ORIGINAL ARTICLE

Factorial design approach for optimization of floating microspheres of diltiazem hydrochloride

Mangal Singh Panwar, Yuvraj Singh Tanwar

Department of Pharmaceutics, Bhupal Nobles, College of Pharmacy, Udaipur, Rajasthan, India

The aim of this study was to perform optimization of floating microspheres of diltiazem hydrochloride for the prolongation of gastric residence time. The microspheres were prepared by a nonaqueous solvent evaporation method using polycarbonate. A full factorial design was applied to optimize the formulation. Preliminary studies revealed that the concentration of polymer and stirring speed significantly affected the characteristics of floating microspheres. The optimum batch of microsphere exhibited smooth surfaces with good flow and packing properties, prolonged sustained drug release, remained buoyant for more than 10 h, high entrapment efficiency up to 97% w/w. Scanning electron microscopy confirmed the hollow structure with particle size in the order of 190 μm. The studies revealed that the increase in concentration of polycarbonate increased the drug release from the floating microspheres. The results of two third full factorial design revealed that the concentration of polycarbonate (X1) and stirring speed (X2) significantly affected drug entrapment efficiency, percentage release.

Key words: *Diltiazem hydrochloride, factorial design, floating microspheres, polycarbonate*

INTRODUCTION

Drugs that are easily absorbed from the gastrointestinal tract and having a short half-life are eliminated quickly from the blood circulation. To avoid this problem, the oral controlled-release formulations have been developed as these will release the drug slowly into the gastrointestinal tract and maintain a constant drug concentration in the serum for a longer period of time. Such oral drug delivery devices have a restriction due to the gastric retention time (GRT), a physiological limitation.[1]

An incomplete release of the drug and shorter residence time of the dosage forms in the upper gastrointestinal tract, a prominent site for the absorption of many drugs, will lead to lower bioavailability.[1] Therefore, prolonged gastric retention is important in achieving control over the GRT because this helps to retain the controlled release system in the stomach for a longer and predicted time.

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.[2,3] In contrast, multiple-unit particulate dosage forms (e.g., microspheres) have the advantages that they pass uniformly through the gastrointestinal tract to avoid the vagaries of gastric emptying and provide an adjustable release, thereby reducing the inter-subject variability in absorption and risk of local irritation. Recently, hollow microspheres with a lower density than that of the gastrointestinal fluids were adopted.[3]

Diltiazem hydrochloride is a calcium channel blocker, an anti-hypertension, and anti-anginal drug, diltiazem Hydrochloride undergoes an extensive biotransformation, mainly through cytochrome P-450 CYP3A, which results in <4% of its oral dose being excreted unchanged in urine. Suffers from poor bioavailability (~30–40%) owing to an important first pass metabolism. It has an elimination half-life of 3.5 h and an absorption zone from the upper intestinal tract. Thus, the present work was aimed to formulate sustain release floating microsphere of diltiazem hydrochloride for gastroretentive drug delivery system.[4,5]

Address for correspondence:
Mr. Mangal Singh Panwar,
BNPG College, Udaipur, Rajashhan, India.
E-mail: mangalchemistry@gmail.com

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MATERIALS AND METHODS

Materials

Diltiazem hydrochloride was obtained as a gift sample from Zydus Cadila Ltd. (Mumbai, India), and Polycarbonate were kindly supplied by Puneet Enterprises (Ratlam, India). All other ingredients were of analytical grade. An ultraviolet (UV)/visible spectrophotometer (Shimadzu-1700) was used for drug analysis.

Factorial design for optimized floating microsphere formulation

Experiments were carried out systematically to analyze the effect of factors such as stirring rate revolutions per minute (rpm) and varying the concentration of polycarbonate using a response surface methodology and to develop an optimized formulation. A central composite design was employed to prepare experimental trials using different concentration of polycarbonate at varying rpm.

