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Statistical Evaluation and Optimization of Influence of Stirring Speed and Polymer Concentration on Hollow Microspheres of Diltiazem HCl

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ABSTRACT

The purpose of the present study was to develop an optimized gastroretentive drug delivery system of Diltiazem HCl Hollow microspheres by the optimization technique. The Diltiazem HCl microspheres were prepared by non aqueous emulsion solvent evaporation method using different grades of Eudragit (Eudragit RS 100 and Eudragit RL 100) in combination. Response Surface Analysis data was obtained using Software STAT-EASE, design expert, 7.0, Trial Version. A 3^2 factorial design was employed in formulating the GRDDS with Stirring speed(X_1) and polymer concentration (X_2) as independent variables and Two dependent variables were particle size and Drug entrapment. The main effect and interaction terms were quantitatively evaluated using a mathematical model. Regression analysis and numerical optimization were performed to identify the best formulation. The result of analysis of variance test for both effects indicated that the test is significant. The best batch exhibited a high entrapment efficiency and particle size.

Keywords: Hollow microspheres, 3^2 factorial design, polymethacrylic acid(Eudragit)

INTRODUCTION

The present investigation is aimed at using eudragit(Eudragit RS 100 and Eudragit RL 100) polymer in combination for sustained drug delivery of water soluble drug for microsphere. Camphor added for forming the cavity in the microspheres. where as Eudragit RS 100 being water insoluble polymer retards drug release. So the objective of the present study was to develop gastroretentive system of Diltiazem HCl for sustained release and evaluate the effect of stirring speed and polymer concentration on entrapment efficiency and particle size.

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 inter-subject variability in absorption and risk of local irritation. Recently, hollow microspheres with a lower density than that of the GI fluids were adopted.[1] The Hollow microspheres were prepared by the non aqueous emulsion solvent evaporation technique using different polymer solution systems.[2,3]

Diltiazem HCl, a benzodiazepine, voltage sensitive Ca^{2+} channel blocker with a high therapeutic potential but with a very short biological half life was encapsulated within microsphere. Diltiazem is a calcium ion influx inhibitor (calcium entry blocker or calcium ion antagonist). The antihypertensive, antianginal and antiarrhythmic effects of diltiazem is believed to be related to its specific cellular action of selectively inhibiting transmembrane influx of

calcium in cardiac muscle, coronary arteries, and systemic arteries and in cells of the intra cardiac conduction system.[4] Given orally, 90–100% of diltiazem is absorbed, but due to high first pass metabolism, bioavailability is much lower (40–60%), half life is 4-5 hours (with chronic dosages) and not cleared by hemodialysis.[5]

The Eudragits are biocompatible copolymers which were synthesized from acrylic and methacrylic acid esters. These polymers are well tolerated by the skin and have been used in the formulation of dosage forms especially matrix tablets for oral sustained release[6,7] and in tablet coating. They have also been used in the microencapsulation of drugs.[8-10]

MATERIALS AND METHODS

Material

Diltiazem HCl was obtained as a gift sample from Lincoln Pharmaceutical Ltd, Kalol, Gujarat. Eudragit RS100 and Eudragit RL100 were obtained from Crystal Chemical Pvt. Ltd, Himatnagar (Gujarat). All other chemicals / reagents used were of analytical grade available commercially.

Method of preparation

Microspheres containing highly water-soluble Diltiazem HCl(DTZ) were prepared by non-aqueous emulsion solvent evaporation method using two structurally different poly(trimethyl ammonioethyl methacrylate) copolymers: Eudragit RL100 and RS100. Polymers were used combined(1:1). In this case polymers, weighed quantities of Eudragit RL100 and RS100 were completely dissolved in Dichloromethane and ethanol (1:1), Magnesium stearate and DTZ were then added and stirred using a magnetic stirrer (10 min). Camphor is added as pore forming agent(1%). Especially with 10% magnesium stearate, the microspheres were nearly uniform. The drug polymer mixture was then slowly introduced into liquid paraffin(100 ml) previously emulsified with 1% Span 80, while stirring at 400,600 and 800 rpm held by the mechanical stirrer (Remi, Mumbai) equipped with a three-blade propeller, at room temperature. The whole system was stirred for 2hr to allow the complete evaporation of organic solvent. The oil layer was decanted and microspheres were washed with n-hexane(10times×20ml). The method is pictorially represented in figure 1.

