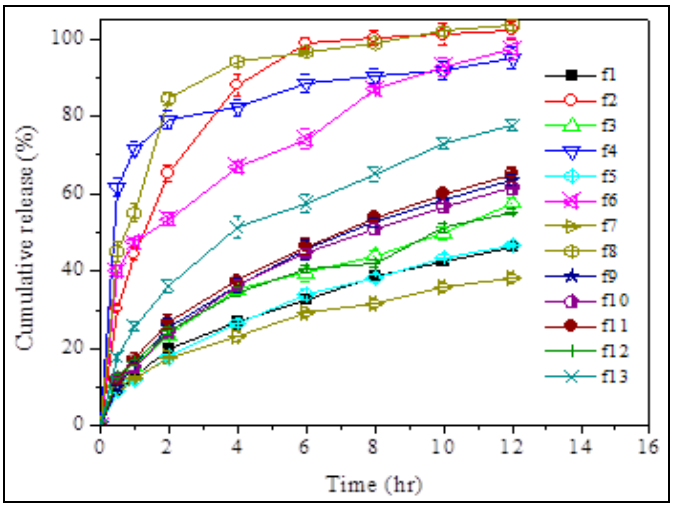

FIGURE 1: EFFECT OF EC/HPMC RATIO AND DRUG LOADING ON THE *IN VITRO* DRUG RELEASE FROM FUROSEMIDE FLOATING MICROSPHERES

Dissolution profiles of all the formulations were compared using dissolution efficiency and results of ANOVA from the dissolution efficiency values of the formulations revealed that there was a significant difference ($p < 0.0001$) in release profiles of the formulations. These differences in release profiles evidenced that changes in values of the investigated formulation variables had significant influence on the pattern of release and hence optimization was required to achieve a controlled drug release over predetermined duration.

Drug release kinetics: The *in vitro* drug release data were fitted to five popular release kinetic models: zero order, first order, Higuchi and Hixson–Crowell model equations to describe the *in vitro* drug release kinetics, and Korsemeyer-Peppas model to describe the drug release mechanism from the polymeric system [15]. On subjecting all 13 formulations to the four kinetic models described, Higuchi square root model showed the best fit with high linearity of $R^2 > 0.98$ for all formulations except f_2, f_4, f_6 and f_8 (**Table 2**).

Formulations composed of small amount of drug (f_6) and small amount of ethylcellulose (f_2, f_4 and f_8) exhibited poor fit with Higuchi model due to their initial burst release. Formulations f_2 and f_6 fitted relatively well to Hixson-Crowell cube root release kinetics whereas formulations f_4 and f_8 showed relatively better fit to first order release kinetics (Table 2).

Generally, the results indicate that the drug release rate in 12 h from the formulations fitted comparatively better to the Higuchi model.

In the Korsemeyer-Peppas model, the value of 'n' illustrates the type of release mechanism. For spherical particles; 'n' close to 0.43 indicates Fickian diffusion, 'n' between 0.43 and 0.85 suggests non-Fickian (anomalous) transport and 'n' close to 0.85 shows erosion (Case II) release [15, 18]. As shown in the table, 'n' values for formulations f_2, f_4, f_6 and f_8 were less than 0.43 indicating quasi-Fickian diffusion [19]. For all the remaining formulations, the values of 'n' were in the range of 0.435 to 0.621, which indicates that drug release from furosemide-loaded EC/HPMC microsphere formulations generally follows non-Fickian (anomalous) transport mechanism. The results also showed, on average, that the 'n' values are close to 0.5, which supports Higuchi square root model (Table 2) as the best fit kinetic model for drug release from furosemide loaded EC/HPMC microspheres.

Therefore, release rates of all 13 formulations were calculated using Higuchi square root model (**Table 3**). With the goal of sustaining the release of furosemide from the formulated floating microspheres for 12 h period, range of release rate expected to achieve 90 to 100% release of the drug in 12 h was also calculated using the Higuchi square root model. The range of release rate calculated was 26 to 29 $h^{-1/2}$, and was used in the optimization study.

TABLE 2: RESULTS OF MODEL FITTING FOR FUROSEMIDE LOADED EC/HPMC FLOATING MICROSPHERES

	Zero-Order Model	First-Order Model	Higuchi Square-root Model	Hixson-Crowell Cube-root Model	Korsemeyer-Peppas model	
	R^2	R^2	R^2	R^2	Exponent (n)	R^2
f1	0.888	0.926	0.988	0.914	0.501	0.994
f2	0.730	0.962	0.917	0.993	0.387	0.945
f3	0.922	0.966	0.994	0.955	0.513	0.992
f4	0.468	0.852	0.698	0.728	0.125	0.974
f5	0.939	0.971	0.997	0.962	0.540	0.997
f6	0.809	0.954	0.951	0.962	0.283	0.983
f7	0.903	0.939	0.995	0.928	0.435	0.995
f8	0.610	0.970	0.834	0.883	0.261	0.904
f9	0.943	0.990	0.996	0.978	0.621	0.996
f10	0.932	0.980	0.997	0.967	0.535	0.993
f11	0.948	0.990	0.998	0.982	0.548	0.997
f12	0.906	0.955	0.991	0.941	0.460	0.991
f13	0.862	0.955	0.984	0.930	0.478	0.995

