Innovare Academic Sciences

International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 6, Issue 7, 2014

Original Article

PREPARATION AND EVALUATION OF ETHYLCELLULOSE MICROSPHERES PREPARED BY SOLVENT EVAPORATION TECHNIQUE

HUSSAIN MOHAMMED ASIF[1*], RENUKUNTLA ARUN KUMAR [1], T. RAMA RAO[2], MAIMUNA ANJUM[1]

[1]Blue Birds College of Pharmacy, Bheemaram, Hanamkonda-506015, Andhra Pradesh, India, [2]Avanthi Institute of Pharmaceutical Sciences, Gunthapally (V), Hayathnagar- 501512, Ranga Reddy Dist, Andhra Pradesh, India.
Email: asifhussainp@yahoo.com

Received: 19 Feb 2014 Revised and Accepted: 26 Mar 2014

ABSTRACT

Losartan is high specific angiotensin 2 type receptor antagonist with antihypertensive activity was formulated as microspheres by using Ethyl cellulose as carrier. These Ethyl cellulose microspheres were prepared by the solvent evaporation method. The prepared microspheres were subjected to various evaluation and *in vitro* release studies. Highest percentage of loading was obtained by increasing the amount of Losartan with respect to polymer. The particle sizes of the prepared microspheres were determined by optical microscopy and SEM analysis. The prepared microspheres had good spherical geometry with smooth surface as evidence by SEM. The *in vitro* release studies showed that Losartan microspheres of 1:5 ratios showed better sustained effect over a period of 12 hours.

Keywords: Ethylcellulose microspheres, Solvent evaporation, Losartan.

INTRODUCTION

Microspheres are carriers for Control Release and can be defined as solid spherical monolithic free flowing particles having size range between 1-1000 micrometres; typical size is 1-500 micrometres. Microspheres are used to sustain the release of drug and for localized effect. They also give protection to the drug from chemical and enzymatic degradation. In addition they reduce toxicity and improves therapeutic efficacy of drug and for localized effect.

Losartan potassium is a highly specific angiotensin 2 type receptor antagonist with antihypertensive activity. The drug is readily absorbed from the GI tract, following oral administration. It has significant first pass metabolism hence its bio availability is 25-35.

Since the drug has low elimination half life (i.e. 1.5-2 hrs), it is suitable candidate for oral controlled release. Administration of Losartan in a controlled release dosage form with an extended release over 8 h, would be more desirable as these characteristics would allow a rapid onset followed by protracted anti-hypertensive effects by maintaining the plasma concentration of the drug well above the therapeutic concentration. The daily dose of Losartan is 25- 100 mg. However, it still has significant side-effects like dizziness, constipation, fatigue and headache; these side effects can be minimized by fabricating a sustain release dosage form. Losartan has short biological half-life (1.5 to 2 hours) and it is rapidly eliminated from the body[1,2]. Hence there is a need to develop a system which provides control release of drug. The objective of present investigation is to prepare Losartan microspheres by using solvent evaporation method Microspheres were prepared by using Ethyl cellulose in various drug and polymer ratios (1:2, 1:3, 1:4). Various physicochemical characteristics and *in vitro* release rates from these microspheres were then examined.

MATERIALS AND METHODS

Losartan Potassium was received as a gift sample from Aurabindo Pharma Pvt.Ltd, Hyderabad. Ethyl cellulose was purchased from Loba chemicals Pvt. Ltd. Liquid paraffin and Span 80 were purchased from Finar chemical, Ahmadabad and all other ingredients used were of analytical standard.

Preparation of Losartan Potassium microspheres by using solvent evaporation method

The drug and polymer in different proportions (1:2, 1:3, 1:4, 1:5 and 1:6) were weighed & dissolved at room temperature into Methanol with vigorous agitation to form uniform drug polymer dispersion. This was slowly poured into the dispersion medium consisting of light liquid paraffin (200 ml) containing 0.1% Span 80.