A two-third full factorial designs were quiet often used to evaluate main effects and interaction effects of the formulation ingredients on the *in-vitro* release of drugs from the formulations and to arrive at an optimum formula with desired drug release characters. Independent variables studied were, polycarbonate (X_1) and stirring speed (X_2). The dependent variables studied were percentage drug release from microsphere and percentage entrapment efficiency. An optimized model was to be identified for each response and also to validate such model developed statistically by comparing theoretically obtained values with the experimental values. The full factorial experimental design layout is given in Table 1. The actual and coded values of the independent factors are given in Table 2.

The two-third full factorial designs, construction of response surfaces and polynomial equations were planned to be done using the trial version of design expert software. The linear computer-generated model equation for each response will be in the format as given below:

$$Y = b_0 + b_1X_1 + b_2X_2 + b_{12}X_1X_2 + b_{11}X_1^2 + b_{22}X_2^2$$

Where, b_1 is the estimated coefficient for the factor X_1, while Y_1 is the measured response. The coefficients corresponding linear effects (b_1 and b_2), interaction (b_{12}) and the quadratic effects (b_{11} and b_{22}) were determined from the results of the experiments. The model, a comparison between the experimental and predicted values of the responses is also presented in terms of %bias.[6,7]

Bias was calculated by the following equation:

$$\%\text{Bias} = \frac{\text{Predicted value - Experimental value}}{\text{Predicted value}} \times 100$$

Preparation of optimized floating microspheres of diltiazem hydrochloride

Microspheres containing diltiazem hydrochloride as a core material were prepared by a nonaqueous solvent evaporation method. Briefly, drug (diltiazem hydrochloride) and Polycarbonate was mixed individual in acetone at various ratios. The slurry was slowly introduced into 40 ml of liquid paraffin while being stirred at varying rpm by a mechanical stirrer equipped with a three-bladed propeller at room temperature (27 ± 0.5°C). The solution was stirred for 2 h to allow the solvent to evaporate completely, and the microspheres were collected by filtration. The microspheres were washed repeatedly with petroleum ether (40–60°C) until free from oil. The collected microspheres were dried for 1 h at 30–40°C temperature and subsequently stored in a desiccator over fused calcium chloride. The polymer used was polycarbonate and its composition was tabulated in Table 1.[8,9]

Evaluation of optimized floating microspheres of diltiazem hydrochloride

Determination of particle size

The mean particle size of the drug and the formulations was analyzed by laser light scattering technique using Ankersmid CIS-50.[10]

Drug content

For the determination of drug content, formulations (50 mg) were triturated with 0.1N hydrochloric acid and finally the volume was made up to 50 ml with the same. The solution was filtered through Whatmann No. 1 filter paper, and suitable dilutions were carried out with 0.1N hydrochloric acid. The concentration of diltiazem hydrochloride was then determined using UV spectrophotometer at a wavelength of 237 nm.[10]

Table 1: Full factorial experimental design layout

Formulation	Variable level in coded form	
	X_1	X_2
OF1	−1	−1
OF2	0	−1
OF3	1	−1
OF4	−1	0
OF5	0	0
OF6	1	0
OF7	−1	1
OF8	0	1
OF9	1	1

Table 2: Actual and coded values of the independent factors

Code	Polymer (polycarbonate) (X_1)	Stirring speed (rpm) (X_2)
−1	125	900
0	150	1300
1	175	1800

Drug entrapment efficiency

Estimation of drug content in floating microspheres can be carried out by dissolving the weight amount of crushed microspheres in required quantity of 0.1N hydrochloric acid and analyzed spectrophotometrically at a particular wavelength using the calibration curve. Each batch should be examined for drug content in a triplet manner. The entrapment efficiency of floating microspheres was calculated by dividing the actual drug content by the theoretical drug content of microspheres.[10]

Buoyancy studies

This study is carried out by USP Type I dissolution test apparatus by spreading the floating microspheres on a simulated gastric fluid (pH 1.2) containing surfactant. The media is stirred at 100 rpm at 37 ± 0.5°C. After specific interval of time, both the fraction of microspheres (floating and settled microspheres) were collected, and buoyancy of the floating microspheres is determined using formula:

$$\text{Buoyancy (\%)} = \frac{Q_f}{Q_f - Q_s}$$

Where, Q_f is floating microspheres, and Q_s is settled microspheres.[10]