Buoyancy: Investigation on floatation test showed that there was a significant difference ($p<0.0001$) in percentage buoyancy of the microsphere formulations at the end of 12 h (Table 3). The significant increase in buoyancy upon increasing drug loading could be due to the poor solubility of furosemide in acidic medium. As the composition of an insoluble component is increased in the formulation, permeability of the microspheres for the dissolution medium is decreased, resulting in prolonged buoyancy of the hollow microspheres. The continuous increase in percentage buoyancy with an increase in ethylcellulose proportion could be due to the hydrophobic nature of the polymer that decreases the penetration of the medium into the microspheres. On the other hand, microspheres with high level of HPMC were least buoyant which is attributed to the highly permeable property of HPMC and tendency of HPMC to increase the wettability of formulations [20]. Consequently, the increased amount of absorbed liquid medium replaces the air inside the floating microspheres, thus rendering them less buoyant.

The calculated release rates and measured percentage buoyancies of all 13 formulations in Table 3 were entered into the Design-Expert 8.0.4 software for optimization analysis.

TABLE 3: CCD MATRIX IN TERMS OF BOTH ACTUAL AND CODED FACTOR LEVELS AND SUMMARY OF EXPERIMENTAL VALUES OF RELEASE RATE AND BUOYANCY

Formulation	Point type	Factors		Responses	
		Furosemide (mg)	EC/HPMC (w/w)	Release rate ($h^{-1/2}$)	Buoyancy (%)
f1	factorial	600 (+1)	11 (+1)	13.21	73.19
f2	factorial	600 (+1)	2.8 (-1)	35.27	13.27
f3	factorial	120 (-1)	11 (+1)	16.20	64.17
f4	factorial	120 (-1)	2.8 (-1)	34.12	11.30
f5	axial	699.41 (+α)	6.9 (0)	13.49	88.17
f6	axial	20.59 (-α)	6.9 (0)	30.91	32.70
f7	axial	360 (0)	12.7 (+α)	11.47	87.80
f8	axial	360 (0)	1.1 (-α)	36.61	10.60
f9	center point	360 (0)	6.9 (0)	19.31	74.82
f10	center point	360 (0)	6.9 (0)	17.87	78.03
f11	center point	360 (0)	6.9 (0)	19.42	81.17
f12	center point	360 (0)	6.9 (0)	16.10	79.70
f13	center point	360 (0)	6.9 (0)	22.40	75.70

Mathematical model development: Fit summary statistics was used to choose a suitable model for a response comparing the models based on p-values and R-squared values. A suitable model is one with the highest order polynomial where the model is significant and R-squared values closer to one. A model is considered significant if the p-value is less than 0.05 or at least less than 0.1. Accordingly, linear model ($p = 0.0002$, $R^2 = 0.821$) and quadratic model ($p < 0.0001$, $R^2 = 0.964$) were selected as best fit models for release rate and buoyancy, respectively.

Model adequacy checking tests such as ANOVA, adequacy of precision, adjusted R^2 and predicted R^2 values and, normal probability and residuals versus predicted plots were examined. ANOVA is an important tool for the evaluation of significance and goodness of fit of the regression model and significance of individual model coefficients. As shown in **Table 4**, models of both responses were significant.

ANOVA result also revealed that the main effects of drug amount and EC/HPMC ratio were significant model terms for the linear model of release rate whereas both the main effects and the quadratic effects of the drug amount and EC/HPMC ratio were highly significant model terms for the quadratic model of buoyancy (Table 4).

However, the second order interaction effect of drug amount and EC/HPMC was not a significant model term for the quadratic model of buoyancy at both 5% and 10% levels of significance.

As reducing insignificant model terms, without including those required to support the hierarchy, could improve the model predictive efficiency [21], the model term that was not significant in the quadratic model of buoyancy was reduced automatically by selecting the backward elimination procedure.

TABLE 4: SUMMARY OF ANOVA RESULTS OF RESPONSE SURFACE LINEAR MODEL FOR DRUG RELEASE RATE AND SURFACE RESPONSE REDUCED QUADRATIC MODEL FOR BUOYANCY

