

**Research Article****STATISTICAL EVALUATION OF LOSARTAN MICROSPHERES PREPARED BY W/O EMULSION METHOD USING FACTORIAL DESIGN AND RESPONSE SURFACE METHODOLOGY**

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**ABSTRACT**

Present investigation describes statistically the influence of variables viz. the amount of drug and the amount of polymer using  $3^2$  factorial designs. Microspheres containing Losartan potassium were prepared by W/O emulsion solvent evaporation method. The morphology, particle size distribution, total entrapment of Losartan potassium into the micro particles and their release profiles were investigated. The mean geometric particle size of microspheres prepared by W/O emulsion solvent evaporation method was found in a range of 126-150  $\mu\text{m}$  respectively. The drug entrapment efficiency of all the formulations was found to be more than 80 %. Response surface were plotted to elucidate the effect of variables on 50 % drug release ( $t_{50}$ ) and the amount of drug release ( $X_{120}$ ). The amount of polymer affected the drug release. Drug polymer interaction was absent as evidenced by FTIR study.

**KEYWORDS** Losartan potassium, Microspheres, W/O emulsion, 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 and w/o emulsion 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.

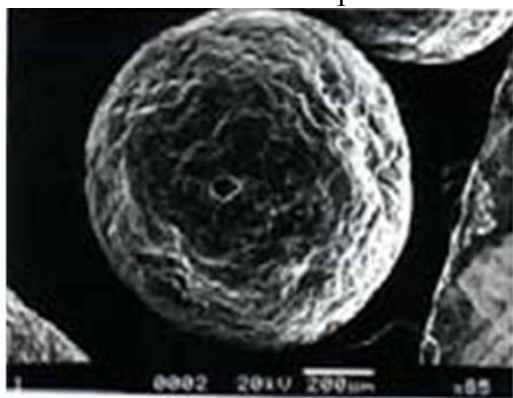
**MATERIALS AND METHODS****Materials**

Losartan potassium was procured as a gift sample from Macleod's Pvt. Ltd, Mumbai (India). Sodium alginate was obtained from LOBA chemicals, Kolkata. Acycoat E30D 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  $Y_i$  were measured for each trial. A statistical model incorporating interactive and polynomial terms was utilized to evaluate the response.



**FIG. 1. Scanning electron micrograph of microspheres (F12) prepared by solvent evaporation method at a resolution of 20kv × 4000.**

**Table 1- Formulation of losartan potassium microsphere by factorial design.**

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

Formulation Code	Variables (levels)	
	Drug $X_1$ (g)	Polymers (AL+AcE30D) $X_2$ (g)
F10	1(-1)	1(-1)
F11	1(-1)	2(0)
F12	1(-1)	3(+1)
F13	2(0)	1(-1)
F14	2(0)	2(0)
F15	2(0)	3(+1)
F16	3(+1)	1(-1)
F17	3(+1)	2(0)
F18	3(+1)	3(+1)

Where, AL = Sodium alginate, Ac = Acrycoat and (-1), (0) and (+1) are three different levels.  $X_1$  and  $X_2$  are two variables.

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

Where,  $\beta_0$  is the arithmetic mean response of 9 runs and  $\beta_1$  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  $X_1 X_2$ ,  $X_1^2 X_2$ ,  $X_1 X_2^2$  and  $X_1^2 X_2^2$  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.

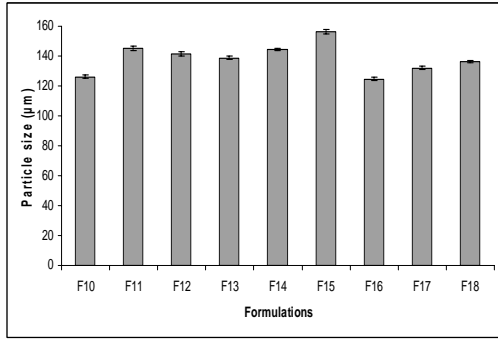
### Method of Preparation

#### W/O emulsion solvent evaporation method

Microspheres were prepared by the water-in-oil (W/O) emulsification solvent evaporation technique. The drug was dissolved in each polymeric aqueous solution. The solutions were

poured into 200 ml of paraffin liquid containing 0.5 % span 80 as an emulsifying agent. The aqueous phase

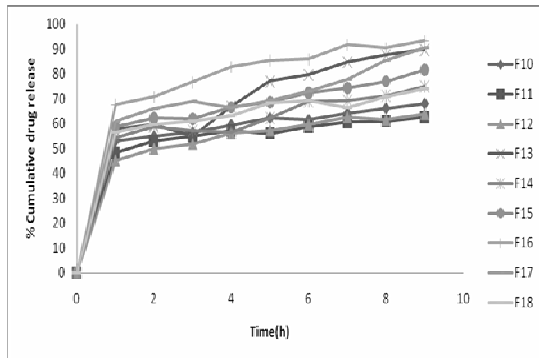
was emulsified into the oily phase by stirring the system in a 500 ml



