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Research Article

PREPARATION AND IN VITRO EVALUATION OF CHITOSAN MICROSPHERES OF EPLERENONE

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ABSTRACT

Objective: To develop and evaluate Eplerenone (EP) microspheres using natural polymer like Chitosan. Eplerenone is a suitable candidate for sustained-release (SR) administration as a result of its dosage regimen (twice or thrice a day), with rapid elimination and unavailability in the market as SR dosage form necessitate the use of Eplerenone as sustained release formulation for prolonged action and to improve patient compliance.

Methods: Microspheres of EP were prepared by emulsification techniques by using Chitosan as a polymer. Various evaluation parameters were assessed, with a view to obtain oral controlled release of EP. The prepared EP microspheres were then subjected to FTIR, SEM, particle size, % yield, entrapment efficiency, *in vitro* dissolution studies and release kinetics.

Results: The microspheres were evaluated for physical characteristics such as particle size, particle shape and surface morphology by scanning electron microscopy, drug entrapment efficiency, *in vitro* drug release characteristics. The drug release was for more than 10 h. The microspheres had a mean particle size of 100-370 µm. Electron microscopy revealed that microspheres were spherical with nearly smooth surface morphology. Application of *in vitro* drug release data to various kinetic equations indicated that the drug release from chitosan microspheres was diffusion Controlled.

Conclusion: The present study conclusively demonstrates the feasibility of effectively encapsulating EP into Chitosan microspheres to form potential Sustained release drug delivery system.

Keywords: Eplerenone, Microspheres, Chitosan, emulsification, Cross linking.

INTRODUCTION

Eplerenone (EP), an aldosterone receptor antagonist is used in treatment of hypertension and heart failure. Eplerenone is a suitable candidate for sustained-release (SR) administration as a result of its dosage regimen (twice or thrice a day) [1]. Good absorption in the entire GIT, relatively short plasma half-life of approximately 4 h [2] with rapid elimination and unavailability in the market as SR dosage form necessitate the use of Eplerenone as sustained release formulation for prolonged action and to improve patient compliance [3,4]. The purpose of this study is to formulate and evaluate chitosan microspheres and also to study the influence of the process variables in the preparation of microspheres [5].

MATERIALS AND METHODS

Materials

EP was a gift sample from Maithri Laboratories Pvt. Ltd, Hyderabad. Chitosan was a gift sample from Central Institute of Fisheries and Technology, Cochin, India. Glutaraldehyde was obtained from Agro-Vel (India) Pvt. Ltd, Secunderabad. Liquid paraffin was procured from Parth Pharma chemicals, Kolkata. All other chemicals used were of pharmaceutical grade.

Preparation of microspheres using Glutaraldehyde cross linking [6,7]

Chitosan (400 mg) was dissolved in 2% (V/V) aqueous acetic acid and the drug (100 mg) was dispersed in it. This dispersed phase was added to continuous phase of light liquid paraffin containing 0.5 % (w/v) span 80 to form w/o emulsion with stirring on Remi three-blade stirrer at high speed (2500 rpm). After 30 min of stirring, 25% aq. glutaraldehyde solution (0.8 ml) was added drop by drop at 30 and 60 min. Stirring was continued for 2.5 h to obtain microspheres. Microspheres obtained were filtered by vacuum and washed several times with petroleum ether to remove oil, and finally washed with distilled water to remove adhering liquid paraffin and glutaraldehyde respectively. The microspheres were then dried at room temperature and stored in desiccators [8].

Effect of process variables on microsphere properties

Chitosan microspheres were prepared at different stirring rates, volume of dispersion medium and volume of cross linking agent with various drug: polymer ratios as stated in Table1.

Table 1: Process variables

Process variable	Different Values
Stirring rates (rpm)	500, 1500, 2000
Cross linking agent amount (ml)	0.5, 0.8
Drug: polymer ratios	1:1, 1:2, 1:3, 1:4, 1:5

Evaluation of Microspheres [9,10]

Particle size

Determination of average particle size of EP microspheres was carried out by optical microscopy. A small quantity of EP microspheres was spread on a clean glass slide and average of 200 EP microspheres was determined in each case.

