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STUDIES ON EFFECT OF VARIABLES BY RESPONSE SURFACE METHODOLOGY FOR LOSARTAN MICROSPHERES

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ABSTRACT : Microspheres of Losartan potassium were formulated using combination of Ethyl cellulose and Acycoat L30D polymer by solvent evaporation method. A 3^2 factorial design was used to elucidate the effect of variables viz. the amount of drug and the amount of polymer. Discrete spherical microspheres in the range of 40-50 µm were produced with the encapsulation efficiency of more than 80 %. Polynomial equations and response surface plots were generated for all dependent variables. It was observed that both the factors had significant influence on all dependent variables studied. It was observed that as the amount of polymer increases, the rate of release decreases. So t_{50} increases and the amount of drug released in 2 h (X_{120}) decreases. Drug polymer interaction study was absent as evidenced by HPLC and FT-IR studies.

Keywords: Losartan potassium, Microspheres, Factorial Design, Response Surface.

INTRODUCTION

The efficiency of any drug therapy can be described by achieving desired concentration of the drug in blood or tissue, which is therapeutically effective and non toxic for a prolonged period. This goal can be achieved on the basis of proper design of the dosage regimen. Microspheres have potential to deliver drug in a controlled fashion. Losartan potassium is an effective antihypertensive drug but is extensively bound to plasma proteins and also causes gastrointestinal disorders, neutropenia, acute hepatotoxicity, migraine and pancreatitis. It may therefore be more desirable to deliver this drug in a sustained release dosage form. The present study was focused on development of sustained release Losartan microspheres using solvent evaporation method. A 3^2 factorial design was employed to study two important factors viz. the amount of drug and the amount of polymer. Response surface methodology was used to evaluate the effect of various parameters.

MATERIALS AND METHODS

Materials

Losartan potassium was procured as a gift sample from Macleod's Pvt. Ltd, Mumbai (India). Ethyl cellulose was purchased from SD-Fine Chemicals, Mumbai. Sodium alginate was obtained from LOBA chemicals, Kolkata. Acycoat L30D was purchased from Corel Pharma Ahmadabad (India). All chemicals were of analytical grade and were used without further purification.

A 3^2 Full Factorial Design

Two factors were evaluated each at three levels and experimental trials were performed at all possible nine combinations. In the present investigation the amount of drug (X_1) and the amount of polymer (X_2) were selected as independent variables. The experimental design with corresponding formulations is outlined in Table 1. The responses Yi were measured for each trial. A statistical model incorporating interactive and polynomial terms was utilized to evaluate the response.

$$Yi = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_{12} X_1 X_2 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{112} X_1^2 X_2 + \beta_{122} X_1 X_2^2 + \beta_{1122} \quad X_1^2 X_2^2$$

Where, β_0 is the arithmetic mean response of 9 runs and β_1 is the coefficient of factor X_1. The main effects X_1 and X_2 represent the average result of changing one factor at a time from its low to high value. The term X_1^2 and X_2^2 indicate curvilinear relationship. The interaction X_1X_2, $X_1^2X_2$, $X_1X_2^2$ and $X_1^2X_2^2$ shows how the dependent variable changes when two or more factors are simultaneously changed. Microsoft Excel with DOEPRO software was used for multiple regression analysis.

Method of Preparation:

Solvent evaporation method[1]

This is the method widely used in the microencapsulation process. Concisely the polymer ethyl cellulose was dissolved in methanol to get a clear solution. The drug Losartan was added and dissolved in the polymer solution. The resultant mixture was then stirred at 900 rpm for 1 hto evaporate the volatile substance. The formed microspheres were collected and air dried for 3 hours and stored in desiccator for further use.

EVALUATIONS

Percentage yield (% yield)[2]

The yield was calculated as the weight of the microspheres recovered from each batch divided by total weight of drug and polymer used to prepare that batch multiplied by 100.