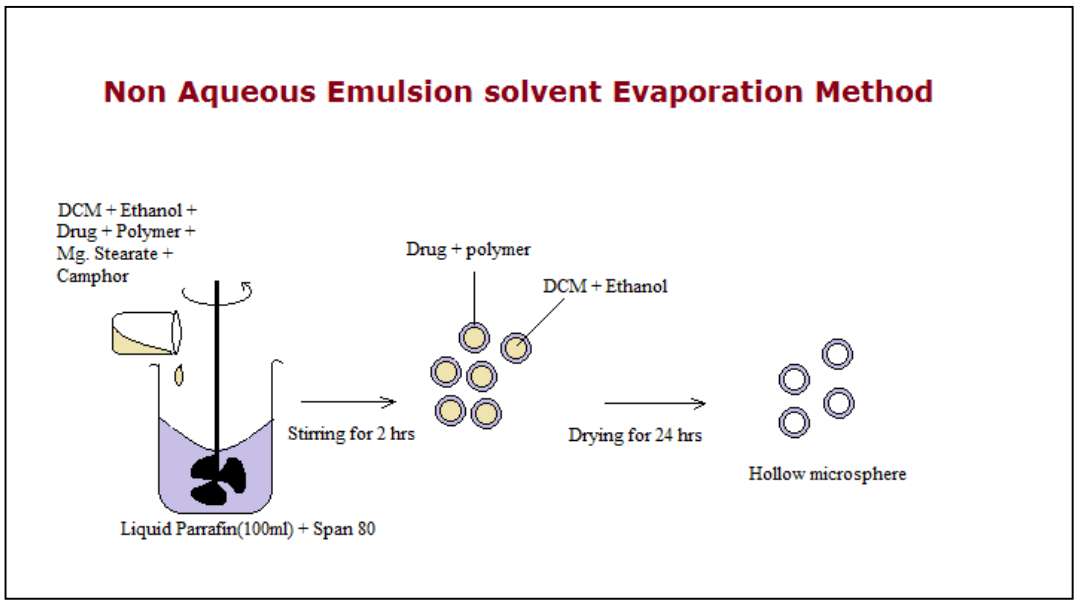

Figure 1. Method of Preparation Hollow microspheres

Preliminary trial:

The Hollow microspheres of Diltiazem HCl using Eudragit RS 100 and Eudragit RL 100 were prepared by non aqueous emulsion solvent evaporation technique. Both the polymer for the preparation of Hollow microspheres owing to its pH independent and control release properties. Different Speed of mechanical stirrer from 300 to 1000

rpm. Results showed that Increase Speed of mechanical stirrer decrease the particles size and the drug entrapment efficiency. At 300 rpm, formulation of microsphere but not Hollow and irregular shape of microspheres was observed. While at 600 rpm particles size and drug entrapment efficiency were 82%, 210 µm and at 1000 rpm spherical shape of microspheres was observed but particles were coalesced to beaker wall and also decrease the particle size. Therefore,400, 600 and 800 rpm was used for further study.

One of the Important factors related to microspheres as reported by Lee et al[11] is the viscosity of the polymer solution. Polymers concentrations of 120mg, 180mg, and 240mg were selected for preliminary trials. Flake formation was observed when Eudragit RS 100 and Eudragit RL 100 concentration was used at a low level, whereas maximum sphericity was observed at the 180mg. Preliminary trial batches were prepared to study the effect of the time for stirring, stirring speed on the % drug entrapment efficiency and characteristics of the microspheres. Increase in the time for stirring from 1 to 3 hrs, showed decrease in % drug entrapment efficiency and particles size. After 2 hr not formulate the hollow microsphere and long term particle become smaller so increase the surface area and release the more drug. Thus, 2 hrs of stirring time was selected for further study. Since stirring speed has significant effect on % drug entrapment efficiency and particles size, it was selected as one an important factor for further study.