Response	Source	Sum of Squares	df	Mean Square	F-value	p-value
Release rate	Model	800.78	2	400.39	22.86	0.0002
	Furosemide (X_1)	87.62	1	87.62	5.00	0.0493
	EC/HPMC (X_2)	713.16	1	713.16	40.71	<0.0001
	Residual	175.16	10	17.52		
	Lack of fit	153.64	6	25.61	4.76	0.0764
	Pure error	21.52	4	5.38		
	Core total	975.94	12			
Buoyancy	Model	10480.25	4	2670.06	48.35	<0.0001
	Furosemide (X_1)	*1635.25*	1	*1635.25*	*30.18*	*0.0006*
	EC/HPMC (X_2)	6456.30	1	6456.30	119.14	<0.0001
	Furosemide^2 (X_1^2)	*803.84*	1	*803.84*	*14.83*	*0.0049*
	EC/HPMC^2 (X_2^2)	*1863.50*	1	*1863.50*	*34.39*	*0.0004*
	Residual	433.51	8	54.19		
	Lack of fit	*405.24*	4	*101.31*	*14.33*	*0.0122*
	Pure error	28.27	4	7.07		
	Core total	10913.76	12			

	Release rate	Buoyancy
R-Squared	0.8205	0.9603
Adjusted R-Squared	0.7846	0.9404
Predicted R-Squared	0.6746	0.8244
Adequate Precision	13.283	19.385

The ANOVA table also shows that the value of R^2 for the linear model of release rate and reduced quadratic model of buoyancy were 0.821 and 0.960, respectively, indicating adequate degree of correlation between the experimental and the predicted values [22]. The results also indicated that the adjusted R^2 and predicted R^2 values of both responses were in reasonable agreement, and the value of adequate precision (signal to noise ratio) of 13.28 for release rate and 19.39 for buoyancy obtained were very high compared to the desirable value of greater than 4 [21]. The important information on the model performance is summarized in residuals (i.e., difference between observed and predicted values) providing a clear view for any discrepancy in fit to the model. Hence, two plots related to residuals: the normal probability plot of residuals and the plot of internally studentized residuals versus predicted values are considered as additional tests of model adequacy checking tools [23]. A check on the normal probability plots in **Figure 2a and Figure 3a** show that points or point clusters are placed closely to the diagonal line implying that the errors are distributed normally for both responses.

Figure 2b and Figure 3b indicate that the points are randomly scattered, with no obvious pattern or structure indicating the models proposed are adequate for their respective responses and there is no reason to suspect any violation of the independence or constant variance assumption.

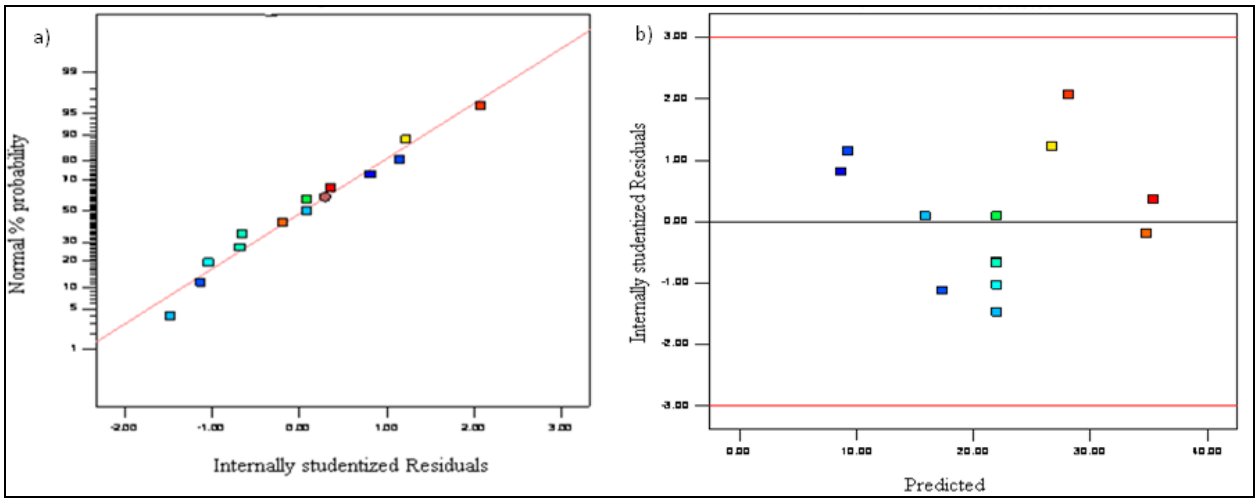

FIGURE 2: NORMAL PROBABILITY PLOT OF RESIDUALS (A), AND PLOT OF RESIDUALS VERSUS PREDICTED VALUES (B) FOR RELEASE RATE DATA