Drug content estimation[2, 3]

Drug loaded microspheres (100 mg) were powdered and suspended in 100 ml methanolic: water (1:99 v/v) solvent. The resultant dispersion was kept for 20 min for complete mixing with continuous agitation and filtered through a 0.45 µm membrane filter. The drug content was determined spectrophotometrically (UV-1700, Shimadzu Japan) at 205.6 nm using a regression equation derived from the standard graph (r^2=0.9954).

Drug Entrapment Study[2, 3]

The drug entrapment efficiency (DEE) was calculated by the equation,

$$DEE = (Pc / Tc) \times 100 \quad \text{……..... 1}$$

Where, Pc is practical content, Tc is the theoretical content. All the experimental units were analyzed in triplicate (n=3).

Particle size analysis[2, 3, 4]

The microsphere size distribution was determined by the optical microscopy method using a calibrated stage micrometer (µm) was calculated by using equation,

$Xg = 10 \times [(n_i \times \log X_i) / N]$ 2

Where, Xg is geometric mean diameter, n_i is number of particle in range, xi is the midpoint of range and N is the total number of particles. All the experimental units were analyzed in triplicate (n=3).

Percentage of moisture loss[2, 3]

The Losartan loaded microspheres of different polymers were evaluated for percentage of moisture loss which sharing an idea about its hydrophilic nature. The microspheres weighed initially and kept in desiccator containing calcium chloride at 37 °C for 24 h. The final weight was noted when no further change in weight of sample.

$$\% \text{ of moisture loss} = \frac{\text{initial weight-final weight}}{\text{Initial weight}} \times 100 \quad \ldots\ldots\ldots\ldots\ldots\ldots 3$$

Drug Polymer Interaction Study:

Fourier Transform Infrared Radiation measurement (FTIR)[5]

The FTIR spectral measurements were taken at ambient temperature using IR spectrophotometer (shimadzu, model 840, Japan). Two mg of pure drug, empty microspheres and drug loaded microspheres were selected separately.

High Performance Liquid Chromatography (HPLC) measurement[6]

The HPLC (Model LC20AT, SHIMADZU, Japan) was used for the study of drug and polymer interaction. About 10 µg/ml concentration of drug and formulations were measured to study the interaction. The mobile phase used was water-acetonitrile-methanol (50+30+20 v/v), the retention reported in standard literatures were 6.4-6.63 min.

Scanning electron microscopy (SEM)[6]

Scanning electron microscopy (Zeiss DSM 962, Zeiss, Oberkochen, Germany) was carried out to study the morphological characteristics of Losartan microspheres. The dried microspheres were coated with gold (100 A°) under an argon atmosphere in a gold coating unit and Scanning electron micrographs of both higher and lower resolutions were observed.

In-vitro drug release[4]

In vitro drug release study was carried out in USP XXI paddle type dissolution test apparatus using Phosphate buffer pH 6.8 as dissolution medium, Volume of dissolution medium was 900 ml and bath temperature was maintained at (37±1)°C throughout study. Paddle speed was adjusted to 50 rpm. An interval of 1 hour, five ml of sample was withdrawn with replacement of 5 ml fresh medium and analyzed for Losartan content by UV-Visible spectrophotometer at 205.6 nm. All the experimental units were analyzed in triplicate (n=3).

In vitro drug release kinetics

In order to study the exact mechanism of drug release from microspheres, drug release data was analyzed according to Zero Order[7] , First Order[7] , Higuchi square root[8] , Hixon Crowell[9], Koresmeyer model[10]. The criterion for selecting the most appropriate model was chosen on the basis of goodness of fit test.

Statistical analysis [12]

All the results obtained during evaluation, were verified with different statistical methods like one way ANOVA, standard deviation, and probability log scale plotting (for measurement of particle size).

RESULTS AND DISCUSSIONS

Formulations of microspheres by applying factorial design are given in Table 1. The percentage yield of all the formulation was found to be more than 81% except F1 as given in Table 2. It can be due to minimum involvement of process parameters and smaller amount of drug loss during manufacturing. Drug entrapment efficiency (DEE) of all formulations were found to be more than 75 % except F4 and F7 as the drug is fully dispersed in the polymer phase by continuous stirring for a longer period represented in Table 2.

