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Research article

EFFECT OF METHOD OF PREPARATION ON PHYSICAL PROPERTIES AND *IN VITRO* DRUG RELEASE PROFILE OF LOSARTAN MICROSPHERES – A COMPARATIVE STUDY

PRASANT K ROUT*, AMITAVA GHOSH, UDAYA K NAYAK AND BHABANI S NAYAK

*E.mil: bhabani143@yahoo.co.in, Cell No: +919938860284 (M), Fax no- 06854-246955.
Department of Pharmaceutics, Faculty of Pharmacy, Jeypore
College of Pharmacy, Rondapalli, Jeypore – 764002, Koraput, Orissa, India.
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ABSTRACT

Present investigation describes preparation of microspheres by solvent evaporation and W/O emulsion solvent evaporation methods followed by *in vitro* characterization of microspheres to evaluate the effect of method of preparation on physical properties and drug release profile of microspheres. The microspheres were found to be discrete, spherical with free flowing properties. The morphology (Scanning Electron Microscopy), particle size distribution, entrapment efficiency and their release profiles were investigated. The yield was found to be maximum in case of solvent evaporation method. The mean geometric particle size of microspheres prepared by solvent evaporation method was found in the ranges of 40-50 µm and the microspheres prepared by W/O emulsion solvent evaporation method was found in a ranges of 126-150 µm, respectively. The microcspheres formulation prepared by solvent evaporation method has shown greater encapsulation efficiency than W/O emulsion solvent evaporation method. The drug carrier interactions were investigated in solid state by Fourier Transform Infrared (FT-IR) spectroscopy study. *In vitro* drug release rate for microspheres was found to be sustained over 8 hours. Hence, it can be concluded that the formulation prepared by solvent evaporation method, 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 and can be adopted for a successful oral delivery of Losartan potassium for safe management of hypertension. All data are verified as statistically significant by using one way ANOVA at 5 % level of significance ($p < 0.05$).

Keywords : Losartan, Microspheres, *In vitro* drug release, SEM

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 and to study the effect of method of preparation on physical properties and drug release profile of Lorsatan potassium microspeheres.

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 and Acycoat E30D were purchased from Corel Pharma Ahmadabad (India). All chemicals were of analytical grade and were used without further purification.

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 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.

W/O emulsion solvent evaporation method [2]

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 beaker. Constant stirring at 2000 rpm was carried out using mechanical stirrer and its content was heated by a hot plate at 80ºC. Stirring and heating were maintained for 2.5 h until the aqueous phase was completely removed by evaporation. 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 50ºC for 2 h and stored in a desiccator at room temperature.

Evaluations

Percentage yield (% yield)[3, 4]

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[3, 4]

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[3,4]

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

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[3,4,5]

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

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

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[3, 4]

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. When no further change in weight of sample was observed, the final weight was noted down.

% of moisture loss =

$$\frac{\text{initial weight - final weight}}{\text{Initial weight}} \times 100$$

Scanning electron microscopy (SEM)[2]

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 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.

***In vitro* drug release**[5]

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 the study. 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[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.

RESULTS

The Losartan loaded microspheres were prepared by two selected methods 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.

Table 1 : Formulation design of Lorsatan potassium microspheres.

Solvent evaporation method			W/O Emulsion solvent evaporation method		
Formulation code	**Drug (g)**	**Polymers(EC+ AcL30D) (g)**	**Formulationc code**	**Drug X_1 (g)**	**Polymers (AL+AcE30D) (g)**
F1	1	1	F10	1	1
F2	1	2	F11	1	2
F3	1	3	F12	1	3
F4	2	1	F13	2	1
F5	2	2	F14	2	2
F6	2	3	F15	2	3
F7	3	1	F16	3	1
F8	3	2	F17	3	2
F9	3	3	F18	3	3

Where, EC = Ethylcellulose, AL = Sodium alginate, Ac = Acrycoat.