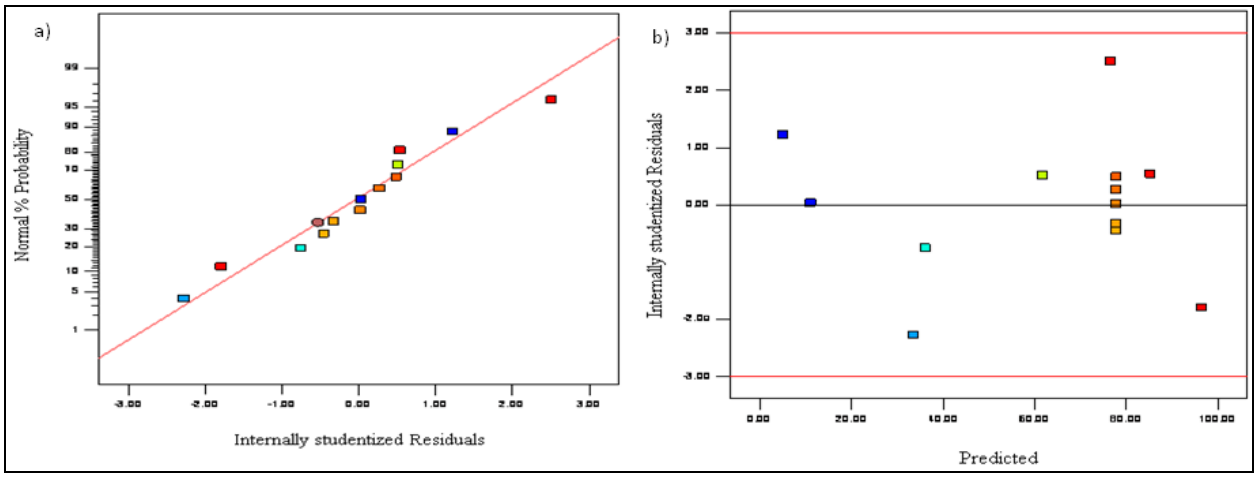

FIGURE 3: NORMAL PROBABILITY PLOT OF RESIDUALS (A), AND PLOT OF RESIDUALS VERSUS PREDICTED VALUE (B) FOR BUOYANCY

Therefore, with evidence of the adequacy checking tests, it was concluded that the selected models were fairly accurate and could be used for further analysis. Thus, the final polynomial equations of response variables in terms of coded coefficients of the factors were developed as:

$$\text{Release rate}\,(Y_1) = 22.03 - 3.31X_1 - 9.44X_2 \quad \text{.....Eq. 14}$$

$$\text{Buoyancy}\ (Y_2) = 77.88 + 14.30X_1 + 28.41X_2 - 10.75X_1^2 - 16.37X_2^2 \quad \text{....Eq. 15}$$

Figures 4 and 5 depict graphical representations of the developed mathematical models. The series of parallel straight lines of the contour plot (**Figure 4a**) and the flat plane of the response surface plot (**Figure 4b**) indicate that there is no interaction effect of EC/HPMC ratio and furosemide amount on the release rate. However, the plots show that the linear model components individually affect the release rate significantly, with a comparatively more significant effect of EC/HPMC ratio than the furosemide amount on the response.

The contour and response surface plots of the quadratic model of buoyancy (**Figure 5**) show that both EC/HPMC ratio and furosemide amount play very important roles in influencing the response buoyancy. However, the curvilinear contours of Figure 5a indicate the interactive effect of the two variables is not significant. A perfect interaction between the independent variables is characterized by formation of elliptical contours, where the maximum predicted value is identified by the surface confined in the smallest ellipse in the contour diagram [22]. The plots also showed the effect of EC/HPMC ratio appeared to be more pronounced as compared to the amount of furosemide on the response buoyancy.

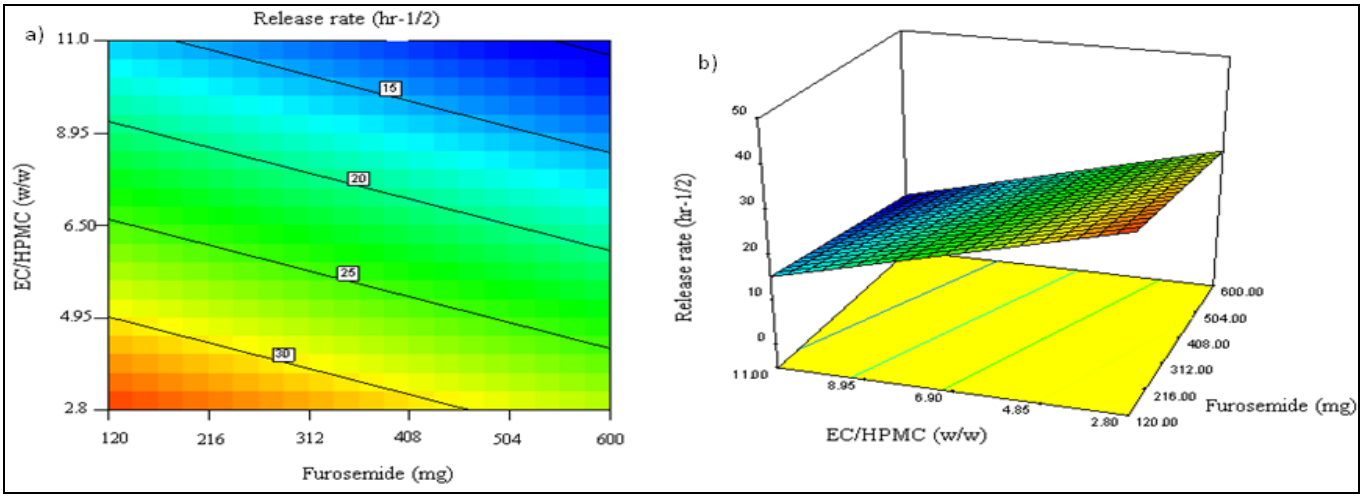

FIGURE 4: CONTOUR PLOT (A) AND RESPONSE SURFACE PLOT (B) SHOWING THE EFFECTS OF POLYMERS RATIO (EC/HPMC) AND AMOUNT OF DRUG ON DRUG RELEASE RATE IN 12 H