Surface morphology

The surface characteristics of the drug powder and that of the formulations before and after the dissolution were studied by scanning electron microscope (SEM) at × 1600. The samples were mounted on double-sided tape that has previously been secured on copper stubs and then analyzed at different magnifications.[9,11]

In-vitro dissolution study

The *in vitro* dissolution studies were carried out using USP Type I dissolution apparatus. The study was carried out in 900 ml of 0.1N hydrochloric acid. The dissolution medium was kept in a thermostatically controlled water bath, maintained at 37 ± 0.5°C. The basket was rotated at 100 rpm. At predetermined time intervals, that is, 1, 2, 4, 8, 12, 14, and 16 h 5 ml of sample was withdrawn and replaced with fresh media. The drug concentration was analyzed using UV/visible spectrophotometer at 237 nm.[10]

RESULTS AND DISCUSSIONS

Optimization data analysis for the diltiazem hydrochloride microsphere

Observed responses of nine formulations were fitted to various models using Design-Expert software trial version 9.0.1 (Stat-Ease, Inc., USA). It was seen that the quadratic models were best-fitted for the studied responses, that was, % drug release and % entrapment efficiency. The quadratic equations generated for responses were given as:

$$\%\text{Drug release} = +94.12 - 2.21\,X_1 - 0.89\,X_2 - 0.89 + 0.65\,X_1X_2 - 0.96\,X_1^1 + 2.63\,X_2^2$$

$$\%\text{Entrapment efficiency} = +78.41 + 3.02\,X_1 + 0.52\,X_2 - 0.41\,X_1X_2 - 0.33\,X_1^1 - 2.00\,X_2^2$$

Where X_1 and X_2 represent the coded values of the polycarbonate and stirring speed (rpm), respectively. The positive value of a factor in the above equations point outs the enhancement of that response and vice versa. All values of the correlation coefficient (R^2), standard deviation, % coefficient of variation, and results of ANOVA are shown in Tables 3 and 4. A value of R^2 and results of ANOVA for the dependent variables confirmed that the model was significant for observed response variables.

Fraction of design space (FDS) evaluation helps experimenter's size constrained response surface (RSM) and mixture designs, for which the normal power calculations lose relevance. Supply the "signal" and the "noise" and the graph will show the amount of the design region that can estimate with that precision. An FDS >80% is generally acceptable to ensure that the majority of the design space is precise enough for your purpose.

Experimental design

Based on the preliminary experiments and our previous studies, two factors (polycarbonates and speed of stirring speed) were identified key factors responsible for % entrapment efficiency and % drug release of microspheres. The polymer polycarbonate was chosen because it decreases the release of drug from the microsphere at optimum concentration. The polymer caused a coarse covering, likely due to drug's residue that has not been surrounded by polymer, thoroughly. When increasing the concentration of polymer, it forms stiff layer over the drugs, and this hindered the release of drug from the microsphere.

Predicted optimum ranges of the independent variables are listed in Table 5. The variation in drug release and entrapment efficiency was observed on changing the concentration of polymer and stirring speed. The fitting results point out that the optimized microsphere formulation with high entrapment efficiency and low drug release percentage was obtained at the stirrer speed of 900 rpm and polycarbonates of 176 mg. The OF6 exhibited lowest drug release and highest drug entrapment efficiency compared to other formulations. The OF1 exhibited highest drug release and lowest drug entrapment efficiency. The regression analysis for responses and analysis of variance for drug release and entrapment efficiency of optimized formulation are tabulated in Tables 3 and 4, respectively. Table 6 shows the observed values of the prepared batch with the optimized formula was very close to the predicted values, with low percentage bias, suggesting that the optimized formulation was trustworthy and rational.

