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Formulation Design, Preparation of Losartan Potassium Microspheres by Solvent Evaporation Method and It's *In Vitro* Characterization

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Present investigation describes preparation of microspheres prepared by solvent evaporation method followed by *in vitro* characterization statistically. Microspheres containing Losartan potassium were prepared by solvent evaporation method by using ethyl cellulose and Acycoat L30D as rate controlling polymer. The microspheres were found to be discrete, spherical with free flowing properties. The morphology (Scanning Electron Microscopy), particle size distribution, total entrapment of Losartan potassium into the microparticles and their release profiles were investigated. The mean geometric particle size of microspheres was found in the range of 40-50 µm. The drug entrapment efficiency of all the formulations was found to be more than 80 %. The drug carrier interactions were investigated in solid state by FT-IR spectroscopy and HPLC study. *In vitro* drug release rate for microspheres was found to be sustained over 8 hours obeying zero order kinetic with good entrapment efficiency. Hence it can be concluded that the formulation F3 has potential to deliver Losartan potassium in a controlled manner in regular fashion over extended period of time in comparison to other formulations and can be adopted for a successful delivery of Losartan potassium for oral use for safe management of hypertension.

KEYWORDS: Losartan potassium, Microspheres, solvent evaporation, Entrapment efficiency.

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.

MATERIALS AND METHOD:

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. Acycoat L30D was purchased from Corel Pharma Ahmadabad (India). All chemicals were of analytical grade and were used without further purification.

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Method of Preparation (Solvent evaporation method):

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 hour to evaporate the volatile substance. The formed microspheres were collected and air dried for 3 hours and stored in desiccator for further use[1].

EVALUATIONS:

Percentage yield (% yield)[1]

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

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)[2,3].

Drug Entrapment Study

The drug entrapment efficiency (DEE) was calculated by the equation[2,3]

$$DEE = (Pc / Tc) X 100$$

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,

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

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

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[2,3].

$$\% \text{ of moisture loss} = \frac{\text{Initial weight-final weight}}{\text{Initial weight}} X 100$$

Table 2. Percentage Yield, Drug Content and Encapsulation Efficiency of Losartan Loaded Microspheres.

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.

Scanning electron microscopy (SEM)[5]

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.

Drug Polymer Interaction Study by FTIR[6]

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.

Table 1. Formulation Design of Lorsatan potassium microspheres.

Formulation Code	Drug (g)	Polymers (EC+AcL30D) (g)
F1	1	1
F2	1	2
F3	1	3
F4	2	1
F5	2	2
F6	2	3
F7	3	1
F8	3	2
F9	3	3

Where, EC = Ethylcellulose, Ac = Acrycoat.

High Performance Liquid Chromatography (HPLC) measurement

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.

Table 3. HPLC Chromatogram of pure losartan potassium and formulation.

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

In vitro drug release

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 hr, 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[4]. 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 Crowel[9], Koresmeyer model[10]. The criterion for selecting the most appropriate model was chosen on the basis of goodness of fit test.

Statistical analysis[11]

All the results obtained during evaluation, were verified with different statistical methods like one way ANOVA, standard deviation, standard error mean.

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
F1	0.9377	0.9387	0.8647	0.9435
F2	0.8102	0.826	0.8664	0.789
F3	0.8962	0.8068	0.8571	0.775
F4	0.8464	0.9451	0.9304	0.8255
F5	0.9257	0.9325	0.920	0.9066
F6	0.8258	0.7651	0.7657	0.8429
F7	0.9436	0.9832	0.9836	0.9321
F8	0.9453	0.9275	0.9306	0.9626
F9	0.9303	0.8773	0.8742	0.9239

RESULTS

The Losartan loaded microspheres were prepared by solvent evaporation method using different combination of primary viz. ethyl cellulose and alginate and secondary polymers viz. different grades of acryl coats as described in the Table 1. The microspheres obtained under these conditions were mostly spherical and without aggregation. The percentage yield of all the formulation was found to be satisfactory and drug entrapment efficiency (DEE) of all formulations were found to be more than 80 % as summarized in Table 2. The mean geometric particle size of microspheres was found in a range of 40 to 50 µm as represented in Table 2. The percentage of moisture loss was determined for all the formulations prepared by various methods and tabulated in Table 2. To detect the surface morphology of the microspheres, SEM of the microspheres were done as given in Fig 2. The interaction study between the drug and polymers in different formulations were performed using FTIR spectrophotometer and

HPLC. The pellets were prepared on KBr press. The spectra were recorded over the wave

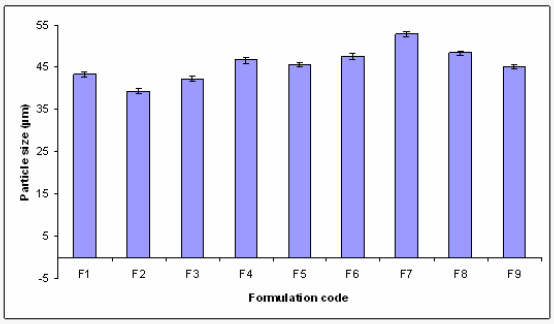

Fig 1. Mean geometric size (Diameter) of different microsphere formulations of Lorsatan Potassium.