On the basis of the preliminary trials a 3^2 full factorial design was employed to study the effect of independent variables (i.e. stirring speed [X_1] and the polymer concentration[X_2]) on drug entrapment efficiency, particle size. The results shows that all the dependent variables are strongly dependent on the selected independent variables as they show a wide variation among the 9 batches. The fitted equations (full models) relating the responses (i.e., drug entrapment efficiency, particle size). The polynomial equations can be used to draw conclusions after considering the magnitude of coefficient and the mathematical sign it carries (i.e., positive or negative). The high values of correlation coefficient for the dependent variables indicate a good fit. The equations may be used to obtain estimates of the response since small error of variance was noticed in the replicates. Parameters for the preparation of microspheres were optimized from preliminary studies and are summarized in **Table 1**. 3^2 full factorial design with coded form and actual form of variables for each batch is described in **Table 2** and **3**.

Table 1. Parameter For Preparation of Hollow Microspheres

Parameter	Condition
Stirring speed	400, 600, 800 (RPM)
Polymer concentration	120, 180, 240 (mg)
Temperature	40°C
Time for stirring	2 hr

Table 2. 3^2 full factorial design with actual form of variables for each batch

Coded value	Actual value	
	X_1	X_2
-1	400	120
0	600	180
+1	800	240

X_1: Stirring Speed (RPM); X_2: Polymer Concentration(mg)

Table 3. 3^2 full factorial design with coded form of variables for each batch

Batch	Coded value	
	X_1	X_2
1	-1	-1
2	-1	0
3	-1	1
4	0	-1
5	0	0
6	0	1
7	1	-1
8	1	0
9	1	1

Response

Particle size:

The particle size was measured by microscopic technique. In this method suspension of floating microspheres was prepared using castor oil. A drop of suspension was mounted on a slide and observed under optical microscope about 300 particles were measured with the help of the eye piece micrometer. All the Microspheres in a field were counted.

Entrapment efficiency:

Floating microspheres were dissolved in a minimum amount of methanol and drug was extracted into 0.1 N hydrochloric acid by evaporating methanol. The solution was filtered through whatman filter paper, diluted suitably and analyzed for drug content spectrophotometrically at 236 nm using 0.1N hydrochloric acid as blank.

Response Surface Analysis:

The results are expressed as second order polynomial equation of the following term

$$Y= \beta_o+\beta_1X_1+\beta_2X_2+\beta_{12}X_1X_2+\beta_{11}X_{11}+\beta_{22}X_{22}$$

Where Y is the predicted response, β_o is the arithmetic mean response of 9 runs (Table 4). The main effects (X_1 and X_2) represent the average result of changing one factor at a time from its low value to its high value. The interaction shows how the particle size and entrapment efficiency value changes when two factors are simultaneously changed, and the exponential terms (and) represent curvature. The coefficients corresponding linear effects (β_1 and β_2), interaction (β_{12}) and the quadratic effects (β_{11} and β_{12}) were determined from the results of the experiment (STAT-EASE, Design expert, 7.0, Trial Version).[12-14]

RESULT AND DISCUSSION

Experimental design and Statistical analysis Factorial design enables all factors to be varied simultaneously, allowing quantification of the effects caused by independent variables and interactions between them. In this study, A 3^2 full factorial experimental design was used to optimize the formulation of microspheres. Initial studies were undertaken to decide the excipient and their levels in the experimental design.

The particle size of prepared Microspheres was in the range of 220±13.43 to 388±9.43 as shown in figure 2. The data obtained showed that, the mean diameter was increased significantly (P=0.001) as the drug: polymer ratio was varied from 1:1, 1:1.5 and 1:2. Low concentration of polymer resulted in a low viscosity of the polymer solution which in turn resulted in smaller emulsion droplets in the aqueous phase. The results also indicated that as the stirring rate increased from 400 rpm-800 rpm, the mean particle size was decreased significantly. This may be attributed to the formation of an emulsion of small droplets by increasing the mechanical stress at high stirring rate. The particle size of the microspheres decreases with decrease in the drug-to-polymer ratio. Nagda et al[15] and Motlekar et al[16] also reported that there is increase in drug-to- polymer ratio increases particle size which may be due to increased viscosity of feed solution which influence the interaction between disperse phase and dispersion medium that affects the size distribution of particle.

