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RESEARCH ARTICLE

Development and in vitro evaluation of floating rosiglitazone maleate microspheres

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Abstract

Background: Various approaches have been used to retain the dosage form in stomach as a way of increasing the gastric residence time, including floatation systems; high-density systems; mucoadhesive systems; magnetic systems; unfoldable, extensible, or swellable systems; and superporous hydrogel systems. *Aim*: The objective of this study was to prepare and evaluate floating microspheres of rosiglitazone maleate for the prolongation of gastric residence time. *Method*: The microspheres were prepared by solvent diffusion–evaporation method using ethyl cellulose and hydroxypropylmethylcellulose. A full factorial design was applied to optimize the formulation. *Results*: Preliminary studies revealed that the polymer:drug ratio, concentration of polymer, and stirring speed significantly affected the characteristics of microspheres. The optimum batch exhibited a prolonged drug release, remained buoyant for >12 hours, high entrapment efficiency, and particle size in the order of 350 µm. *Conclusion*: The results of 32 full factorial design revealed that the concentration of ethylcellulose 7 cps (X_1) and stirring speed (X_2) significantly affected drug entrapment efficiency, percentage release after 8 h and particle size of microspheres.

Key words: *Ethyl cellulose; factorial design; floating microspheres; rosiglitazone maleate; solvent diffusion–evaporation*

Introduction

Several approaches have been developed to prolong the residence time of dosage forms in the stomach[1]. Various approaches have been used to retain the dosage form in the stomach as a way of increasing the gastric residence time (GRT), including floatation systems; high-density systems; mucoadhesive systems; magnetic systems; unfoldable, extendible, or swellable systems; and superporous hydrogel systems[2]. Floating drug delivery systems (FDDS) or hydrodynamically balanced systems (HBS) are among the several approaches that have been developed to increase the GRT of dosage forms. Both single and multiple unit systems have been developed. The single-unit floating systems are more popular but have a disadvantage owing to their 'all-or-nothing' emptying process, leading to high variability of the gastrointestinal transit time[3,4]. In contrast, multiple-unit particulate dosage forms (e.g., microspheres) have the advantages that they pass uniformly through the gastrointestinal tract (GIT) to avoid the vagaries of gastric emptying and provide an adjustable release, thereby reducing the intersubject variability in absorption and risk of local irritation. Recently, hollow microspheres with a lower density than that of the GI fluids were adopted[5]. The floating microspheres were prepared by the emulsion solvent diffusion–evaporation technique using different polymer solution systems[6,7]. Rosiglitazone maleate (RGM) acts as an agonist at peroxisome proliferator-activated receptors (PPARs) in target tissues for insulin action, such as adipose tissue, skeletal muscle, and liver. Activation of the PPAR-γ regulates the transcription of insulin-responsive genes involved in the control of glucose production, transport, and utilization. In this manner, RGM enhances tissue sensitivity to insulin[8].

RGM has all the requisite characteristics suitable for developing an FDDS, which includes solubility of RGM decreases with increasing pH. Hence, floating microspheres were prepared to improve the bioavailability and achieve steady-state plasma concentration of the drug.

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Materials and methods

Materials

RGM was obtained as a gift sample from Cipla Pharmaceutical Ltd. (Pune, India). Ethyl cellulose 7 cps (EC 7 cps) and hydroxypropyl methylcellulose (HPMC K100M CR) was supplied by Colorcon Asia Ltd. (Goa, India). All solvents used were of analytical grade.

Preparation of floating microspheres

Floating microspheres with a central hollow cavity were prepared by using a solvent diffusion–evaporation technique[6]. In the preliminary trials, weighed quantities of drug, EC 7 cps, and HPMC K100M CR (Table 1) were dissolved in a mixture of ethanol (ETN) and dichloromethane (DCM) (1:1 solvent ratio) at room temperature. This solution was poured into 100 mL distilled water containing 0.1% Tween 80 maintained at a temperature of 30°C–40°C. The resultant emulsion was stirred with a propeller type agitator at 800 rpm for 45 minutes to allow the volatile solvent to evaporate.

Design of experiments

A 3^2 full factorial was applied to design the experiments. Concentration of EC (7 cps) and stirring speed were used as independent variables, whereas % entrapment efficiency, % drug release after 8 hours, and particle size were kept as dependent variables. Formulations F_1 to F_9 were prepared using three different levels of EC concentration and stirring speed. Microspheres thus obtained were filtered, washed with water, and dried overnight at room temperature. The responses of the dependent variables were evaluated. The polynomial equations were generated for each responses using Design Expert Software (7.1.4) and intensive grid search was performed over the experimental domain to locate five optimum formulations (S_1–S_5). These five formulations were then formulated and used to validate the obtained polynomial equation model. The summary of the formulations is shown in Table 2.