Table 1. Formulation of Losartan Potassium microsphere by Factorial Design.

Two variables were studied in three levels of concentration to achieve 3^2 factorial design for the experimental batches.

Formulation Code	Variables (levels)	
	Drug X_1 (g)	Polymers (EC+AcL30D) X_2 (g)
F1	1(-1)	1(-1)
F2	1(-1)	2(0)
F3	1(-1)	3(+1)
F4	2(0)	1(-1)
F5	2(0)	2(0)
F6	2(0)	3(+1)
F7	3(+1)	1(-1)
F8	3(+1)	2(0)
F9	3(+1)	3(+1)

Where, EC = Ethylcellulose, Ac = Acrycoat and (-1), (0) and (+1) are three different levels. X_1 and X_2 are two variables.

To determine the surface morphology of the microspheres, SEM of the microspheres were performed. Scanning electron microphotographs of Losartan loaded microspheres shows that microspheres obtained were discrete, spherical and uniform as shown in Fig 1. The particle sizes of all the formulations were found to be satisfactory. Particle sizes of the formulation were within the range of 39.36 to 52.84 µm represented in Table 2 and Fig 2.

Table 2. Percentage Yield, Drug Content and Encapsulation Efficiency of Losartan Loaded Microspheres Prepared by Different Techniques.

Formulation code	Yield (%) (X±S.D)	Actual Drug content (mg) (X±S.D)	Drug Entrapment Efficiency (%) (X±S.D)	Particle Size $D_{\text{geometric mean}}$ (µm) (X ± S.D)	% moisture loss (X ± S.D)
F1	73.16±0.412	54.26±0.542	79.22±0.790	43.24±0.593	4.32±0.324
F2	89.45±0.326	32.31±0.423	86.59±1.10	39.36±0.623	2.98±0.423
F3	90.35±0.156	23.25±0.489	84.05±1.71	42.27±0.682	3.94±0.411
F4	84.78±0.842	52.78±0.754	67.12±0.963	46.65±0.707	3.09±0.254
F5	87.89±0.743	43.54±0.826	76.54±1.45	45.58±0.526	3.70±0.359
F6	89.28±0.584	35.85±0.564	80.04±1.25	47.59±0.684	4.23±0.452
F7	84.42±0.187	60.12±0.456	67.67±0.845	52.84±0.568	3.65±0.325
F8	88.54±0.386	52.56±0.854	77.57±1.53	48.32±0.572	4.11±0.289
F9	87.60±0.423	43.77±0.522	76.69±0.920	45.11±0.632	4.86±0.326

All values are represented as mean ± standard deviation (n=3).Standard error mean < 0.988.

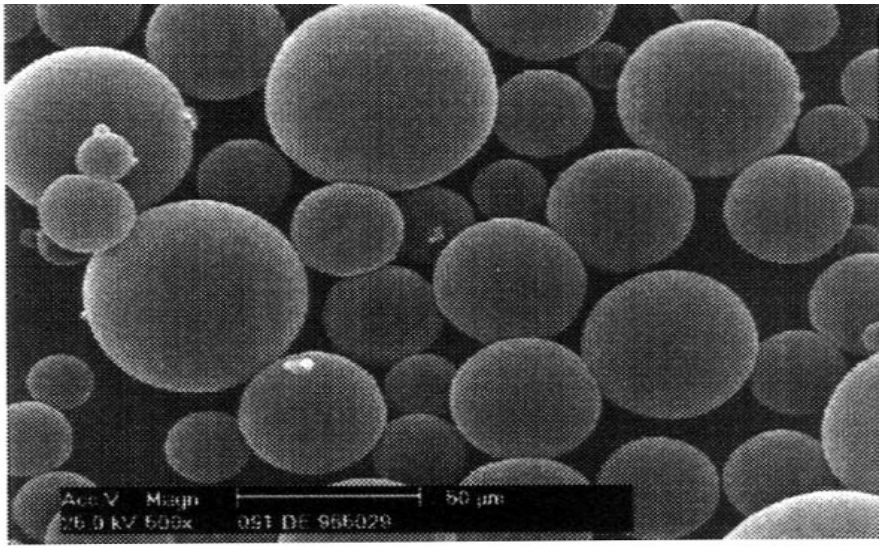

Fig 1. Scanning electron micrograph of microspheres prepared by solvent evaporation method under higher resolution.