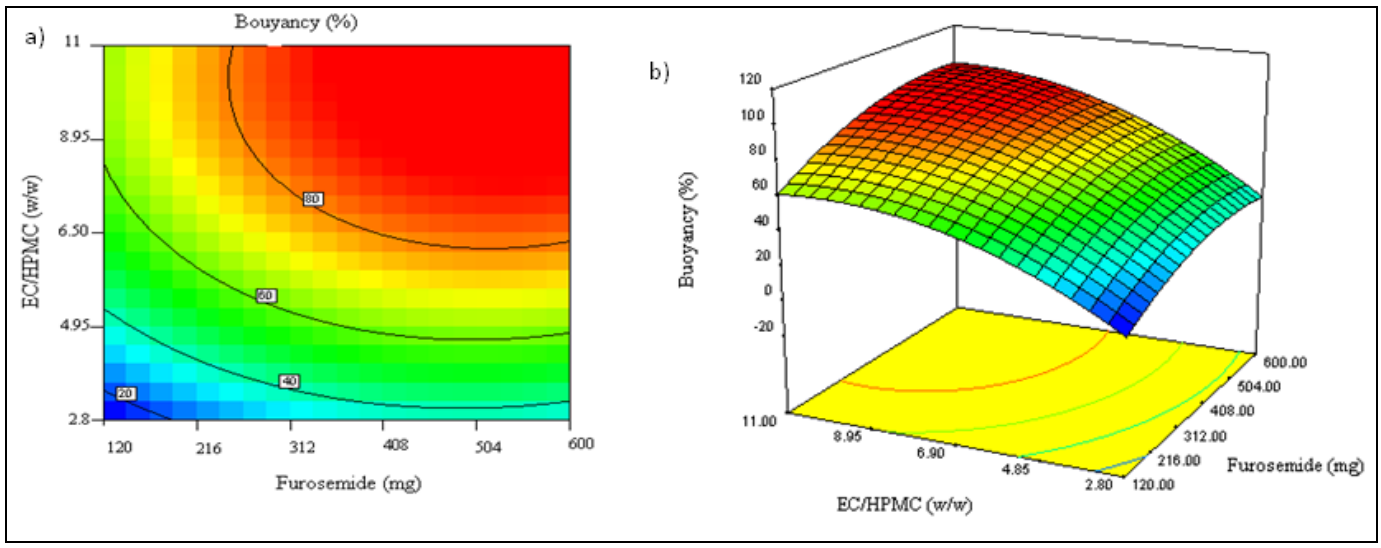

FIGURE 5: CONTOUR PLOT (A) AND RESPONSE SURFACE PLOT (B) SHOWING THE EFFECTS OF POLYMERS RATIO (EC/HPMC) AND AMOUNT OF DRUG ON BUOYANCY

Simultaneous optimization of buoyancy and release rate: After generating the model polynomial equations to relate the dependent and independent variables, the formulation was optimized for the two responses simultaneously. The final optimal experimental parameters were obtained using both numerical and graphical optimization techniques of Design-Expert 8.0.4 software, which allows the compromise among various responses and searches for a combination of factor levels that jointly optimize a set of responses by satisfying the requirements for each response in the set. **Table 5** presents the criteria defined for factors and responses during optimization by both numerical and graphical techniques.

TABLE 5: CRITERION SETTINGS OF FACTORS AND RESPONSES FOR FORMULATION OPTIMIZATION BY NUMERICAL AND GRAPHICAL OPTIMIZATION

Factor constraints				
Factor	Low	High		
Furosemide (mg)	120	600		
EC/HPMC (w/w)	2.8:1	11:1		
Response constraints				
Response	Goal	Lower limit	Upper limit	Importance
Release rate ($h^{-1/2}$)	Target = 27	26	29	4
Buoyancy (%)	Maximize	50	90	5

Numerical optimization: Numerical optimization is used in order to find the specific point that maximizes the global desirability function. In the numerical optimization of this study, the desired goals for responses were chosen from the menu and importance to each response was assigned. To find the global (overall) desirability function, the software performed thousands of iterations and calculations and finally came up with the maximum desirability score of 0.422 (**Figure 6a**).