Table 3: Summary of results of regression analysis for responses and analysis of variance for drug release

Parameters	df	SS	MS	*F*	*P*	R^2	SD	Coefficient of variance percentage
Model	5	51.48	10.30	34.13	0.0076 significant	0.9827	0.55	0.58
Residual	3	0.91	0.30	-	-	-	-	-
Total	8	52.39	-	-	-	-	-	-

df: Degree of freedom, SS: Sum of squares, MS: Mean square, SD: Standard deviation

Table 4: Summary of results of regression analysis for responses and analysis of variance for entrapment efficiency

Parameters	df	SS	MS	*F*	*P*	R^2	SD	Coefficient of variance percentage
Model	5	65.27	13.05	55.06	0.0038 significant	0.9892	0.49	0.63
Residual	3	0.71	0.24	-	-	-	-	-
Total	8	65.98	-	-	-	-	-	-

df: Degree of freedom, SS: Sum of squares, MS: Mean square, SD: Standard deviation

Table 5: Independent variables along with their coded level and respective responses values of different batches of microspheres

Formulation	X_1	X_2	Drug release	Entrapment efficiency
OF1	−1	−1	99.62	72.25
OF2	0	−1	97.25	75.42
OF3	1	−1	94.15	79.37
OF4	−1	0	95.34	75.19
OF5	0	0	94.67	78.68
OF6	1	0	90.43	80.72
OF7	−1	1	96.42	73.76
OF8	0	1	95.71	77.14
OF9	1	1	93.54	79.24

The relationship between the dependent and independent variables is further elucidated by constructing the response surface plot. The three-dimensional (3D) response surface graphs generated by the design-expert software (trial versions version 9.0.1) for the most statistical significant variables on the evaluated parameters are presented in Figures 1-6. The 3D response surface curves are used for studying the interaction patterns. The half-normal plot is used to select effects to be included in the model [Figures 1 and 4]. Large effects (absolute values) appear in the upper-right section of the plot. The lower-left portion of the plot contains effects caused by noise rather than a true effect.

On the basis of 3D response surface graphs, it can be said that the polycarbonates concentration and rotation speed of the stirrer produces a significant effect on entrapment efficiency and drug release percentage [Figures 3 and 6]. Figure 2 interaction plot exhibited prediction value 0.94403 for drug release, and it evoke that the model was best for use. Figure 5 interaction plot exhibited prediction value 0.971613 for entrapment efficiency and it suggest that the model was best for use. Figure 3 exhibits significant decrease in drug release on changing the concentration of polycarbonate and stirring speed. Figure 6 displays significant increase in the percentage of entrapment efficiency on varying the concentration of polycarbonate and stirring speed. At last, according to the final results, this program suggested some formulations and also predicted their responses containing a probability factor named "desirability" that ranged between 0 and 1.

Graphs show that with increasing the concentration of polycarbonates in formulation, percentage of entrapment efficiency increase but percentage of drug release was decreased and vice versa. In case of second factor (rotation speed of stirrer) was responsible for higher percentage of entrapment efficiency and low percentage of drug release.

Evaluation of optimized formulation

The design expert software produced optimizes formulation which can furnish better result compared to other formulations. Hence considering, we further prepared formulation of selected optimized formulations. The microsphere size, percentage entrapment efficiency, percentage drug content, and percentage buoyancy of optimized formulation are depicted in Table 7. The result exhibited that the increased in microsphere size, percentage entrapment efficiency, percentage drug content, and percentage buoyancy compared to trail formulations.

The mean size of the optimized formulation was 148 μm, which is slightly higher than trial formulation, but it is acceptable. It indicates that on increasing the concentration of polymer and decreasing the stirring speed can enhance the size of microspheres. It justifies the trial results that on decreasing the concentration of polymer it decreases the microsphere sizes.

The entrapment efficiency and drug content were 79.52% and 49.28%, respectively. The finding demonstrated that entrapment efficiency and drug content increased compared with selected trial formulations. It implies that the on increasing the concentration of polymer and decreasing the stirring speed it improves the entrapment efficiency and drug content in microspheres. Researches demonstrated that the lower concentration of polymer makes the microspheres fragile, and higher stirrer speed breaks the microsphere and produced irregular shapes. It decreased the drug content and entrapment efficiency of drug loaded microspheres.