The system was stirred using 3 blade homogenizer at 500 rpm, at room temperature over a period of 2-3 hours, to ensure complete evaporation of the solvent. The liquid paraffin was then decanted & the microspheres were separated by filtration through a Whatmann filter paper, washed thrice with 180 ml of n- hexane and air dried for 24 hours[3,4]. Five batches of microspheres were prepared & labelled as F1, F2, F3, F4 and F5.

Table 1: Drug and polymer ratio of losartan potassium loaded microspheres of all formulations

Formulation code	Drug &polymer Ratio	Amount of Drug taken (mg)	Amount of Polymer taken (mg)
F1	1:2	100	200
F2	1:3	100	300
F3	1:4	100	400
F4	1:5	100	500
F5	1:6	100	600

Evaluation of microspheres

Particle size measurement

The microspheres size was determined by the optical microscopy method using a calibrated stage micrometer (µm) and the size of particle was calculated by using equation[5].

$$D_{mean} = \frac{\sum nd}{\sum n} \quad \text{..... (Eqn 1)}$$

Percentage yield

Percentage practical yield is calculated to understand the efficiency of any method, as it helps in selection of appropriate method of production. Practical yield was calculated as the weight of LP microspheres obtained from each batch in relation to the amount of drug and polymer used in the preparation [6].

Percentage yield was calculated using the formula:

$$\% \text{ Yield} = \frac{\text{Weight of microspheres obtained after solvent evaporation}}{\text{Amount of drug and polymer used}} \times 100 \quad \text{...(Eq. 2)}$$

Encapsulation efficiency

Drug loaded microspheres (100 mg) were powdered and suspended in 100 ml methanolic: water (5:95 v/v) solvent. The resultant dispersion was kept for 20 min for complete mixing with continuous agitation using magnetic stirrer and filtered using a wattmann filter. The drug content was determined spectrophotometrically at 234 nm using a regression equation derived from the standard graph (r^2=0.998) [7].

$$\text{Encapsulation Efficiency} = \frac{\text{Practical Drug content}}{\text{Theoretical Drug content}} \times 10 \quad \text{...(Eq. 3)}$$

Effect of drug-polymer ratio on particle size, %yield and %EEof Losartan Potassium Loaded Microspheres

For this study five different batches were prepared. The particle size was measured and %yield and %EE were studied by increasing the polymer. Depending on parameters particle size and drug release the optimized ratio (formulation) was selected.

I. R. Spectroscopy

IR spectroscopy was carried out to check the compatibility between drug and polymers. The IR spectra of drug with polymer were compared with the standard I.R. spectrum of the pure drug [8].

Surface Morphology (SEM)

Scanning electron microscopy was used to determine particle size distribution, surface topography, texture, and to examine the morphology of fractured or sectioned surface. SEM is the most commonly used method for characterizing drug delivery systems, owing in large to simplicity of sample preparation and ease of operation.

SEM studies were carried out by using JEOL JSM T-330A scanning microscope (Japan). Dry LP microspheres were placed on an electron microscope brass stub and coated with in an ion sputter. Picture of LP microspheres were taken by random scanning of the stub.

***In vitro* drug release studies**

The drug dissolution tests of microspheres were carried out in pH 6.8 Phosphate buffer 900ml in the rotating basket method specified in the US Pharmacopoeia. Microspheres were weighed (weight equivalent to 50 mg of drug) and filled in capsules then these microspheres were placed in the basket of dissolution medium with rotation speed of 100 rpm and thermostatically controlled at 37°C. Perfect sink conditions were maintained during the dissolution tests. The sample solution was withdrawn at a suitable interval from the dissolution vessel and analysed spetrophotometrically at 234 nm[9].

RESULTS AND DISCUSSIONS

Particle size measurement

The particle size analysis was carried out using optical microscope for all the five formulations. F1 showed the smallest particle size and highest Particle size was observed in F5 formulation. The average particle size was observed between 18 to 86 µm. (Table 2)

Table 2: Particle Size Measurement of Losartan Potassium Loaded Microspheres of all the formulations.