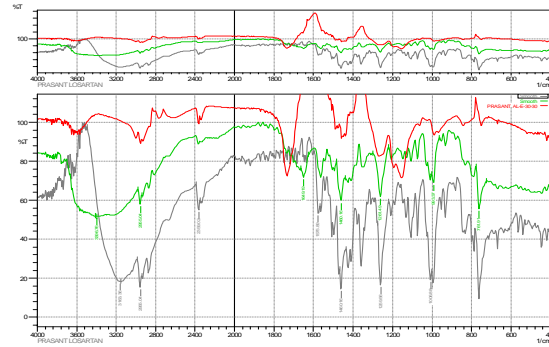
**FIG. 2 Mean geometric size (diameter) of different microsphere formulations of lorsatan potassium.**

Each bar is represented as mean ± standard deviation (n=3).

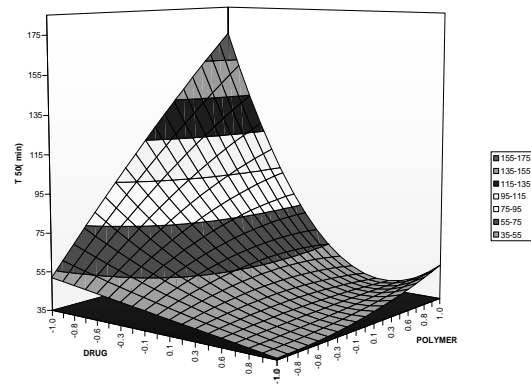
beaker. Constant stirring at 2000 rpm was carried out using mechanical stirrer the beaker and its content were heated by a hot plate at 80 °C. Stirring and heating were maintained for 2.5 hour until the aqueous phase was completely removed by evaporation<sup>1</sup>. The light oil was decanted and collected microspheres were washed three times with 100 ml aliquots of n-hexane, filtered through whatman filter paper, dried in an oven at 50 °C for 2 hour and stored in a desiccator at room temperature.



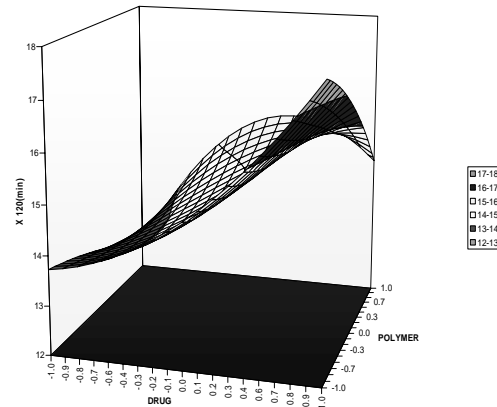
**FIG. 3. In-vitro drug release profile of Lorsatan microspheres prepared by w/o emulsion solvent evaporation method.**



**FIG. 4. Drug polymer interaction study by FT-IR (drug + AL.ACE30D + formulation, F12).**



**FIG.5. Response surface for time required for 50% (t<sub>50</sub>) drug release of the formulations prepared by w/o emulsion solvent evaporation method.**



**FIG 6. Response surface for amount of drug release in 2 hours (x<sub>120</sub>) of the formulations prepared by w/o emulsion solvent evaporation method.**

**EVALUATIONS****Percentage yield**

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<sup>2</sup>.

**Drug content estimation**

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<sup>2,3</sup>. 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**

The drug entrapment efficiency (DEE) was calculated by the equation<sup>2,3</sup>,

$$DEE = (Pc / Tc) \times 100 \dots\dots\dots$$

(1)

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

**Table 2- Percentage yield, drug content, particle size and encapsulation efficiency of losartan potassium loaded microspheres.**

Formulation code	Yield (%) (X±S.D)	Actual Drug content (mg) (X±S.D)	Drug Entrapment Efficiency (%) (X±S.D)	Particle Size D <sub>geometric mean</sub> (µm) (X ± S.D)	% moisture loss (X ± S.D)
F10	69.76±0.812	55.26±0.764	77.10±1.62	96.23±0.857	4.91±0.341
F11	84.85±0.716	33.45±0.682	85.15±1.16	95.52±1.451	3.41±0.368
F12	88.38±0.464	22.98±0.531	81.25±1.96	98.67±1.532	3.85±0.623
F13	81.66±0.368	54.23±0.735	66.43±1.54	88.73±0.949	3.57±0.425
F14	89.13±0.575	43.12±0.548	74.87±1.33	94.54±0.868	3.88±0.235
F15	90.13±0.147	37.83±0.674	80.19±1.12	86.37±1.241	3.94±0.232
F16	84.93±0.378	58.97±0.721	66.78±1.48	94.62±1.123	3.89±0.298
F17	88.31±0.567	52.42±0.754	77.17±1.86	92.18±0.923	4.16±0.264
F18	88.43±0.182	42.63±0.589	75.41±1.65	96.26±0.982	5.06±0.354

All values are represented as mean ± standard deviation (n=3). Standard error mean < 1.135.