Hence, the 900 rpm stirrer speed for stirring of formulation was optimum speed to form a smooth surface and uniform size of microspheres.

The buoyancy study indicates floatability property of microspheres. The final formulation produces 85.31% buoyancy for drug loaded microspheres [Table 7]. The microspheres floated for a prolonged time over the surface of the dissolution medium without any apparent gelation. The nature of the polymer influenced the floating behavior of the microspheres.

This buoyancy may be attributed to the common hollow structure. It may also deduced that these hollow microspheres can float in full gastric fluid, retarding the passage of the spheres (and, therefore, the drug contained in them) into the intestinal region and prolonging their presence in the stomach.

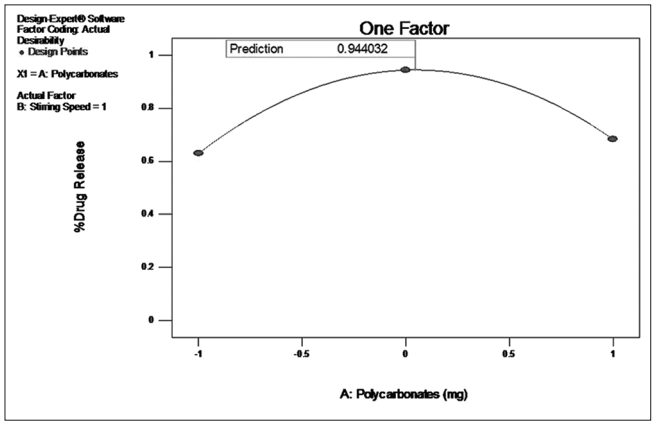

Figure 1: Plot showing the main effect of polycarbonate on %drug release

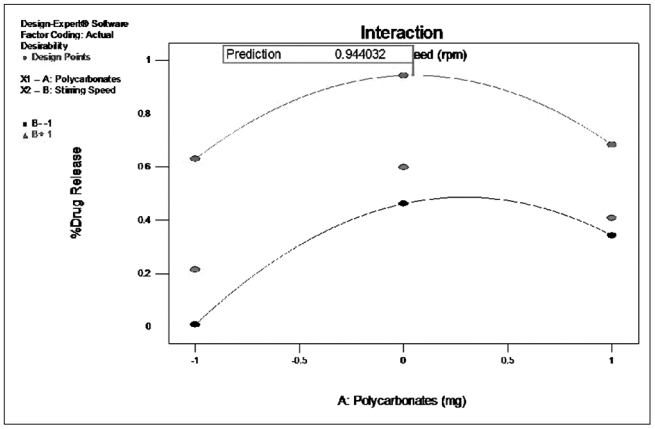

Figure 2: Interaction plots showing the effect of polycarbonate and stirring speed on %drug release