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 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	82.04±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
F10	69.76±0.812	55.26±0.764	77.10±1.62	126.23±0.857	4.91±0.341
F11	84.85±0.716	33.45±0.682	85.15±1.16	145.52±1.451	3.41±0.368
F12	88.38±0.464	22.98±0.531	81.25±1.96	141.67±1.532	3.85±0.623
F13	81.66±0.368	54.23±0.735	66.43±1.54	138.73±0.949	3.57±0.425
F14	89.13±0.575	43.12±0.548	74.87±1.33	144.54±0.868	3.88±0.235
F15	90.13±0.147	37.83±0.674	80.19±1.12	156.37±1.241	3.94±0.232
F16	84.93±0.378	58.97±0.721	66.78±1.48	124.62±1.123	3.89±0.298
F17	88.31±0.567	52.42±0.754	77.17±1.86	132.18±0.923	4.16±0.264
F18	88.43±0.182	42.63±0.589	75.41±1.65	136.26±0.982	5.06±0.354

All values are represented as mean ± standard deviation (n=3). Standard error mean < 1.131

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups and column	12366.48	35	9425.497	1.0896	0.04112	4.1300

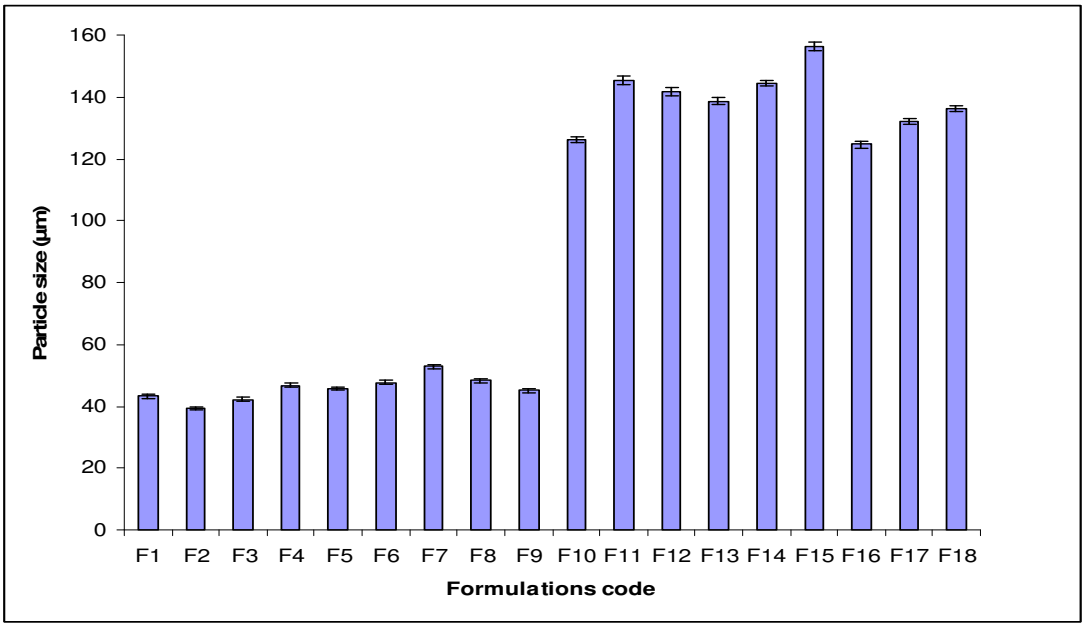
The mean geometric particle size of microspheres prepared by solvent evaporation method was found in a range of 40 to 50µm and the microspheres prepared by W/O emulsion solvent evaporation method was found in a range of 126.23 to 150µm represented in Table 2. The percentage of moisture loss was determined 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. Scanning electron microphotographs of microspheres prepared by solvent evaporation and W/O emulsion solvent methods is represented in Fig 7 and 8. The interaction study between the drug and polymers in different formulations were performed using FTIR spectrophotometer.