S. No.	Formulation code	Range of particle size(µm)	Average particle size(µm)	SD
1	F1	14.23 - 24.8	18.04	±2.43
2	F2	31.43 - 34.65	32.33	±3.03
3	F3	53.24 - 58.67	56.45	±1.82
4	F4	63.18 - 69.91	68.15	±1.60
5	F5	84.24 - 88.42	86. 83	±3.61

Percentage Yield

The percentage yield of microspheres of Losartan Potassium using Ethyl cellulose ranged from 89% to 96%.

Encapsulation Efficiency

The amount of drug encapsulated in the microspheres gives an estimate of encapsulation efficiency which is an important parameter in the selection of microspheres for further characterization. Three batches for each formulation were prepared, among the different formulations, one best formulation was selected on the basis of *in vitro* drug release and the concentration of Span80 of this formulation was further changed to see the effect on encapsulation. Maximum encapsulation of 91% was observed in F5, followed by 88% in F4, 85%in F3, 78% in F2 and 73%in F1. The encapsulation efficiency of the five formulations is in the following order F5 > F4 > F3 > F2 > F1. Hence it was observed that as the weight of the polymer increased, there was an increase in the encapsulation efficiency.

Effect of drug-polymer ratio on particle size, % yield and %EE

Effect of drug-polymer ratio on particle size, % yield and % EE was studied. As the polymer ratio increased the particle size, % yield and % EE also increased. Results were shown in the (Table 3).

Table 3: Effect of drug-polymer ratio on particle size, %yield, and %EE of Losartan Potassium Loaded Microspheres of all the formulations.

S. No.	Drug:polymer	LLP (ml)	Speed (rpm)	Yield (%)	EE (%)
1	1:2	200	500	89.99±1.37	73.15±1.28
2	1:3	200	500	90.25±1.80	78.54±0.59
3	1:4	200	500	94.50±2.18	85.12±1.31
4	1:5	200	500	95.39±2.30	88.31±0.78
5	1:6	200	500	96.33±0.69	91.32±2.92

I.R. Spectroscopy

FT-IR spectra of pure Losartan Potassium, Ethyl Cellulose were recorded on Perkin Elmer spectrophotometer. The scans were evaluated for presence of principal peaks of drug, shifting and masking of drug peaks due to presence of polymer. No significant change in peaks was observed hence there are no drug and polymer interactions.

Surface morphology of Losartan potassium microspheres (SEM)

The surface morphology of the LP microspheres was studied by SEM. SEM photograph of the Formulation F5 was shown in the Fig1. Surface smoothness of the LP microspheres was increased with the increase in the polymer concentration, which was confirmed by SEM. At lower polymer conc. (1:2) surface was rough and crumpled and at higher polymer conc. (1:6) the LP microspheres with smooth surface were obtained

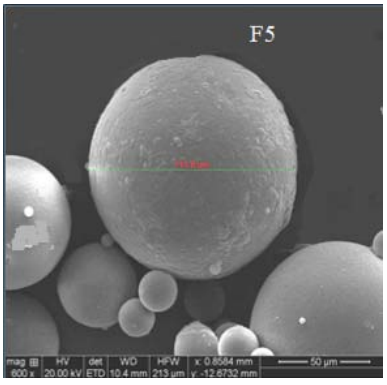

Fig.1: SEM photograph of formulation F5

In Vitro Drug Release Studies

The *in vitro* performance of LP microspheres showed prolonged and sustained release of LP. The results of the *in vitro* dissolution studies of formulations F1 to F5 are shown in Table 5.

The study indicated that the amount of drug release decreases with an increase in the polymer concentration. The formulations F1 showed a maximum of 98.84% and F5 showed a maximum of 64.98% cumulative drug release.

Table 4: *In Vitro* Drug Release Studies of all the formulations.