This narrow range of particle size can be attributed to the effect of stirring time, stirring speed and rate of solvent evaporation during preparation of microspheres. The percentage of moisture loss was found to be minimum in all the formulations as shown in Table 2. This leads to draw a conclusion that the stability of internal water phase in all the formulations is high facilitating prolonged storage of formulation due to less water content in them. Formulations F2, F3 and F5 show sustained release of drug for more than 9 hours as shown in Fig 3.

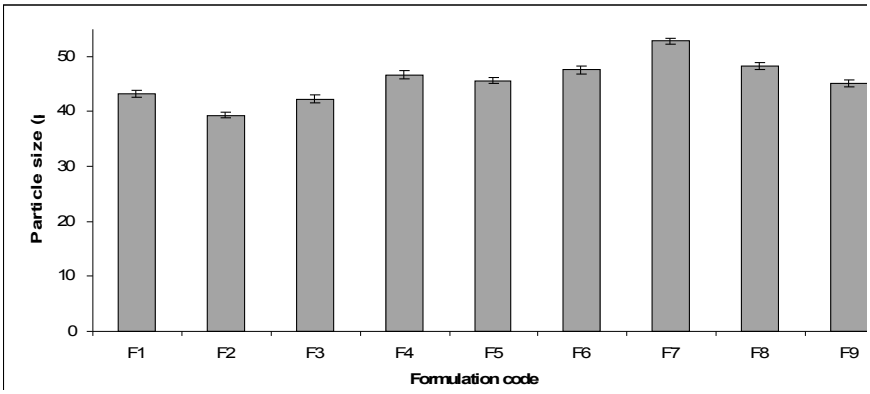

Fig 2. Mean geometric size (Diameter) of different microsphere formulations of Lorsatan Potassium. Each bar is represented as mean ± standard deviation (n=3).

Table 3. Step-Wise Multiple Regression Analysis for measured Response.

Coefficient	Parameters	
	Solvent evaporation method	
	t 50(min)	X120(mg)
β0	133.0	10.15
β1	-85.00	4.205
β2	16.00	-2.997
β12	7.75	-0.79675
β11	-8.00	2.368
β22	-93.00	6.67
β112	50.25	0.26525
β122	81.25	-2.487
β1122	77.75	-5.68

Table 4. Mathematical modeling of different formulation of losartan loaded microspheres solvent evaporation method.

Code	Ka value				n	t 50 (min)	X120 (mg)
	Zero order	First order	Higuchi	Hixon Crowell			
F1	2.456	0.029	9.650	0.033	0.1355	55	13.725
F2	3.962	0.034	16.763	0.064	0.3580	210	8.313
F3	3.052	0.028	12.954	0.046	0.2570	172	9.855
F4	2.380	0.066	10.21	0.026	0.1810	24	19.817
F5	3.141	0.030	12.811	0.046	0.2231	133	10.150
F6	3.8312	0.064	15.091	0.047	0.1907	56	13.823
F7	2.663	0.068	11.136	0.029	0.1415	32	18.754
F8	3.083	0.061	12.517	0.0388	0.1519	40	16.723
F9	3.275	0.0345	12.991	0.046	0.2071	180	11.697