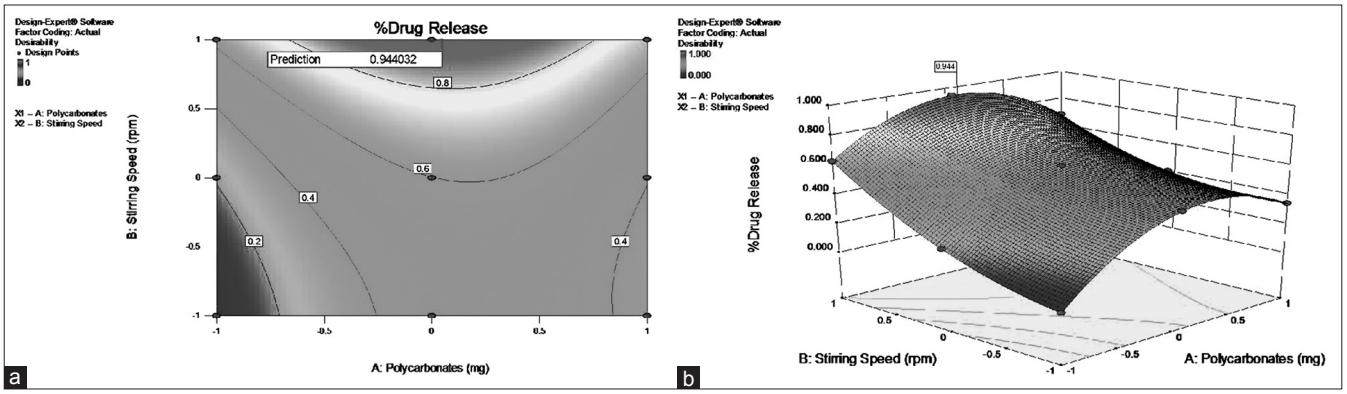

Figure 3: (a) Surface plots showing the effect of variables on %drug release of diltiazem hydrochloride microsphere. (b) Surface plots showing the effect of variables on %drug release of diltiazem hydrochloride microsphere

Table 6: Comparison of the observed and predicted values in the floating microsphere prepared under predicted optimum conditions

Responses variable	Predicted optimum range		Predicted value	Observed value	Bias %
	X_1	X_2			
Percentage drug release	176	900	90.94	90.43	0.56
Percentage entrapment efficiency	176	900	81.11	80.72	0.48

Table 7: Particle size analysis of formulations

Formulation	Particle size	Percentage drug content	Percentage entrapment efficiency	Percentage buoyancy
Final formulation	148.23±1.25	49.28±1.65	79.52±1.42	85.31±1.78

Values are mean±SD. SD: Standard deviation

Scanning electron micrograph of optimized microsphere

The surface morphology of the optimized drug loaded microsphere was investigated by SEM. Studies using SEM provided a better understanding of the morphological characteristics of the microspheres. The optimized microsphere produces a smooth surface of microspheres [Figure 7].

When the inner water phase is evaporated the crust is destroyed, the outer surface collapses and, as a result, small pores are formed. The entrapped substance is drained, affecting the loading efficiency. Furthermore, it will concentrate toward the microparticle surface contributing to the initial burst release. Surface hollows could be attributed to the subsequent shrinkage of the microspheres after solidification.

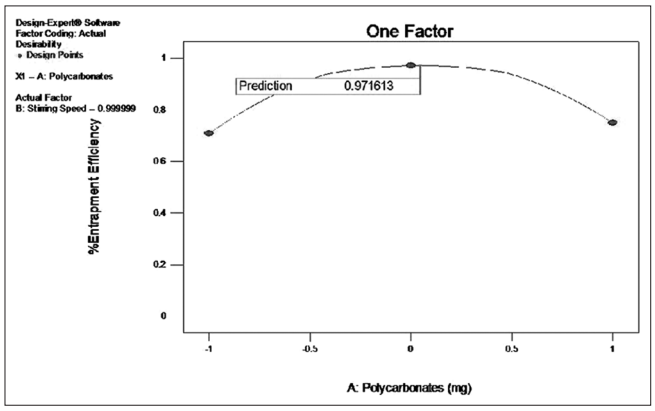

Figure 4: Plot showing the main effect of polycarbonate on %entrapment efficiency

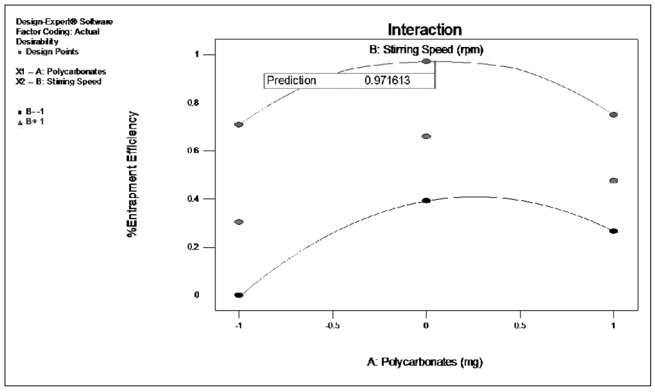

Figure 5: Interaction plots showing the effect of polycarbonate and stirring speed on %entrapment efficiency