Evaluation of formulations subjected to optimization

% Drug retained and yield of floating microspheres

Ten milligrams of floating microspheres were dissolved in 10 mL ETN. The samples were assayed for drug content using UV spectrophotometry (Jasco V550; Jasco, Tokyo, Japan) at 247 nm after suitable dilution. No interference was found due to the other floating microspheres components at 247 nm. The percentage drug entrapment efficiency and yield were calculated as follows:

$$\% \text{ Drug entrapment efficiency} = \frac{\text{Calculated drug concentration}}{\text{Theoretical drug concentration}} \times 100.$$

$$\% \text{ Yield} = \frac{\text{Total weight of floating microspheres}}{\text{Total weight of drug and polymer}} \times 100.$$

Scanning electron microscopy

The external and internal morphology of the microspheres were studied using scanning electron microscopy (SEM). The samples for SEM were prepared by lightly sprinkling on a double adhesive tape stuck to an aluminum stub. The stubs were then coated with platinum to a thickness of about 10 Å under an argon atmosphere using a gold sputter module in a high-vacuum evaporator. The stub containing the coated samples was placed in the scanning electron microscope (JSM-6360A; JEOL, Tokyo, Japan) chamber. The samples were then randomly scanned, and photomicrographs were taken at the acceleration voltage of 20 kV, original

Table 1. Formulation of preliminary trials of floating microspheres.

Ingredients	P_1	P_2	P_3	P_4	P_5	P_6	P_7	P_8	P_9	P_{10}
Ehtyl cellulose 7 cps (%)	7	10.5	14	17.5	7	10.5	14	17.5	14	14
HPMC K100M CR (%)	—	—	—	—	0.5	0.5	0.5	0.5	1	1.5
Solvent ratio (ETN:DCM)	1:1	1:1	1:1	1:1	1:1	1:1	1:1	1:1	1:1	1:1

All batches contained 0.35 g of RGM, 10 mL ethanol, and 10 mL dichloromethane.

Table 2. 3^2 full factorial design layout.

Ingredients	F_1	F_2	F_3	F_4	F_5	F_6	F_7	F_8	F_9
EC 7 cps (%)	11.5	11.5	11.5	14	14	14	16.5	16.5	16.5
Stirring speed (rpm)	800	1200	1600	800	1200	1600	800	1200	1600

All batches contained 0.35 g of RGM, 1% of HPMC K100M CR, 10 mL ethanol, 10 mL dichloromethane, 0.1% Tween 80, and 100 mL distilled water.

magnification 30× to investigate the internal morphology, and microballoons were divided into two pieces by using a knife.

Particle size

The particle size of the microspheres was measured using an optical microscope and the mean particle size was calculated by measuring 100 particles with the help of a calibrated ocular micrometer[9].

Measurement of flow properties

Angle of repose (θ) of different formulation, which measures the resistance to particle flow, was determined by using a fixed funnel method and calculated as follows:

$$\tan\theta = \frac{2H}{D},$$

where $2H/D$ is the surface area of the free standing height of the microspheres heap, which is formed on a graph paper after making the microspheres flow from the glass funnel.

The tapping method was used to determine the tapped density and percent compressibility index as follows:

$$\text{Tapped density} = \frac{\text{Mass of microspheres}}{\text{Volume of microspheres after tapping}},$$

$$\%\ \text{Compressibility index} = \left[1 - \frac{V}{V_0}\right] \times 100,$$

where V_0 and V are the volumes of the sample before and after the standard tapping, respectively[9].

In vitro evaluation of floating ability

Floating behavior of hollow microspheres was studied using a USP dissolution test apparatus I by spreading the microspheres (100 mg) on 900 mL of 0.1 mol/L HCl containing 0.02% Tween 80 as surfactant. The medium was agitated with a paddle rotating at 100 rpm and maintained at 37°C. After 12 hours, both the floating and the settled portions of microspheres were collected separately. The microspheres were dried and weighed. The percentage of floating microspheres was calculated using the following equation[10]:

$$\%\ \text{Floating microspheres} = \frac{\text{Weight of floating microspheres}}{\text{Initial weight of floating microspheres}} \times 100.$$

In vitro drug release study

The release rate of RSM from microspheres was determined using USP dissolution testing apparatus I (Basket type). The dissolution test was performed using 900 mL of 0.1 N HCl, at 37 ± 0.5°C and 100 rpm. Microspheres equivalent to 8 mg RSM were used for the test. Aliquots (5 mL) were withdrawn at hourly intervals for 12 hours. Samples were replaced by its equivalent volume of dissolution medium. The samples were filtered through Whatman filter paper and solutions were analyzed at 228 nm using UV spectrophotometer (Jasco V530; Jasco, Tokyo, Japan). Cumulative percentage drug release was calculated using PCP Disso v2.08 Software (Poona College of Pharmacy, Pune, India).