**Table 3-Step-wise multiple regression analysis for measured response.**

Coefficient	Parameters	
	W/O emulsion solvent evaporation method	
	$t_{50}(\text{min})$	$X_{120}(\text{mg})$
$\beta_0$	56.00	14.693
$\beta_1$	-37.50	1.702
$\beta_2$	5.50	0.337
$\beta_{12}$	-25.25	-0.3865
$\beta_{11}$	21.50	0.2520
$\beta_{22}$	-6.50	0.5310
$\beta_{112}$	28.25	-1.352
$\beta_{122}$	4.25	-0.107
$\beta_{1122}$	6.75	-0.7825

**Table 4- *In vitro* drug release kinetic studies of prepared Losartan loaded microspheres.**

Formulation code	$r^2$ (Regression co-efficient)				
	Zero Order	First Order	Higuchi	Hixon-Crowell	n
<b>F10</b>	0.9783	0.9782	0.9715	0.9733	0.1355
<b>F11</b>	0.9225	0.8249	0.9605	0.9239	0.3580
<b>F12</b>	0.9825	0.9663	0.9796	0.9232	0.2570
<b>F13</b>	0.9198	0.9727	0.8852	0.9759	0.1810
<b>F14</b>	0.9048	0.9185	0.848	0.9059	0.2231
<b>F15</b>	0.9821	0.9882	0.9424	0.9843	0.1907
<b>F16</b>	0.9355	0.9674	0.9706	0.9186	0.1415
<b>F17</b>	0.8929	0.8053	0.8198	0.896	0.1519
<b>F18</b>	0.9125	0.9005	0.9166	0.9013	0.2071

The correlation coefficient of different formulations ( $r^2$ ).

**Table 5- HPLC chromatogram of pure Losartan potassium and one of the formulation prepared by w/o emulsion 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
F12	5.957	388.785	28.875	83.3	77.2

### Particle size analysis

The microsphere size distribution was determined by the optical microscopy method using a calibrated stage micrometer ( $\mu\text{m}$ ) was calculated by using equation <sup>2,3,4</sup>.

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

$X_g$  is geometric mean diameter,  $n_i$  is number of particle in range,  $x_i$  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

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 hours. The final weight was noted when no further change in weight of sample <sup>2,3</sup>.

$$\% \text{ of moisture loss} = (\text{initial weight} - \text{final weight} / \text{Initial weight}) \times 100 \dots\dots\dots (3)$$

### Drug Polymer Interaction Study Fourier Transform Infrared Radiation measurement (FTIR)

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 <sup>5</sup>.

### In-vitro drug release

*In vitro* drug release study was carried out in USP XXI paddle type dissolution test apparatus using Phosphate buffer pH6.8 as dissolution medium, Volume of dissolution medium was 900 ml and bath temperature was maintained at (37±1) °C throughout study <sup>3</sup>. Paddle speed was adjusted to 50 rpm. An interval of 1 hour, five ml of sample was withdrawn with replacement of five 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 <sup>6</sup>, First Order <sup>6</sup>, Higuchi square root <sup>7</sup>, Hixon Crowell <sup>8</sup>, Koresmeyer model <sup>9</sup>. The criterion for selecting the most appropriate model was chosen on the basis of goodness of fit test.

### Statistical analysis

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)<sup>10</sup>.

## 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 F10 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 90 % except F13 and F15 as the drug is fully dispersed in the polymer phase by continuous stirring for a longer period represented in TABLE 2. To determine the surface morphology of the microsphere, SEM of the microsphere were performed. Scanning electron microphotographs of Losartan loaded microsphere shows that microsphere obtained were discrete and spherical 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 126-150  $\mu\text{m}$  represented in Table 2 and Fig 2. 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 microsphere. 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 F11 and F12 show sustained release of

drug for more than 9 hours as shown in Fig 3. Putting all data in different release kinetics models and comparing the coefficient of determination ( $r^2$ ), it was found that F11, F16 and F18 releasing drug by diffusion mechanism confirming Higuchi kinetics, whereas F10 and F12 fits with Zero Order kinetic model. Only formulations F15 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 microsphere 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. It was further confirmed by HPLC as we got almost same retention time (6.590 min and 5.957 min) 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 hour obtained after stepwise multiple regression analysis are depicted in Table 3. 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 microsphere, occur. With the higher amount of drug in the formulations prepared by w/o emulsion 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. The response surface for the amount of drug released at 2 hours is depicted in Table 3 and Fig 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.

## CONCLUSION

Both the variables affected the parameters like amount of drug release in 2 hours 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 justification of the regression equation achieved so far.

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