Table 3 : *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
F10	0.9783	0.9782	0.9715	0.9733
F11	0.9225	0.8249	0.9605	0.9239
F12	0.9425	0.9663	0.9796	0.9232
F13	0.9198	0.9727	0.8852	0.9759
F14	0.9048	0.9185	0.848	0.9059
F15	0.9821	0.9502	0.9424	0.9843
F16	0.9355	0.9674	0.9706	0.9186
F17	0.8929	0.8053	0.8198	0.896
F18	0.9125	0.9005	0.9166	0.9013

The pellets were prepared on KBr press. The spectra were recorded over the wave 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 = 1200-1400$cm^{-1}$of benzene which confirms the purity of the drug. FT-IR spectrum of pure Losartan potassium and formulations (F3) is represented in Fig 4 and 5. *In vitro* drug release from Losartan loaded microspheres prepared by solvent evaporation method and W/O emulsion solvent evaporation methods were represented in Fig 6 and 7 respectively. 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 analyzed according to different kinetic equations described in Table 3. Release data of F3, F5 and F9 and F10 obeys zero order kinetic, where as F2, F7, F11, F12, F16 and F18 following Higuchi square root kinetic. Formulations F1, F6, F8, F13, F15 and F17 release drug following Hixon Crowell cube root kinetic

equation and the formulations F4 and F14 obeys first order kinetic. All data are verified as statistically significant by using one way ANOVA at 5 % level of significance ($p < 0.05$).



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

Fig. 1 : mean geometric size (diameter) of different microsphere formulations of Lorsatan potassium.

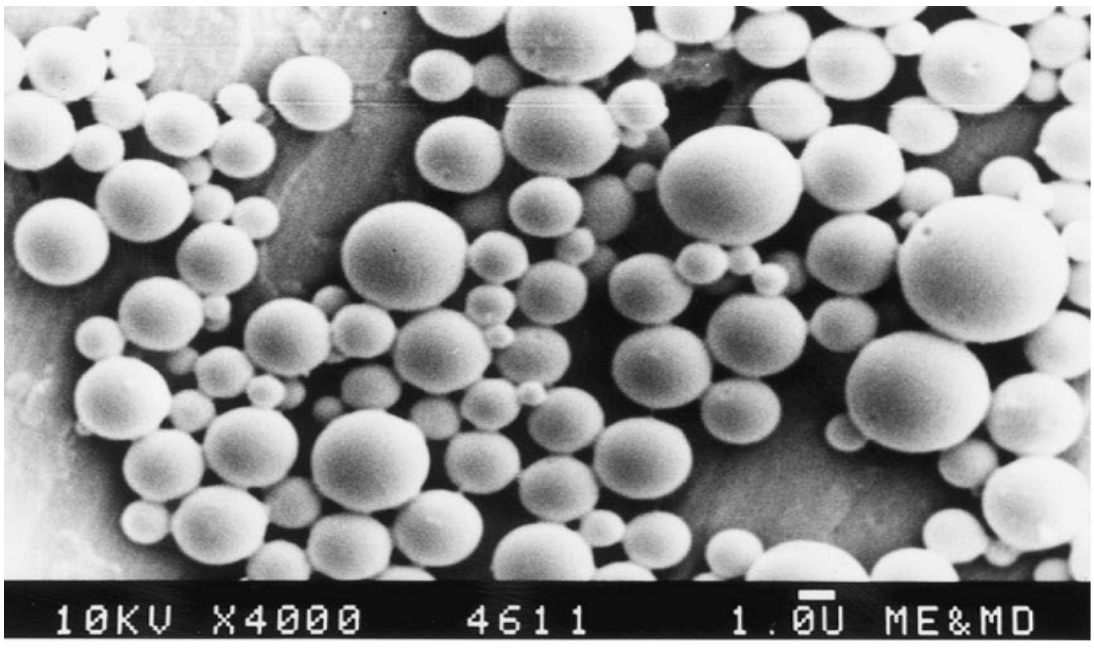


Fig. 2 : Scanning electron micrograph of microspheres (f3) prepared by solvent evaporation method at a resolution of 10kv × 4000.

DISCUSSIONS

The percentage yield of all the formulation was found to be more than 81%. Microsphere formulation F3 prepared by solvent evaporation method 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, F7 , F13 and F16 as the drug is fully dispersed in the polymer phase by continuous stirring for a longer period. Most of the microcspheres formulation prepared by solvent evaporation method showing greater encapsulation efficiency than W/O emulsion solvent evaporation methods. 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. Microcspheres prepared by solvent evaporation method showing lesser size than W/O emulsion solvent evaporation methods. 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.