Data analysis

A statistical model incorporating interactive and polynomial terms was used to evaluate the responses:

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_1 X_2 + \beta_4 X_1^2 + \beta_5 X_2^2 + \beta_6 X_1 X_2^2 + \beta_7 X_1^2 X_2 + \beta_8 X_1^2 X_2^2,$$

where β_0, the intercept is the arithmetic average of all quantities outcomes of nine runs, β_1 to β_8 are the coefficients computed from the observed experimental values of Y, and X_1 and X_2 are the coded levels of the independent variables. The terms X_1X_2 and X_i^2 ($i = 1, 2$) are the interaction and polynomial terms, respectively.

Stability studies

The stability studies were carried out at an optimized formulation, i.e., formulation S_3. The formulation was stored at 40° ± 2°C/75% ± 5% RH for 3 months (Climatic zone IV condition for accelerated testing) to assess their stability. The protocol of stability studies was in compliance with the WHO guidelines for stability testing intended for the global market. After intervals of 7, 15, 30, 60, and 90 days, samples were withdrawn and retested for drug content, floating behavior, and drug release studies.

Results and discussion

Preliminary trials

In the preliminary studies, RGM floating microspheres were prepared by using the solvent diffusion–evaporation method. EC 7 cps was selected as a polymer for the preparation of floating microspheres owing to its film forming, release rate controlling ability, nontoxicity, nonirritancy, stability at GI pH, compatibility with drug, and good mechanical strength property. Different concentrations of EC 7 cps from 7% to 17.5% (w/v) were

Table 3. Result of preliminary trial batches.

Batch code	Yield (%)	Mean particle size (μm)	Drug entrapment efficiency[a] (%)	Drug release[a] (%)	Floating ability (%)
P_1	77.14	375	60.35 ± 3.2	54.40 ± 2.9	83.02
P_2	83.67	447	72.09 ± 1.9	47.06 ± 3.7	87.61
P_3	85.71	505	87.43 ± 2.7	41.26 ± 4.1	93.34
P_4	84.41	580	92.28 ± 4.1	36.14 ± 7.2	96.29
P_5	75.67	348	56.08 ± 2.7	61.23 ± 5.6	79.61
P_6	82.35	395	67.24 ± 2.3	5674 ± 4.3	83.40
P_7	86.15	475	83.65 ± 2.9	49.16 ± 2.4	88.08
P_8	85.56	537	87.81 ± 3.1	43.56 ± 5.1	91.50
P_9	80.59	435	80.64 ± 3.0	62.41 ± 4.2	83.78
P_{10}	69.72	380	69.53 ± 4.7	78.36 ± 3.8	71.31

[a]Mean ± SD; $n = 3$.

used for preparing the polymer solution for formulations P_1 to P_4 (Table 1). Concentration of EC 7 cps was found to have a significant impact on drug entrapment. For formulations P_1 to P_4, as the concentration of EC 7cps increased entrapment efficiency was also found to increase from 60.35% to 92.28% (Table 3). However, the drug release was greatly retarded at higher concentrations of EC. The % drug release ranged from 54.40% at low-EC concentration (7%) to 36.14% at high-EC concentration (17.5%), as shown in Table 3. Therefore, HPMC was selected to be used in combination with EC to increase the drug release from microspheres because of its hydrophilic nature. Formulations P_5 to P_{10} (Table 1) are formulated by using a combination of EC 7 cps (7%–17.5%) and HPMC K100M CR (0.5%–1.5%). It was observed that when the concentration of HPMC increased in formulations (P_9 and P_{10}), drug release increased (from 61.23% to 78.36%), but at the same time drug entrapment efficiency (from 80.64% to 69.53%) and the yield of microsphere (from 80.59% to 69.72%) decreased. As the concentration of EC 7 cps increased (P_5–P_8), the floating ability also increased (from 79.61% to 91.50%); whereas, as the concentration of HPMC increased (P_9 and P_{10}), the floating ability decreased (from 83.78% to 71.31%). Concentrations of EC 7 cps (14%) and HPMC K100M CR (1%) polymers showed adequate drug release along with drug entrapment (P_9). Formulation P_9 showed good values of drug entrapment (80.64%) and drug release (62.41%). From the literature review[4], it was found that stirring speed also shows significant effect on drug entrapment efficiency, drug release, and particle size. Hence, stirring speed was kept constant in the preliminary trial and selected as a second independent variable. From the results of the preliminary studies, the concentrations of EC and stirring speed were selected as independent variables for 3^2 full factorial design, because both of them affected the entrapment efficiency, drug release, and particle size of microspheres. Nine formulations as per 3^2 full factorial designs were prepared as given in Table 2.

Evaluation of formulations subjected to optimization

This floating microparticulate system was developed by emulsion solvent diffusion–evaporation method by using DCM and ETN as solvents. Finely dispersed droplets of the solution of drug and polymer solidify in the aqueous phase due to the diffusion of ETN. Evaporation of DCM from the solidified droplets leaves the cavity in the microspheres filled with water. During the drying procedure, the cavity inside each microsphere becomes filled with air, generating the microballoon[6].