Accordingly, the predicted optimum values and the corresponding levels of parameters at this maximum desirability score were obtained as release rate of 27 $h^{-1/2}$ and buoyancy of 58.45%, and furosemide amount of 344 mg and EC/HPMC ratio of 4.84:1 (w/w), respectively.

Graphical optimization: The methodology essentially consisted of overlaying the curves of the two models obtained from the CCD according to the specific criteria imposed in Table 5. **Figure 6b** shows the overlay plot in which the yellow area represents the area satisfying the imposed criteria. The point identified by the flag was chosen in the graph as representative of the optimized area corresponding to furosemide amount of 344 mg and EC/HPMC ratio of 4.84:1 (w/w). Under these conditions the model predicts release rate of 27 $h^{-1/2}$ and buoyancy of 58.45%.

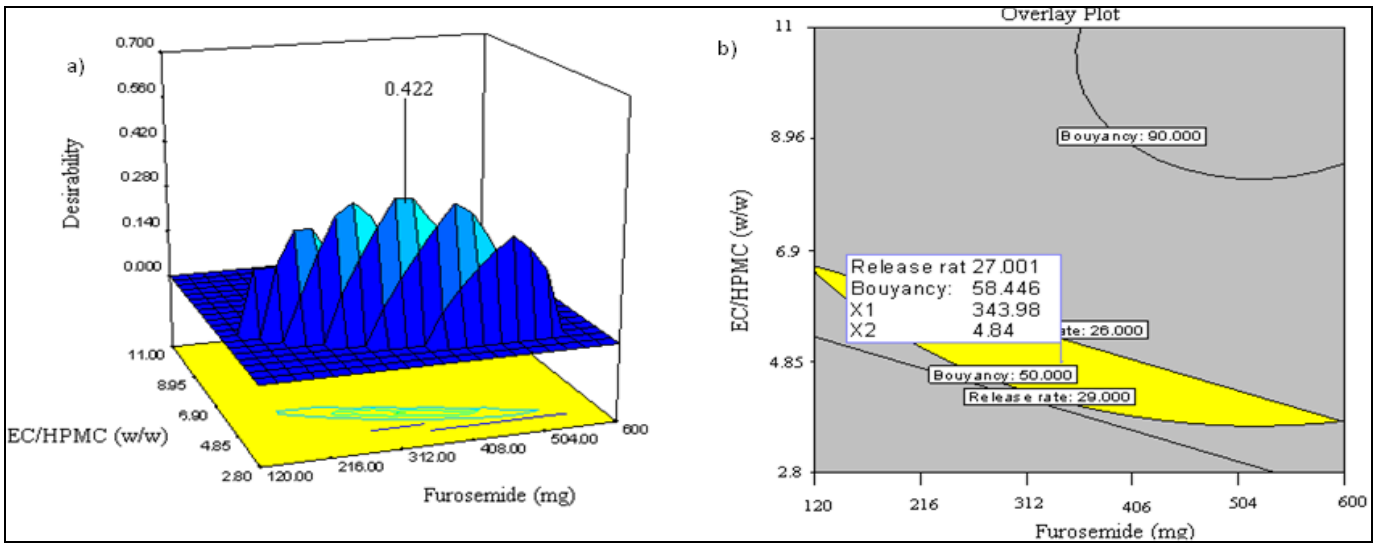

FIGURE 6: GRAPHICAL REPRESENTATION OF THE MAXIMUM GLOBAL DESIRABILITY FUNCTION (A) AND THE OPTIMUM REGION IDENTIFIED BY OVERLAYING PLOTS OF THE TWO RESPONSES (BUOYANCY AND RELEASE RATE) (B) AS FUNCTIONS OF FUROSEMIDE AMOUNT AND EC/HPMC RATIO

Confirmation test: To experimentally confirm the validity of obtained optimal point, confirmation experiments were carried out at the optimal combinations of the factors (X_1 = 344 mg, X_2 = 4.84:1). From the results presented in **Table 6**, the values of percentage errors for release rate and buoyancy have fallen within about 5% indicating that the experimental values of the optimized formulations agreed well with the predicted values [24].

TABLE 6: EXPERIMENTALLY PREPARED FORMULATIONS BASED ON THE PREDICTED VALUES AND THE EVALUATION OF RELEASE RATE AND BUOYANCY

Response	Predicted value	Experimental value	% Error
Release rate (Y_1, $h^{-1/2}$)	27	28.49 ±0.42	5.22
Buoyancy (Y_2, %)	58.448	61.05±0.32	4.26

Evaluation of the optimized floating microspheres of furosemide: The optimized formulation was evaluated for its flow property, particle size, entrapment efficiency, yield and release properties (**Table 7**). The angle of repose and Carr's index values were 35.4° and 10.95%, respectively, indicating that the flowability of the optimized furosemide microspheres could be rated as *'good'* as per the angle of repose and Carr's index general scales of flowability set in the USP 30/NF 25 [25].