Assesment of *in-vitro* drug release of the final formulation

From Table 8, *in-vitro* dissolution studies revealed that 100% of drug release from the formulation at 18 h. The 50% of the drug was released from the microspheres within 8 h. The results of *in-vitro* drug exhibited that on increasing the concentration of polymer it decreased the drug release from microspheres. The high concentration of polymer makes the microsphere stiff. This hardness of microsphere decreases the rate of drug release from microspheres. The finding implies that drug release from the microsphere was reduced compared to selected trial formulations.

The concentration of polymer and stirring speed of the stirrer imparts important role in reducing the drug release from microspheres. As on decreasing the stirrer speed up to 600 rpm produced lower entrapment efficiency of microsphere because it fail to uniform the distribution of drug in solution. When solution was stirred at 900 rpm, it leads to increase in entrapment efficiency because it uniformly

Table 8: Data for percentage cumulative drug release of optimized formulation

Time in hours	Square root of time	Log time	Percentage cumulative drug release
1	1	0.0	11.36±1.25
2	1.414	0.301	19.25±1.05
4	2.0	0.602	32.45±2.18
8	2.82	0.903	58.57±1.96
12	3.46	1.079	69.14±1.48
14	3.74	1.146	78.48±1.67
16	4.00	1.204	89.63±1.73
18	4.24	1.26	100.00±1.37

Values are mean±SD. SD: Standard deviation

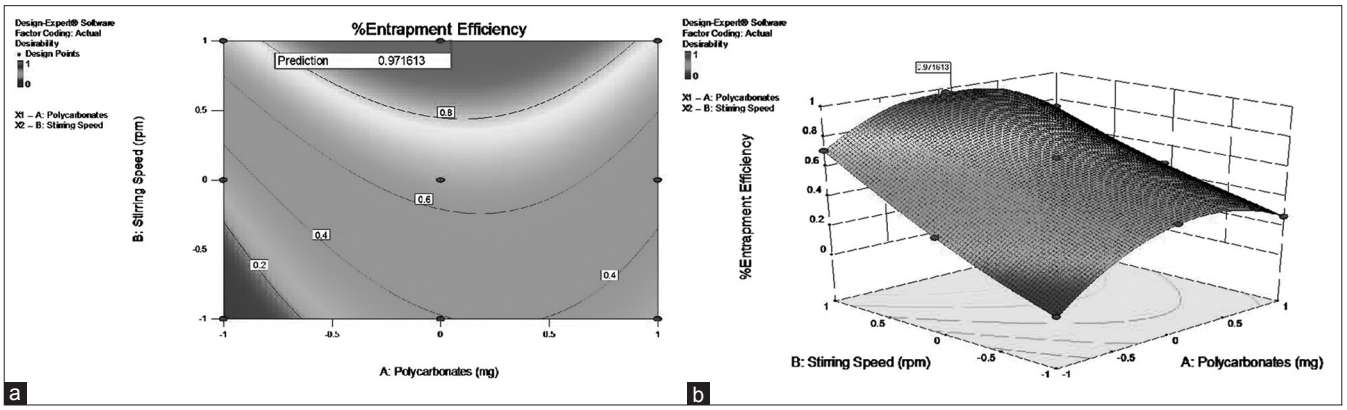

Figure 6: (a and b) Surface plots showing the effect of variables on %entrapment efficiency of diltiazem hydrochloride microsphere