% Drug retained and yield of floating microspheres

The drug entrapment efficiency of microspheres varied from 45.47% to 88.63% (Table 4). Results demonstrated that an increase in the concentration of EC increased the entrapment efficiency of the drug. The drug entrapment efficiency was good because the drug was sparingly soluble in the aqueous medium. Stirring speed had a negative effect on entrapment efficiency. The percentage yield of

Table 4. Results of optimized formulations.

Batch code	Yield (%)	Entrapment efficiency[b] (Y_1)	Mean particle size[a] (μm)	Angle of repose[b]	Tapped density[b] (g/cm^3)	Carr's index[b]	Floating ability[b] after 12 hours (%)
F_1	76.49	64.83 ± 1.9	397 ± 2.3	20.43 ± 0.7	0.4854 ± 1.03	1.44 ± 1.1	80.12 ± 1.2
F_2	83.68	57.34 ± 2.2	306 ± 4.1	18.83 ± 1.2	0.4950 ± 1.2	0.98 ± 1.6	74.63 ± 1.5
F_3	89.47	45.47 ± 1.4	261 ± 3.7	16.59 ± 0.9	0.5102 ± 0.9	1.01 ± 1.1	68.23 ± 1.7
F_4	86.86	84.83 ± 1.3	461 ± 5.7	19.69 ± 2.1	0.4931 ± 1.3	0.85 ± 2.0	87.23 ± 0.9
F_5	92.98	77.1 ± 2.1	328 ± 4.1	16.27 ± 1.4	0.5287 ± 0.7	3.46 ± 0.9	83.41 ± 1.1
F_6	94.77	68.34 ± 2.2	286 ± 5.2	15.54 ± 1.1	0.5312 ± 1.3	2.56 ± 1.5	78.26 ± 1.05
F_7	84.15	88.63 ± 1.4	527 ± 1.9	19.81 ± 2.0	0.5024 ± 1.1	0.75 ± 1.1	89.73 ± 1.1
F_8	90.25	81.04 ± 1.3	418 ± 3.4	16.31 ± 1.5	0.5271 ± 1.12	1.93 ± 1.3	85.44 ± 1.3
F_9	93.5	73.03 ± 2.1	344 ± 2.8	15.25 ± 1.1	0.5297 ± 1.3	1.51 ± 2.4	81.23 ± 1.5

[a]Mean ± SD; $n = 6$. [b]Mean ± SD; $n = 3$.

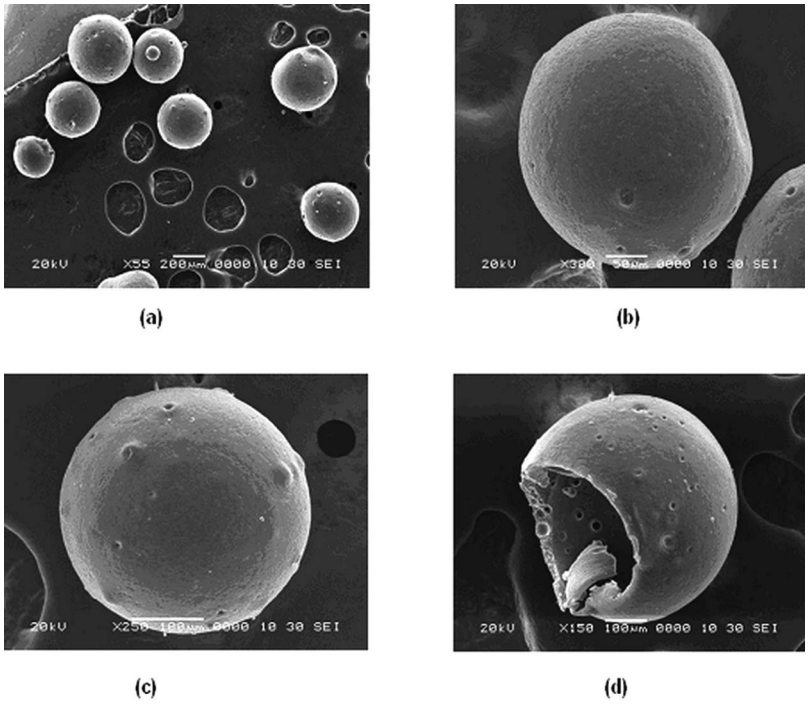

Figure 1. Scanning electron microphotographs of floating microspheres. (a and b) Smoothness of the surface of spherically shaped microspheres; (c) dented surface structure; and (d) internal view of the shell having a porous structure.

microspheres varied from 76.49% to 94.77% (Table 4). To observe the effect of polymer concentration on the percentage yield of the resulting microspheres, formulations were prepared at varying concentrations of EC 7 cps and stirring speed (Table 2). The yield of the resulting microspheres increased with increasing concentration of the polymer. The % yield increased when stirring speed increased from 800 to 1200 rpm, whereas decreased with further increment to 1600 rpm. At low stirring speed, polymer solution was aggregated around the propeller shaft, and the resultant yield of microspheres was relatively low. Again even at higher stirring speed (1600 rpm), the % yield decreases as it leads to the break up of microspheres. Hence, the stirring speed should be optimized to obtain the desired % yield. At a low concentration of the EC, a portion of the polymer solution aggregated into a fiber-like structure, as it solidified before forming droplets, or the transient droplets were broken before solidification was complete resulting in low yield[11].