All Batches show percent encapsulation more than 73 % and it is found that encapsulation of drug increases with an increase in the amount of the polymer. Batch 7 shows maximum entrapment whereas Batch 3 shows minimum entrapment of the DTZ in the polymer as shown in Figure 3.

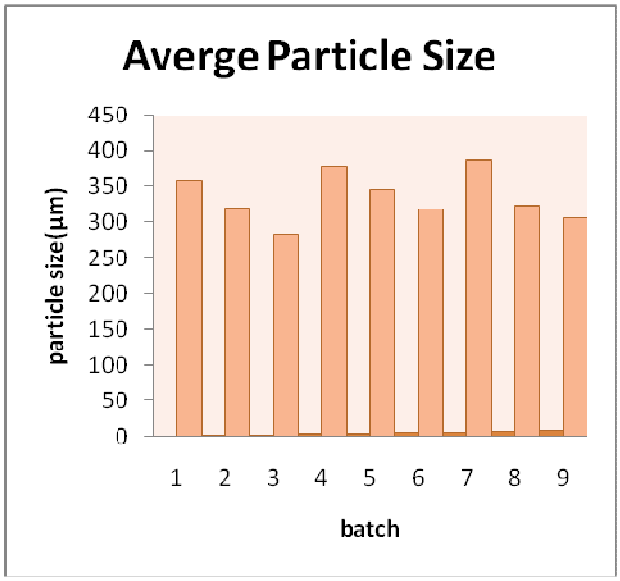

Figure 2. particle size of all batch

Figure 3. Entrapment Efficiency of all batch

Data analysis:

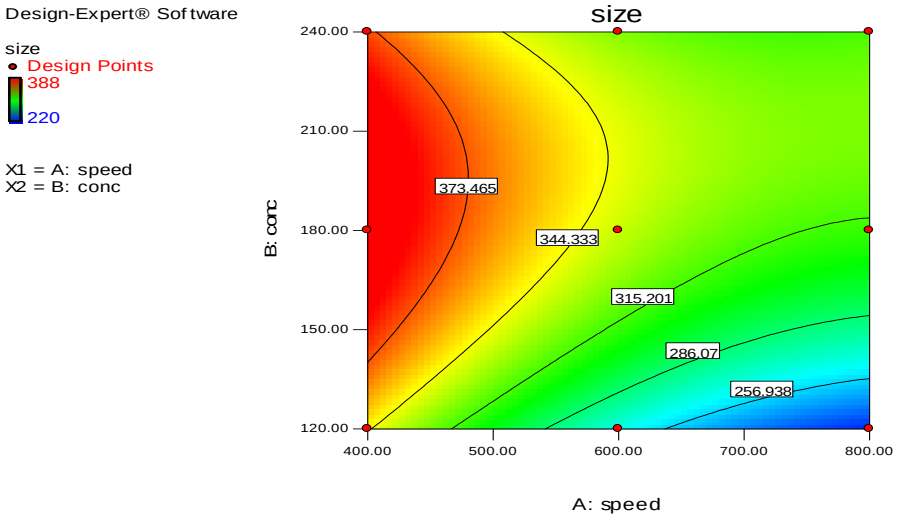

Figure 2. contour plot of particle size

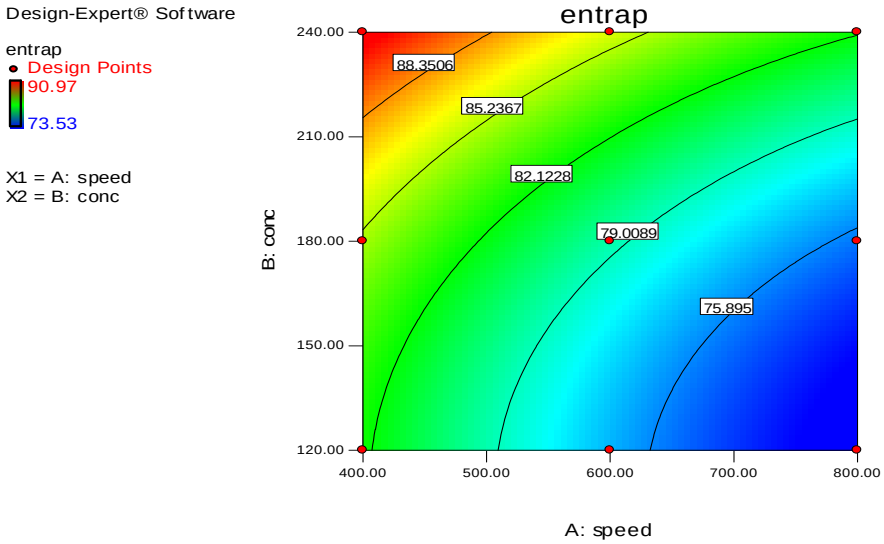

Figure 3. contour plot of entrapment efficiency

Table 4. Final Equation in term of Actual Factors

Response	β_0	β_1	β_2	β_{12}	β_{11}	β_{22}
Particle size(Y_1)	-166.86	-0.18375	5.74028	2.7083	9.166	-0.0138
Entrapment efficiency(Y_2)	42.7422	-0.0133	0.89811	1.7333	7.216	-2.5314

Final Equations in Terms of Coded Factors (Particle Size)

The Y_1 for all 9 Batches 1 to 9 shows good correlation co-efficient of 0.9994. From table 4. Variable X_1 has p value 0.0354 ($p < 0.05$) & variable X_2 has p value <0.0001 ($p<0.05$). Variables which have p value less than 0.05, significantly affect. So here both X_1 (stirrer speed) & X_2 variable (polymer concentration) significantly affects the particle size of the formulation. Here negative sign of X_1 coefficient indicate that as the stirrer speed increase, it will decreases particle size & positive sign of X_2 coefficient indicate that as polymer concentration increase it will increase the particle size of microsphere. X_{11} has p value (0.1022) > 0.05, X_{22} has p value (<0.0001) <0.05, and interaction of X_{12} has p value (0.0615) > 0.05.So, all three have significant affect on particle size. Contour plot for Hollow microspheres particle size figure 4.

$$Y_1=166.86-0.18375*X_1+5.74028*X_2+2.7083*X_1X_2+9.166*X_1X_1-0.0138*X_2X_2$$

Final Equations in Terms of Coded Factors (Entrapment efficiency)

The Y_2 for all 9 Batches 1 to 9 shows good correlation co-efficient of 0.9449. From table 4. Variable X_1 has p value 0.1359 ($p > 0.05$) & variable X_2 has p value 0.0266 ($p < 0.05$). Variables which have p value less than 0.05, significantly affect. So here both X_1 (Volume of internal phase) & X_2 variable (Volume of the external phase) significantly affects the %yield in the formulation. Here negative sign of X_1 coefficient indicate that as the stirrer speed increase, it will decreases the entrapment efficiency & positive sign of X_2 coefficient indicate that as polymer concentration is increase it will increase the entrapment efficiency product. X_{11} has p value (0.2572) > 0.05, X_{22} has p value (0.0217) > 0.05, and interaction of X_{12} has p value (0.2502) > 0.05.So, all three have significant affect on entrapment efficiency. Contour plot of Hollow microspheres entrapment efficiency figure 5.

$$Y_2=42.7422-0.0133*X_1+0.89811*X_2+1.7333*X_1X_2+7.2167*X_1X_1-2.5314*X_2X_2$$

CONCLUSION

The hollow microspheres of Diltiazem HCl using Eudragit RS 100 and Eudragit RL 100 with combination were successfully developed as gastroretentive drug delivery systems. As the Drug: Eudragit ratio was increased from 1:1, 1:1.5, 1:2 particle size of microspheres increased and drug release decreased.

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