Time(hrs)	F1	F2	F3	F4	F5
1	36.41±0.89	24.32±3.09	14.6±0.9	9.64±0.76	7.44±.045
2	45.29±1.54	31.1±2.09	22.28±1.11	21.22±1.86	14.84±1.97
3	59.23±2.09	39.06±.045	28.24±1.54	23.71±3.09	17.49±0.98
4	67.38±1.97	45.06±0.78	32.52±.0.93	30.04±0.88	23.49±1.48
6	73.07±1.32	57.82±0.98	38.48±1.34	56.12±1.97	29.52±2.13
8	86.61±2.45	73.76±3.54	60.92±3.31	63.71±2.65	52.82±1.09
10	93.53±3.08	84.92±0.59	72±0.09	70.43±3.07	59.22±3.09
12	98.84±3.67	93.68±2.99	78.92±3.09	72.56±2.09	64.98±1.29

Release kinetics

Dissolution data of the all formulations were fitted in to different mathematic models (Zero order, First order, Higuchi and Peppas) in order to predict the kinetics of drug release rate.

The model with high regression coefficient value (R^2) was chosen as the most appropriate model.

By comparing the coefficient of determination (r2), it was found that formulations F1 and F4 followed first order kinetics where F2, F3 and F5 followed Zero Order kinetics. Except F1 all the other formulations followed Non - Fickian transport. This can be attributed to the facts that as the polymer ratio increased resulted in the increase in diffusion path and Sigmoidal Pattern (Where drug is more distributed towards the core of the matrix) was another factor which resulted in Zero order release.

Table 5: Kinetic Models applied and the R^2 values for different microsphere formulations

Formulation	Zero order R^2	First order R^2	Higuchi R^2	Peppas R^2	n values
F1	0.8456	0.9231	0.9854	0.9888	0.4118
F2	0.9629	0.0.9474	0.9846	0.9841	0.5658
F3	0.9772	0.0.9664	0.9431	0.9743	0.6874
F4	0.9438	0.973	0.9406	0.9726	0.8379
F5	0.9779	0.9695	0.908	0.9803	0.8843

CONCLUSION

The concept of formulating microspheres containing Losartan Potassium offers a suitable, practical approach to achieve a prolonged therapeutic effect by continuously releasing the medicament over extended period of time. Losartan Potassium microspheres were successfully prepared by solvent evaporation technique using the different concentration of polymer and drug ratios. Among the various methods reported for the preparation of microspheres, this method was chosen as it is a simple and novel method.

CONFLICT OF INTERESTS

Declared None

REFERENCES

1. K., S., By W, O. Prasant Bhabani "Statistical Evaluation Of Losartan Microspheres Prepared Method Using Factorial Design And Response Surface Methodology".Asian J of Pharmand Clinical Res 2009;4(2).
2. Anil G. Prasanthv.v Sapna Desai, "Mucoadhesive Microspheres Of Midazolam:Nose To Brain Delivery". J Res J of Pharm Biological and Chemical Sciences 2011;2(4):382.
3. Sam M, T. Akash Chakraborthy "Microspheres-An Overview" Int J of Res in Pharm and Biomedical Sci 2011;2(2).
4. Majeti NV, Kumar R, J. Nano and microparticles as controlled drug delivery devices. J Pharmaceut Sci 2000;3(2):234-58.
5. T.Sudhamani, K. Noveenkumarreddy, V.R. Ravi Kumar, R.Revathi, V. Ganesan "*Preparation And Evaluation Of Ethyl Cellulose Microspheres Of Ibuprofen For Sustained Drug Delivery".* IJPRD 2010.
6. Sreeramulu J. Aswartha umakanthareddy M, Satyanarayana Punna " Formulation Development of Losartan Potassium Microspheres Using Natural Polysaccharides and Their *In-Vitro* Evaluation". Res J of Pharm Biological and Chemical Sci 2012;734.
7. Khan GM, Lee TW, Robinson JR, Gennaro AR. Controlled release oral dosage forms:Some recent advances in matrix type drug delivery systems2000. 350-4 p.
8. Patrick B, James W. O'Donnell, McGinity "Preparation of microspheres by the solvent evaporation technique" Advanced Drug Delivery Reviews,199. Indian J Pathol Microbiol 28:25-42.
9. Gowda DV, Shivakumar HG. Encapsulation of griseofulvin in wax/fat Microspheres:preparation, characterization and release kinetics of microspheres. J Indian drugs 2005;42(7):453-60.