Scanning electron microscopy

The morphology of microspheres was examined using SEM. The view of the microspheres showed a hollow spherical structure with a smooth surface morphology (Figure 1A and B) and exhibited a range of sizes within each batch. Some of the microspheres showed a dented surface structure (Figure 1C), but they showed good floating ability on the surface of the medium indicating intact surface. The outer surface of the microspheres was smooth and dense, whereas the internal surface was porous. The shell of the microspheres also showed some porous structure (Figure 1D). It may be caused by the evaporation of solvent entrapped within the shell of microspheres after forming a smooth and dense skin layer.

Particle size

The effect of polymer concentration on the particle size of the microspheres was determined. The mean particle size of the microspheres was increased with the increasing EC concentration (Table 2) and was in the range of 261 ± 3.3 to 527 ± 9.8 μm (Table 4). The viscosity of the medium increases at a higher EC concentration resulting in enhanced interfacial tension. Shearing efficiency is also diminished at higher viscosities. This results in the formation of larger particles. To observe the effect of stirring speed on the size of the resulting microspheres[4], formulations were prepared at varying stirring speeds in between 800 and 1600 rpm (Table 2). As reported previously, the size of the resulting microspheres decreased with increasing speed of stirring[4].

Flow properties and tapped density of floating microspheres

The angle of repose of formulations of the microspheres ranged from $15.25 \pm 1.1°$ to $20.43 \pm 0.7°$. The tapped density values of formulations of the microspheres ranged from 0.4854 ± 1.03 to 0.5312 ± 1.31 g/cm^3. The % compressibility index (Carr's index) ranged between

0.75 ± 2.0% and 3.46 ± 0.9%. The values of Carr's index and the angle of repose indicate excellent flow properties. Thus, it is an added advantage while processing the formulation using high-speed packaging equipments. Moreover, the process scale-up is also facilitated because of the excellent flow properties.

Floating ability

The purpose of preparing floating microspheres was to extend the GRT of the drug. The floating ability test was carried out to investigate the floatability of the prepared microspheres. The microspheres were spread over the surface of simulated gastric fluid (SGF) and the fraction of the microspheres settling as a function of time was quantified. The microspheres containing EC showed good floating ability (Table 4) due to the insolubility of EC polymer in the SGF (pH 1.2). The results also showed a tendency that the larger the particle size, the longer the floating time (Table 4). It should be noted, however, that the situation in vivo can be quite different and the residence time may vary widely depending on the phase of gastric motility.

In vitro drug release study

In vitro dissolution studies of RGM from floating microspheres were performed in 0.1 N HCl (pH 1.2) for 12 hours using USP dissolution test apparatus I. It was found that formulations F_1, F_2, and F_3 showed 86.69%–96.44% of rel_{8h} and 100% drug release in 9 hours. As drug release was not sustained considerably, the EC concentration was increased to achieve further retardation in drug release. For formulations F_4, F_5, and F_6, the drug release was 76.24%–90.60% within 8 hours and 91.02%–101.78% in 12 hours. Formulations F_7, F_8, and F_9 showed 62.33%–80.78% of rel_{8h} and 81.84%–92.59% drug release in 12 hours (Figure 2). Moreover, from the results it is also clear that no burst effect was seen and drug release was significantly sustained (Figure 2). It was observed that as the concentration of EC increased, the % cumulative release of RGM decreased. The increase in EC concentration leads to the formation of high-density polymer matrix into the microspheres, which results in an increased diffusional path length and consequent retardation in drug release. The effect of speed stirring on the particle size of microspheres has already been studied. Smaller microspheres were formed at lower concentrations of EC and have larger surface area exposed to the dissolution medium giving rise to faster drug release.

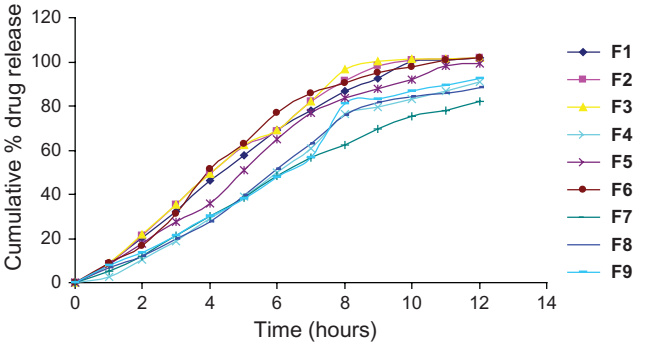

Figure 2. Comparative cumulative % drug release profiles of formulations F_1 to F_9.