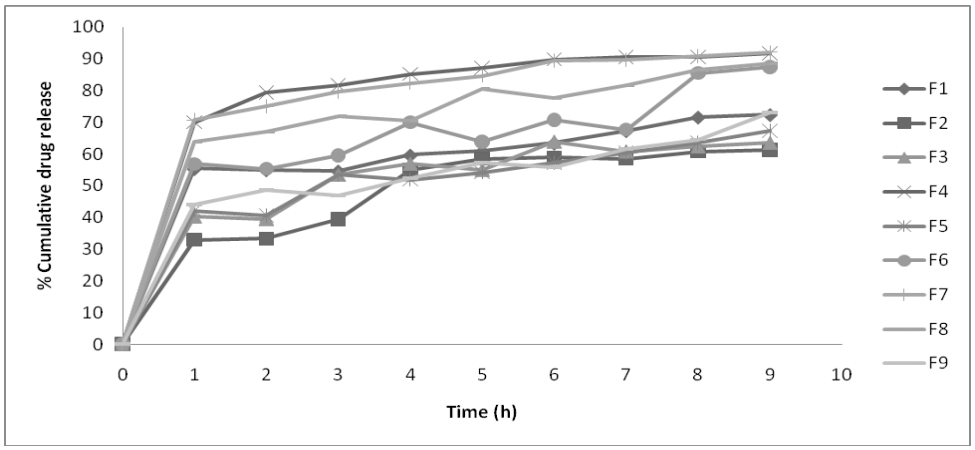

Fig 3. *In vitro* drug release profile of different formulation prepared by solvent evaporation method.

Putting all datas in different release kinetics models and comparing the coefficient of determination (r^2), it was found that F2, F3 and F7 tends to fit with Fickian diffusion model given by Higuchi confirming drug release by diffusion mechanism, whereas F5 and F9 fits with both Zero Order kinetic model. Only formulations F4 follow First Order kinetic model and the rest formulation followed Hixon-Crowell release model confirming the drug release by a complex mechanism as shown in Table 4. To justify the result power law was applied and from the diffusion coefficient value (n), it was found that almost all formulations follow Case I anomalous diffusion transport mechanism. This can be attributed to the fact that the drug release from the microspheres did not follow uniform geometry; instead the drug got released through fractal rearrangements of polymeric chain. Determination of interaction between drug and polymer were performed using FT-IR Spectroscopy as well as in HPLC. FT-IR spectra study showed no change in the fingerprint of pure drug spectra, thus confirming absence of drug to polymer interaction as depicted in Fig 4.

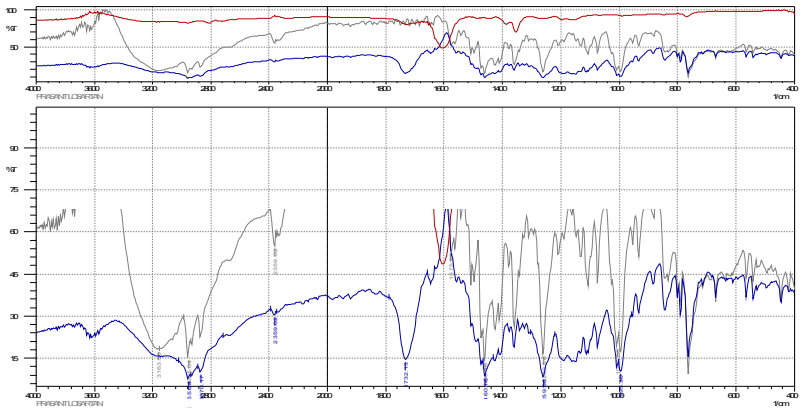

Fig 4. FTIR study of pure drug, polymer and the formulation (F3) prepared by solvent evaporation method.

It was further confirmed by HPLC as we got almost same retention time (6.56) for drug and formulations prepared by solvent evaporation method given in Table 5. The equations for time required for 50 % release and amount of drug released in 2 hours obtained after stepwise multiple regression analysis are depicted in Table 3.

Table 5. HPLC Chromatogram of pure Losartan potassium and one of the formulation prepared by solvent evaporation method.