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

number range of 3600 to 400 cm $^{-1}$. The drug shows different peaks at C-H = 3008, C=C = 1605, 1495, 1466, O-H = 3231, N=N = 1576 and Cl = 1200-1400cm $^{-1}$of benzene which confirms the purity of the drug. FT-IR spectrum of pure Losartan potassium and formulations (F3) is represented in Fig 3. HPLC data of pure Losartan potassium and formulations (F3) is represented in Table 3. *In vitro* drug release from Losartan loaded microspheres were represented in Fig 4. All the formulations found to release Losartan in a controlled manner over six hours. To describe the kinetic of drug release from microspheres, release data was

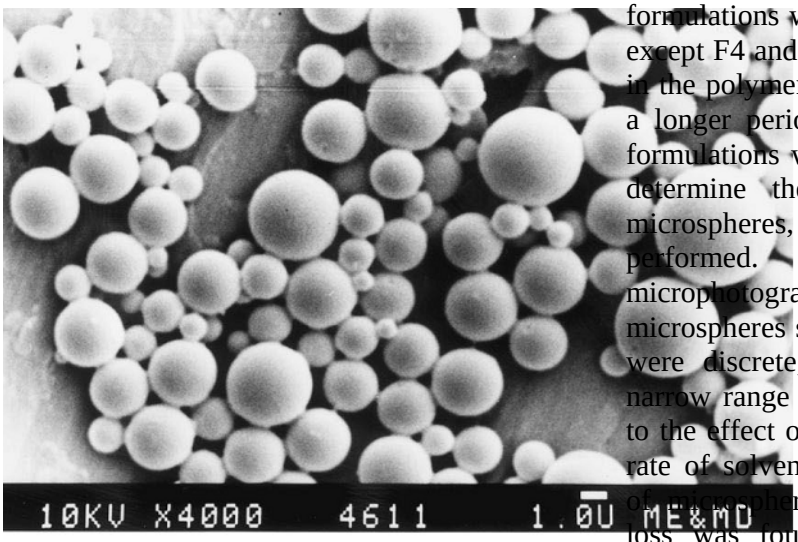

Fig 2. Scanning electron micrograph of microspheres (F3) prepared by solvent evaporation method at a resolution of 10KV × 4000.

analyzed according to different kinetic equations described in Table 4. Release data of F3, F5 and F9 obeys zero order kinetic, where as F2 and F7 following higuchi square root kinetic. Formulations F1, F6, and F8 release drug following Hixon Crowell cube root kinetic equation and the formulations F4 obeys first order kinetic. All data are verified as statistically significant by using one way ANOVA at 5 % level of significance ($p < 0.05$).

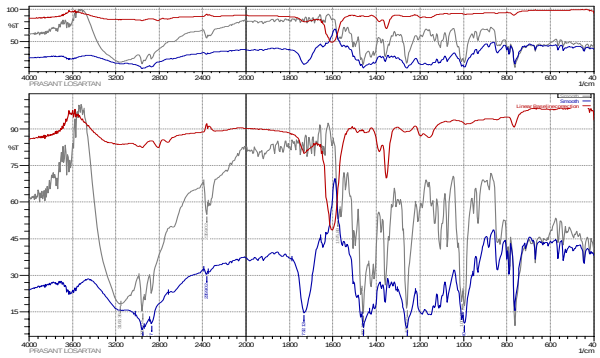

Fig 3. Drug polymer interaction study by FT-IR (Drug+EC.AcL30D+formulation, F3).

DISCUSSIONS

The percentage yield of all the formulation was found to be more than 81 %. Microsphere formulation F3 was found to have maximum yield (90.35%). 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. The particle sizes of all the formulations were found to be satisfactory. 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. 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. 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. FT-IR spectra and HPLC study showed no change in the fingerprint of pure drug spectra, thus confirming absence of drug to polymer interaction.

Formulations F2, F3 and F5 shows sustained release of drug for more than 8 hours clarified from the Fig 7. It is seen that the formulation F3, has potential to deliver Losartan potassium in a controlled manner in a regular fashion over extended period of time in comparison to all other formulations. Putting all data in different release kinetics models and comparing the coefficient of determination (r^2), it was found that F2, F3 and F7 tend to fit with Fickian diffusion model. 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.

CONCLUSION

The polymer combinations of ethyl cellulose, alginate and acrylic release retardant polymers resulted in microspheres with good yield and moderate entrapment. Results of the present study suggest that combinations of both polymers in different ratio shows sustained release microspheres. The novel formulation design facilitated the optimization and successful development of microsphere formulations for enhanced oral drug delivery. Our data concluded that Losartan microsphere protocol may be an effective strategy for the development of easy, reproducible and cost effective method to prove its potential for safe and effective oral drug delivery therapy.

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