Data analysis

On the basis of the data obtained from the formulations subjected to optimization, a general statistical model can be depicted with respect to the above data. The model developed can be characterized by using the polynomial equation representing the respective response data. This can be given as follows:

Drug

$$\text{Entrapment} = 71.18 + 12.51X_1 - 2.79X_1^2 - 8.57X_2 - 0.32X_2^2 + 0.94X_1X_2 - 0.17X_1^2X_2 + 0.33X_1X_2^2 - 0.076X_1^2X_2^2.$$

$$\text{rel}_{8h} = 82.66 - 9.21X_1 - 0.39X_1^2 + 7.09X_2 - 0.48X_2^2 + 2.18X_1X_2 - 0.043X_1^2X_2 - 0.8X_1X_2^2 - 0.23X_1^2X_2^2.$$

$$\text{Particle size} = 369.78 + 54.17X_1 + 5.72X_1^2 - 82.33X_2 + 9.56X_2^2 - 11.75X_1X_2 + 2.58X_1^2X_2 - 0.92X_1X_2^2 - 2.81X_1^2X_2^2$$

From the above polynomial equations, response surface graphs and contour plots of the respective responses were generated, which were then used to predict the responses of dependent variables at the intermediate levels of independent variables.

Figure 3 depicts a quite linear increasing trend in the values of drug entrapment efficiency with increased value of EC and nearly linear decreasing trend with stirring speed. Nevertheless, the influence of EC is distinctly far more significant than that of the stirring speed. The same is confirmed from the corresponding contour plot (Figure 3B), showing linear contour lines. Hence, the higher levels of EC have to be complemented with lower levels of stirring speed to maintain drug entrapment at a constant level.

Figure 4 reveals a decline in the value of rel_{8h} with increase in the concentration of EC. A linear increasing trend is seen in the values of rel_{8h} with increase in stirring speed. Linear descending contour lines in Figure 4B further elucidate that the variation in rel_{8h} is a complex function of the concentration of EC and the effect of stirring speed being less significant.

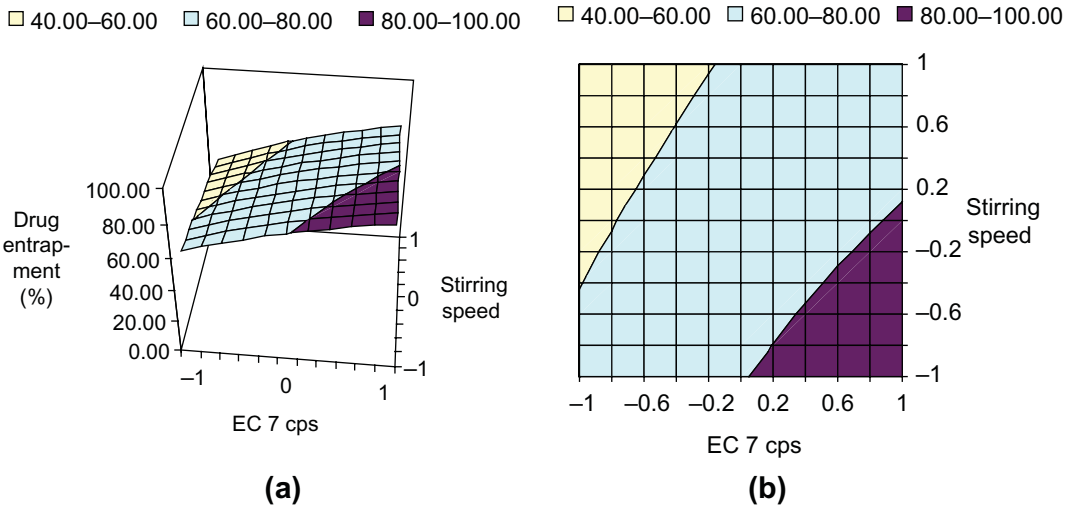

Figure 3. (a) Response surface plot and (b) contour plot showing the influence of EC 7 cps and stirring speed on drug entrapment efficiency (%) of floating microspheres formulations of RGM.