TABLE 7: CHARACTERISTIC PROPERTIES OF OPTIMIZED FUROSEMIDE MICROSPHERE FORMULATION

Parameters	Experimental values
Bulk density (g/cm^3)	0.169 ± 0.002
Tapped density (g/cm^3)	0.189 ± 0.002
Angle of repose (°)	35.40 ± 0.78
Carr's Index (%)	10.95 ± 0.76
Particle size (µm)	812.7 ± 7.95
Yield (%)	92.31 + 2.42
Drug entrapment efficiency (%)	96.54 ± 1.17

The good flow property of the microspheres can be attributed to their spherical shape as seen in **Figure 7**. In addition, the results also show excellent drug entrapment efficiency and good yield of 96.54% and 92.31%, respectively, which could be attributed to the poor solubility of the drug in aqueous medium and higher proportion of the hydrophobic ethylcellulose.

FIG. 7: PHOTOGRAPHIC PICTURE OF OPTIMIZED FLOATING MICROSPHERES OF FUROSEMIDE

The release profiles of the optimized formulation were evaluated using three different batches (**Figure 8**). ANOVA results from comparison of release profiles based on DE values of the three batches, 61.7 ± 3.5, 61.6 ± 0.3 and 60.1 ± 0.7%, revealed that there was no statistically significant difference ($p = 0.302$) in the release profiles of the formulations. The release profile curves presented in the figure also support the ANOVA results of DE that the release patterns are similar among the three batches, indicating the optimal formulation yields reproducible results. The results also confirmed the optimal formulation release the drug over a period of 12 h in a controlled manner.

The release kinetics study for the optimized formulation showed Higuchi square root kinetic model was the best fit model with $R^2 > 0.991$. The 'n' values from the Korsemeyer-Peppas model range from 0.519 to 0.641 indicating drug release from the optimized formulation was mainly non-Fickian (anomalous) transport mechanism.

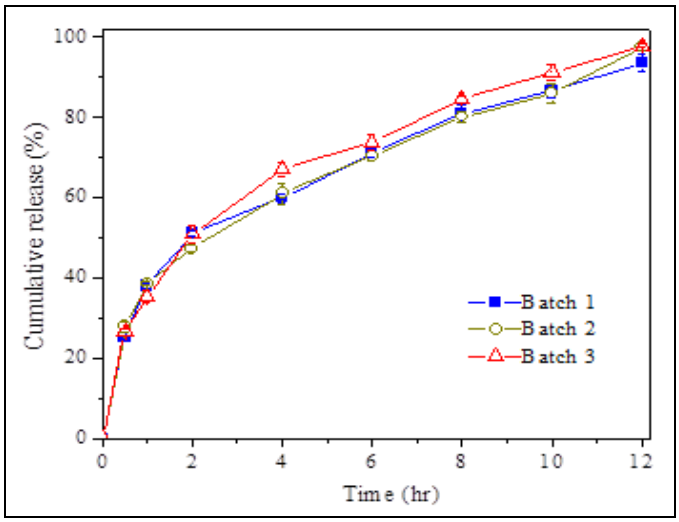

FIGURE 8: RELEASE PROFILES OF OPTIMIZED FUROSEMIDE-LOADED EC/HPMC FLOATING MICROSPHERES

CONCLUSIONS: Furosemide loaded microspheres were successfully prepared by solvent evaporation method using ethylcellulose and HPMC polymer blends. The preliminary studies indicated that drug loading (amount) and EC/HPMC ratio showed significant effects on drug release and buoyancy of microspheres. Statistical models were established to predict the selected responses, buoyancy and drug release, by simultaneously studying the effect of the two variables using the CCD under RSM.

The results of the model adequacy checking tools evidenced that the developed models were fairly accurate and could be used for further analyses. The ANOVA results, model term coefficients and, contour and response surface plots of the developed models revealed the release rate was dependent only on the linear effects whereas buoyancy was affected by the linear and squared (quadratic) effects of the two variables.

However, interaction effects did not exist for release rate and were not significant for buoyancy. The most desirable optimal point was obtained with release rate of 27 $h^{-1/2}$ and buoyancy of 58.45%, under conditions of 344 mg furosemide amount and 4.84:1 (w/w) EC/HPMC ratio. The validity of obtained optimal point was confirmed by the low magnitude of percent prediction error.

Thus, this study has come up with an optimum formula for the preparation of furosemide floating microspheres that could remain buoyant releasing the drug over a period of 12 h in a sustained manner.