Formulation	Retention time(min)	Area (m.Vs)	Height (mV)	Area (%)	Height (%)
Pure drug	6.560	525.691	34.461	82.7	76.7
F3	6.557	378.785	25.875	86.3	81.2

The response surface for $T_{50\%}$ was generated from the data obtained after stepwise multiple regression analysis as shown in the Table 3. When the drug is released it leaves behind pores or channels, through which the diffusion of the drug presents in the interior portion of the microspheres, occur. With the higher amount of drug in the formulations prepared both by solvent evaporation method, more pores or channels are formed and hence a higher release rate and a subsequent decrease in the time required for 50 % release was observed as indicated in the response Fig 5. However the time required for 50 % release increases as the polymer level increases keeping the concentration of drug constant. When the amount of polymer is increased the crosslink density increases which causes barrier for drug diffusion and hence the rate of release decreases and $T_{50\%}$ increases. However this effect was seen at lower levels of drug. At the same factorial level for both drug and polymer, $T_{50\%}$ is observed in a medium range.

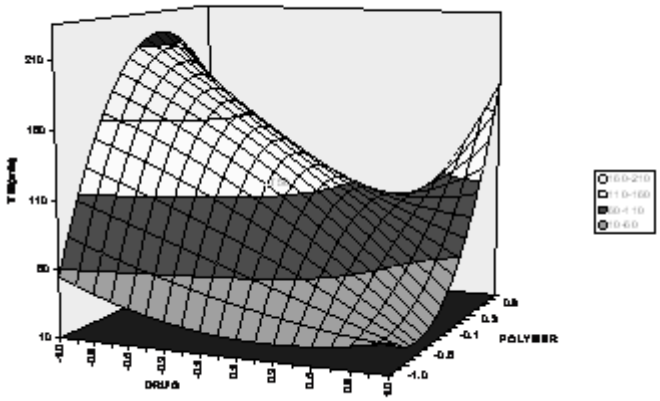

Fig 5. Response Surface for Time required for 50% (t_{50}) Drug Release of the formulations prepared by Solvent evaporation method

The response surface for the amount of drug released at 2 hours is depicted in Table 4 and Figure 6. With the higher amount of drug, the amount of drug released (X_{120}) is high but as the polymer level is increased in comparison to drug X_{120} decreases as indicated by negative value of interaction term. At same levels of drug and polymer X_{120} is increased but not as high as at higher levels of drug as indicated in the Fig 6 for formulation prepared by solvent evaporation.

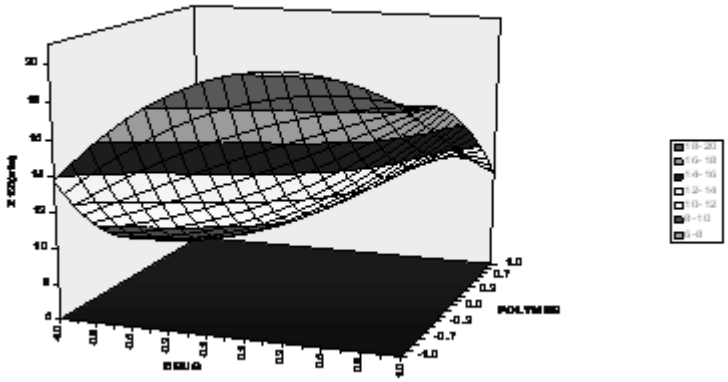

Fig 6. Response Surface for amount of drug release in 2hours (X_{120}) of the formulations prepared by Solvent evaporation method.

CONCLUSION

Both the variables affected the parameters like amount of drug release in 2hours and time required for 50% drug release. Use of factorial design and response surface methodology helps in understanding the effect of variables in a better way. Using the regression equation we can predict our desire response by varying the variable factor in any level. This equation optimizes the formulation statistically reducing the valuable time which could have been required for further experimentation. In future an unknown combination of drug and polymer shall be prepared and both its actual and predicted values shall be compared to estimate percentage prediction error. This will lead to validation of the regression equation achieved so far.

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