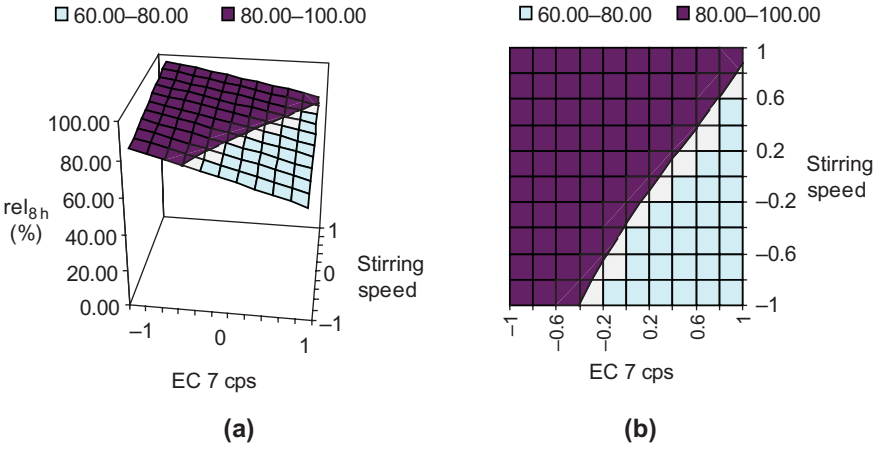

Figure 4. (a) Response surface plot and (b) contour plot showing the influence of EC 7 cps and stirring speed on $rel_{8\,h}$ (%) of floating microspheres formulations of RGM.

Figure 5 shows nearly linear decreasing patterns for the values of particle size as the stirring speed increased. At high levels of the polymers, however, the response surface shows a slightly linear shape. Maximum particle size is observed at high levels of EC. The same is confirmed from the corresponding contour plot (Figure 5B), showing linear increasing contour lines. Nearly vertical contour line also shows that EC influences the particle size significantly.

Validation of optimum floating microspheres formulations

For all five checkpoint formulations, results of the physical evaluation and microspheres drug content were found to be within limits. Table 5 lists the composition of the checkpoints, the predicted and experimental values of all the response variables and the percentage error in prognosis. Figure 6 shows linear correlation plots between the observed and the predicted values of drug entrapment efficiency, $rel_{8\,h}$, and particle size. The linear correlation plots drawn between the predicted and the observed responses demonstrated higher values of R^2 (ranging between 0.9112 and 0.9821), indicating excellent fitting of the model ($P < 0.001$). Upon comparison of the observed responses with that of the anticipated responses, the prediction error varied between –1.21% and 2.64%. Thus, the low magnitudes of error as well as the significant values of R^2 in the current study indicate a high prognostic ability of floating microspheres of RGM using RSM optimization.

Stability studies

The samples subjected to stability studies were then analyzed. The results of the stability studies (Table 6) indicated that the formulations were able to retain their stability for a period of 3 months at 40°C/75% RH.

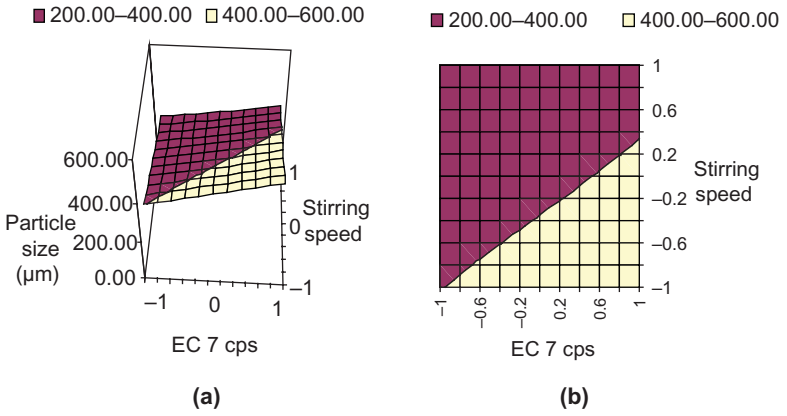

Figure 5. (a) Response surface plot and (b) contour plot showing the influence of EC 7cps and stirring speed on particle size (RPM) of floating microspheres formulations of RGM.

Table 5. Comparison of experimental results with predicted responses of floating microsphere formulations.

Bath code	Composition		Response	Predicted value	Experimental value	Percentage error
	X_1 g	X_2 RPM				
S_1	2.76	1168	Drug entrapment (%)	70.33	70.21	−0.17
			rel_{8h} (%)	83.26	82.91	−0.42
			Particle size (μm)	367.1	362.7	−1.21
S_2	2.92	1352	Drug entrapment (%)	70.81	70.54	−0.38
			rel_{8h} (%)	83.22	83.31	0.11
			Particle size (μm)	352.1	358.4	1.76
S_3	2.93	1320	Drug entrapment (%)	71.72	70.98	−1.043
			rel_{8h} (%)	82.47	82.28	−0.23
			Particle size (μm)	359.5	357.3	−0.62
S_4	2.99	1456	Drug entrapment (%)	70.17	70.26	0.13
			rel_{8h} (%)	83.83	83.74	−0.11
			Particle size (μm)	339.5	348.7	2.64
S_5	3.00	1488	Drug entrapment (%)	69.71	70.02	0.44
			rel_{8h} (%)	84.21	84.37	0.19
			Particle size (μm)	334.5	342.1	2.22
Mean (±SEM) of percentage error						0.2205 ± 1.13