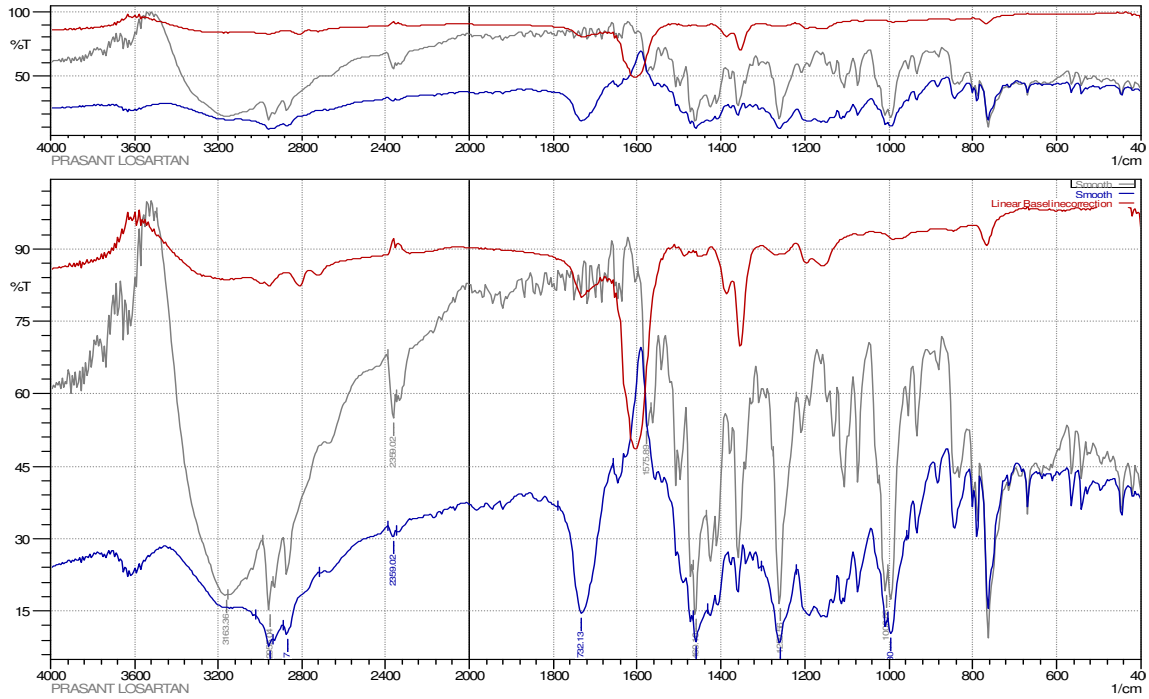


Fig. 3 : Drug polymer interaction study by FTIR (drug + ec.acl30d + formulation).