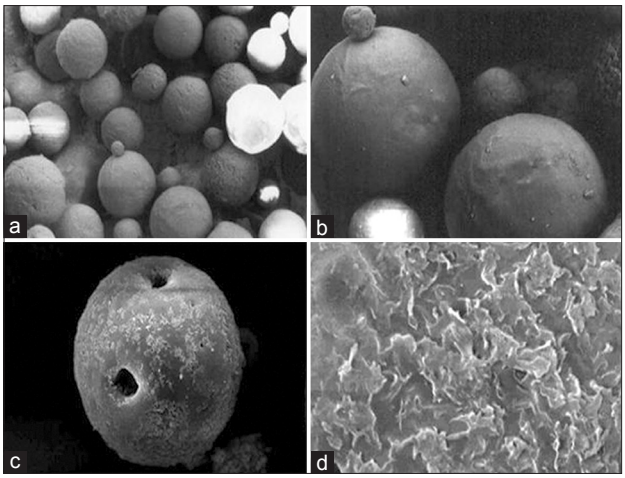

Figure 7: Surface morphology of floating microspheres by scanning electron microscopic photographs – (a) Range of floating microspheres. (b) Smooth texture of floating microspheres. (c) Dents on the surface. (d) Surface morphology of floating microspheres

distributes the drug in solution. Moreover, 1300 rpm indicates the decrease in lower entrapment efficiency and increase rate of drug release. It implies that higher speed of the stirrer can break the microsphere into small pieces or raptures the microspheres. The formulation exhibited the sustained release of drug from microspheres.

CONCLUSIONS

Microspheres containing diltiazem hydrochloride were prepared by a nonaqueous solvent evaporation method to produce maximum entrapment efficiency and desirable drug release. The formulation variable polycarbonate and variable stirring rate exerted a significant influence on the drug entrapment and drug release. The findings acquired suggested that response surface methodology can be successfully used to evaluate the effect of formulation variables and improve an optimized formulation thereby reducing the number of trials, time, and cost of formulation development. Thus, multiple unit systems based on polycarbonate microspheres would be of significance as floating diltiazem hydrochloride loaded microspheres for sustained drug delivery by the oral route. Hence, prepared floating microspheres may prove to be potential candidates to enhance the bioavailability in the upper part of the gastrointestinal tract.

REFERENCES

1. Jain NK. Controlled and Novel Drug Delivery. 1st ed. New Delhi: CBS Publishers and Distributors; 1997. p. 71.
2. Robinson JR, Lee VH. Controlled Drug Delivery: Fundamentals and Applications. 2nd ed. New York: Marcel Dekker; 1987. p. 16.
3. Yeole PG, Shagufta K, Patel VF. Floating drug delivery systems: Need and development. Indian J Pharm Sci 2005;67:265-72.
4. Government of India, Ministry of Health and Family Welfare. Indian Pharmacopoeia. Vol. 2. Delhi: Government of India, Ministry of Health and Family Welfare, Published by Controller of Publications; 1996. p. 797.
5. Ryden T, Jonsson UE. A study of the absorption of diltiazem from the distal parts of the gastrointestinal tract. Eur J Clin Pharmacol 1989;36 suppl: A243.
6. Shirolkar SV, Tawar MG, Gandhi NS, Deore NB. Development and evaluation of floating microspheres of Pioglitazone hydrochloride using ethyl cellulose. Pharm Lett 2010;2:261-77.
7. Pande AV, Nimbalkar UA, Dhoka MV, Sonawane PA. Floating microspheres of cefpodoxime proxetil: Formulation and optimization by factorial design. Int J Res Pharm Chem 2011;1:448-57.
8. Streubel A, Siepmann J, Bodmeier R. Floating microparticles based on low density foam powder. Int J Pharm 2002;241:279-92.
9. Kiyoyama S, Shiomori K, Kawano Y, Hatate Y. Preparation of microcapsules and control of their morphology. J Microencapsul 2003;20:497-508.
10. Yang Z, Song B, Li Q, Fan H, Ouyang F. Preparation of microspheres with microballoons inside for floating drug delivery systems. J Appl Polym Sci 2004;94:197-202.
11. Gandhi S, Bhandari A, Jani GK, Upadhyay N, Pathak R. Optimization of floating microspheres of captopril using full factorial design. Asian J Biomed Pharm Sci 2012;2:69-94.

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