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REFERENCES:

1. Granero G, Longhi M, Mora M, Junginger H, Midha K, Shah V, Stavchansky S, Dressman J and Barends D: Biowaiver monographs for immediate release solid oral dosage forms: Furosemide. J Pharm Sci 2010; 99: 2544-2556.
2. Klausner EA, Lavy E, Stepensky D, Cserepes E, Barta M, Friedman M and Hoffman A: Furosemide pharmacokinetics and pharmacodynamics following gastroretentive dosage form administration to healthy volunteers. J Clin Pharmacol 2003; 43: 711-720.
3. Jackson E: Drugs acting Renal and Cardiovascular functions. In: Brunton L, Lazo J and Parker K. (eds): Goodman and Gilman's The Pharmacological Basis of Therapeutics, 11th edn., McGraw Hill, New York, 2006: 773-771.
4. Chaulang G, Patel P, Hardikar S, Kelkar M, Bhosale A and Bhise S: Formulation and evaluation of solid dispersions of furosemide in sodium starch glycolate. Trop J Pharmaceut Res 2009; 8: 43-51.
5. Mojtahedzadeh M, Ardakani M, Salehifar E, Najafi A, Khajavi M, Moeini M and Sadray M: The relationship between pharmacokinetic variables and pharmacodynamic profiles of bolus versus continuous infusion of furosemide in critically ill patients. Daru 2009; 13: 127-132.

6. Khan K and Bajpai M: Floating Drug Delivery System: An Overview. Int J PharmTech Res 2010; 2: 2497-2505.
7. Gattani Y, Kawtikwar P and Sakarkar D: Formulation and evaluation of gastroretentive multiparticulate delivery system of aceclofenac. Int J ChemTech Res 2009; 1: 1-10.
8. Ghosh A, Nayak K, Rout P, Nag T and Roy P: Preparation, evaluation and *in vitro in vivo* correlation study of lamivudine loaded microspheres. Res J PharmTech 2008; 1: 353-356.
9. Yüce M and Canefe K: Indomethacin-loaded microspheres: preparation, characterization and *in-vitro* evaluation regarding ethylcellulose matrix material. Turk J Pharm Sci 2008; 5: 129-142.
10. Garg R and Gupta G: Gastroretentive floating microspheres of silymarin: Preparation and *in vitro* evaluation. Trop J Pharm Res 2010; 9: 59-66.
11. Karthikeyan D, Karthikeyan M and Ramasamy C: Development of floating microspheres to improve oral bioavailability of cefpodoximeproxetil. Acta Pharma Sci 2010; 52: 101-104.
12. Basu S and Adhiyaman R: Preparation and characterization of nitrendipine loaded Eudragit RL100 microspheres prepared by an emulsion-solvent evaporation method. Trop J Pharmaceut Res 2008; 7: 1033-1041.
13. Sadray S, Honary S, Ebrahimi P, Jafari P and Sezaee R: Dissolution profile comparison: Model dependent and model independent approaches. Int J Pharm and Bio Sci 2010; 2: 1-10.
14. Belete A and Gebre-mariam T: Formulation and evaluation of sustained release paracetamol peroral matrix tablets: optimization and *in vitro* evaluation. Ethiop Pharm J 2004; 22: 27-38.
15. Shoaib M, Tazeen J, Merchant H and Yousuf R: Evaluation of drug release kinetics from ibuprofen matrix tablets using HPMC. Pak J Pharm Sci 2006; 19: 119-124.
16. Nighute A and Bhise S: Preparation and evaluation of rifabutin loaded polymeric microspheres. Res J PharmTech 2009; 2: 371-374.
17. Prabu L, Shirwaikar A, Shirwaikar A and Kumar A: Formulation and evaluation of sustained release microspheres of rosin containing aceclofenac. Ars Pharm 2009; 50: 51-62.
18. Ali J, Arora S, Ahuja A, Babbar A, Sharma R, Khar R and Baboota S: Formulation and development of hydrodynamically balanced system for metformin: *in vitro* and *in vivo* evaluation. Eur J Pharm Biopharm 2007; 67: 196-201.
19. Ali MS, Singh S, Kumar A, Tahir MD and Pattnaik AG: Preparation and *in vitro* evaluation of sustained release matrix tablets of phenytoin sodium using natural polymers. Int J Pharmacy Pharm Sci 2010; 2: 174-179.
20. Freiberg S and Zhu X: Polymer microspheres for controlled drug release. Int J Pharm 2004; 282: 1-18.
21. Aziz H, Kamaruddin A and Abu-Bakar M: Process optimization studies on solvent extraction with naphthalene-2-boronic acid ion-pairing with trioctylmethyl ammonium chloride in sugar purification using design of experiments. Separ Purif Tech 2008; 60: 190-197.
22. Baril N, Alam Z, Muyibi S, Jamal P and Al-Mamun A: Statistical optimization of process parameters for the production of citric acid from oil palm empty fruit bunches. Afr J Biotechnol 2010; 9: 554-563.
23. Kusic H, Jovic M, Kos N, Koprivanac N and Marin V: The comparison of photooxidation processes for the minimization of organic load of colored waste water applying the response surface methodology. J Hazard Mater 2010; 183: 189-202.
24. Mohajeri L, Aziz H, Esa M and Zahed M: A statistical experiment design approach for optimizing biodegradation of weathered crude oil in coastal sediments. Bioresour Technol 2010; 101: 893-900.
25. USP 30/ NF 25: United States Pharmacopoeial Convention, Maryland, Rockville Inc, 2007.

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