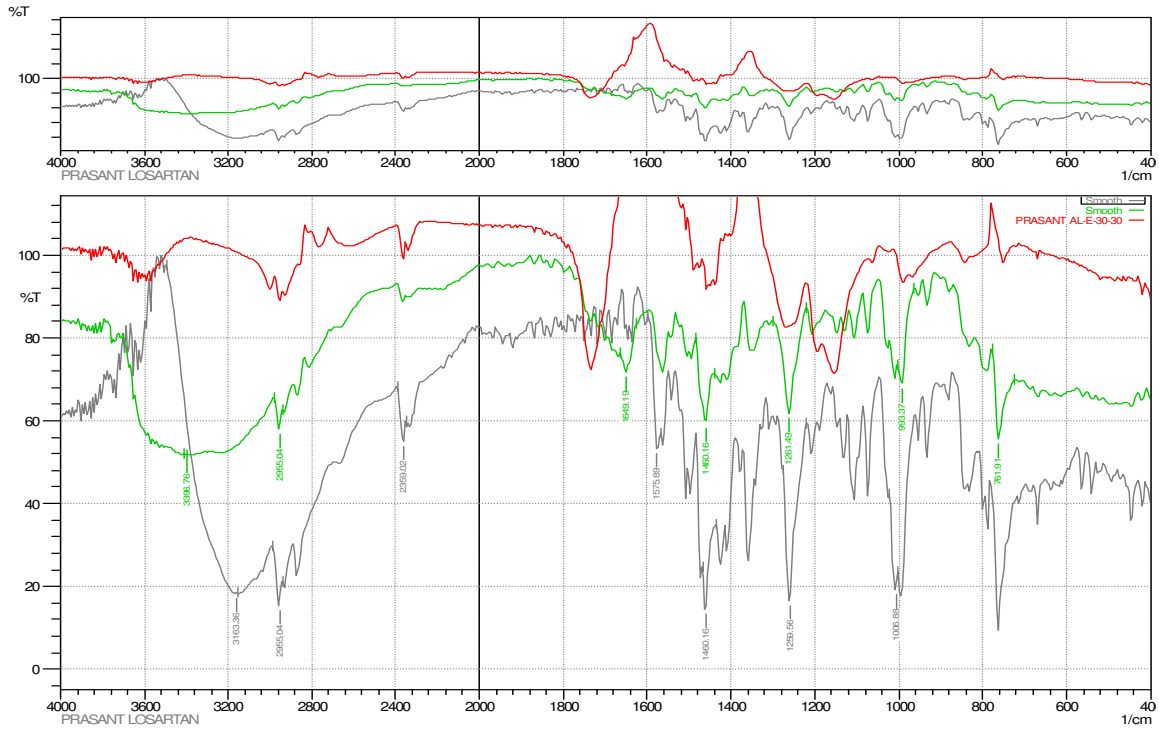


Fig. 4 : Drug polymer interaction study by FTIR (drug + al.ace30d + formulation).

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 study showed no change in the fingerprint of pure drug spectra, thus confirming absence of drug and polymer interaction. Formulations F2, F3, F5, F11, F12 and F14 shows sustained release of drug for more than 8 hours clarified from the Fig 5 and 6. It is seen that the formulation F3 prepared by solvent evaporation method, 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 datas in different release kinetics models and comparing the coefficient of determination (r^2), it was found that F2, F3, F7, F11, F12, F16 and F18 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.

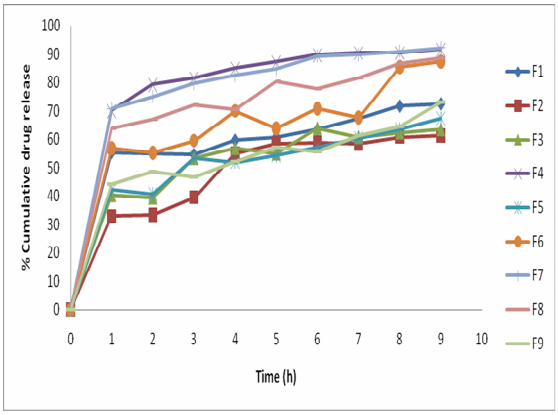


Fig. 5 : in vitro drug release profile of Lorsatan microspheres prepared by solvent evaporation method.

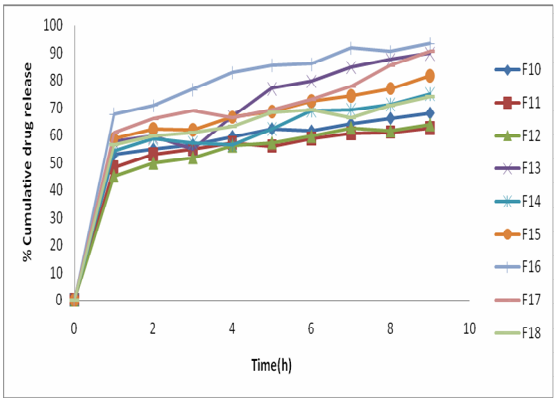


Fig. 6 : *In vitro* drug release profile of Lorsatan microspheres prepared by w/o emulsion solvent evaporation method.

CONCLUSION

The polymer combinations of ethyl cellulose, alginate and acrylic release retardant polymers resulted in microspheres with good yield and entrapment. Results of the present study suggest that combinations of both polymers in different ratio shows sustained release microspheres. From the above study it could be concluded that method of preparation have great effect on better physical properties and drug release profile of microspheres. So selection of appropriate method for preparation of microspheres must be taken in to consideration for designing the best microsphere formulation.

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