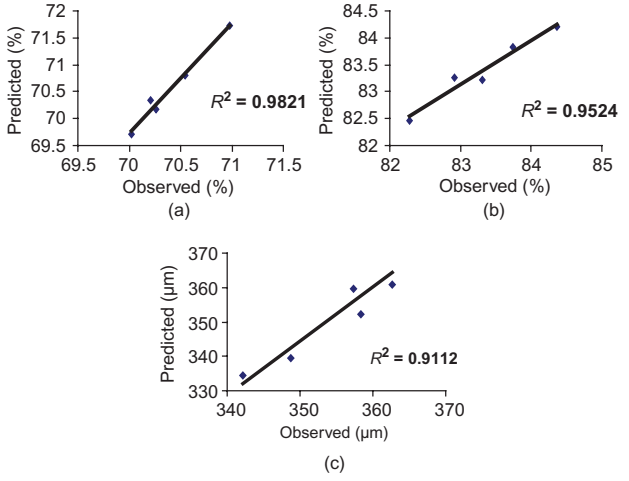

Figure 6. Linear plots between observed and predicted values of (a) drug entrapment, (b) rel_{8h}, and (c) particle size.

Table 6. Stability studies.

Days	Drug context (%)	Floating ability (%)	Drug release (%)
Before storage			
0 day	70.98 ± 2.4	85.23 ± 1.3	82.28 ± 3.6
After storage[a]			
7 days	70.90 ± 1.9	85.23 ± 1.7	82.12 ± 2.4
15 days	70.88 ± 2.1	85.13 ± 1.3	81.90 ± 3.1
30 days	70.43 ± 3.0	84.90 ± 1.1	81.76 ± 2.1
60 days	70.31 ± 2.4	84.82 ± 1.2	81.58 ± 2.4
90 days	70.27 ± 1.8	84.78 ± 1.3	81.39 ± 2.9

[a]Storage at 40°C and 75% RH for 3 months ($n = 3$).

Conclusions

The results of a 3^2 full factorial design revealed that the concentration of EC 7 cps (X_1) and stirring speed (X_2)

significantly affected the dependent variables such as drug entrapment efficiency, drug rel_{8h}, and particle size of the microspheres. The polynomial equation–based optimization model was generated and validated. The accuracy of the model was established on the basis of the magnitude of errors and R^2 values. The microspheres of the optimum batch (F_5) exhibited 77.1% drug entrapment efficiency, mean particle size of 328 μm and 83.47% rel_{8h}. An appropriate balance between the levels of the polymer (EC 7 cps) and stirring speed was imperative to acquire maximum drug entrapment efficiency, sustained release of the drug, and adequate particle size. Hence, it could be established that among the prepared formulations, F_5 was the optimum formulation. In vitro data obtained for the floating microspheres of RSM showed excellent floating ability, good buoyancy, and prolonged drug release.

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References

1. Singh NB, Kim KH. (2000). Floating drug delivery systems: An approach to oral controlled drug delivery via gastro retention. J Control Release, 63:235–59.
2. Arora S, Ali J, Ahuja A, Khar RK, Baboota S. (2005). Floating drug delivery systems: A review. AAPS PharmSciTech, 6(3), E372–90.
3. Deshpande AA, Rhodes CT, Shah NH, Malick AW. (1996). Controlled release drug delivery systems for prolonged gastric residence: An overview. Drug Dev Ind Pharm, 22:531–39.
4. Srivastava AK, Ridhurkar DN, Wadhwa S. (2005). Floating microspheres of cimetidine: Formulation, characterization and in vitro evaluation. Acta Pharma, 55:277–85.
5. Chaurasia H, Jaina K, Prajapatis K, Chaurasia D, Gupta R, Arya R, Bhardwaj P. (2007). Formulation and *in vitro* evaluation of rosiglitazone maleate floating microspheres. Indian Pharm, 6(65):101–03.
6. Kawashima Y, Niwa T, Takeuchi H, Hino T, Ito Y. (1991). Preparation of multiple unit hollow microspheres (microballoons) with acrylic resins containing tranilast and their drug release characteristics (in vivo). J Control Release, 16:279–90.
7. Jain SK, Awasthi AM, Jain NK, Agrawal GP. (2005). Calcium silicate based microspheres of repaglinide for gastroretentive floating drug delivery: Preparation and in vitro characterization. J Control Release, 107:300–09.
8. Hardman JG, Limbird LE (Editors-in-chief). (1996). Goodman &. Gilman's The Pharmacological Basis of Therapeutics. 9th ed. New York: McGraw-Hill Companies, 1487–1517.
9. Martin A, Bustamante P, Chun AHC. (1996). Physical Pharmacy. New Delhi, India: B I Waverly Pvt. Ltd., 423–52.
10. Naggar VF, E-Kamel AH, Sokar MS, Al-Gamal SS. (2001). Preparation and evaluation of Ketoprofen floating oral delivery system. Int J Pharm, 220:13–21.
11. Lee JH, Park TG, Choi HK. (1996). Development of oral drug delivery system using floating microspheres. Microencapsulation, 16(6):715–29.