Scanning electron microscopy (SEM) of microspheres

Scanning electron microscopy (SEM) has been used to determine particle size distribution, surface morphology, texture and to examine the morphology of fractured or sectioned surface. SEM studies were carried out by using JEOL JSM T-330A scanning microscope (Japan).

Percentage yield

Percentage yield helps in selection of appropriate method of production. Practical yield was calculated as the weight of EP microspheres recovered from each batch in relation to the starting material.

The percentage yield of prepared EP microspheres was determined by using the formula

$$\text{Percentage yield} = \frac{practical\ yield}{Theoretical\ yield} \times 100$$

Percentage drug entrapment efficiency [11,12]

Weighed amount of EP microspheres was dissolved in distilled water. This solution was kept overnight for the complete dissolution of the EP in water. This solution was filtered and further dilutions are made. The absorbance of the solutions was measured at 242 nm using double beam UV-Visible spectrophotometer against distilled water as blank and calculated for the percentage of drug present in the sample. Efficiency of drug entrapment for each batch was calculated as per the following formula

$$\text{Drug entrapment efficiency} = \frac{practical\ \ drug\ content}{Theoretical\ \ drug\ \ content} \times 100$$

Fourier Transform Infrared Spectroscopy (FTIR)

Drug-polymer interactions were studied by FTIR spectroscopy. The spectra were recorded for pure drug, physical mixture of drug and polymer and drug-loaded microspheres using FTIR (Model No. IR Presige-21, Shimadzu). Samples were prepared in KBr disks (2 mg sample in 200 mg KBr). The scanning range was 400-4000 cm^{-1}.

***In vitro* drug release study**

The *in vitro* drug release study was carried out in Franz diffusion cell by placing known amount of microspheres on previously soaked gelatin paper. The study was done in 0.1 N HCl medium for 2 h and later with 6.8 pH phosphate buffer up to 12 h. 5 ml of sample was withdrawn at predetermined time intervals and samples were analyzed and the amount of EP released was determined by UV absorption spectroscopy at 242 nm.

Kinetic drug release pattern from microspheres [13]

The cumulative release data were fitted to models representing zero order (Q v/s t), first order [Log (Q_0-Q) v/s t], Higuchi's square root of time (Q v/s √t) and Korsemeyer Peppas double log plot (log Q v/s log t) respectively, where Q is the cumulative percentage of drug released at time t and (Q_0-Q) is the cumulative percentage of drug remaining after time t. In order to define a model which will represent a better fit for the formulation, drug release data was further analyzed by Peppas equation, where fraction of drug release at time t, kinetic constant, diffusion exponent and R^2 values were calculated for the linear curves obtained by regression analysis of the above plots.

RESULTS AND DISCUSSION

From the spectra of EP, physical mixture of EP and polymer and EP microsphere, it was observed that all characteristic peaks of EP were present in the combination spectrum, thus indicating compatibility of the EP and polymer. IR Spectra shown in Fig.1-2.

After optimizing the speed and volume of dispersion medium, the microspheres of Eplerenone were prepared by the emulsification cross linking method using glutaraldehyde as cross linking agent. The microspheres obtained under these conditions were found to be spherical and median size ranges from 100-370 µm. Particle size, percentage yield and drug entrapment efficiency of different batches of microspheres prepared were tabulated in Table2.

Entrapment efficiency increase with increase in the Glutaraldehyde concentration. From the results it can be inferred that there is a proper distribution of EP in the microspheres and the deviation is within the acceptable limits.

The percentage entrapment efficiency was found to be 33.12% to 76.73%. The results obtained are given in Table.2. A maximum of 76.73% drug entrapment efficiency was obtained in the N4 formulation. It is observed that the drug entrapment was proportional to drug: polymer ratio and Glutaraldehyde concentration.

The *in vitro* performance of EP microspheres showed prolonged and controlled release of EP. The results of the *in vitro* dissolution studies of formulations F1 to F5 and N1 to N5 are shown in Table.3-4. F1 formulation showed 94.92 % cumulative drug release at the end of 12 h while F5 formulation showed 78.67 % cumulative drug release at the end of 12 h. This is because with increase in polymer concentration the free drug concentration on the surface of microspheres will be decreased and more drug get entrapped in the microspheres and drug gets released slowly.

The cumulative % drug release from N1 formulation is 70.80% at the end of 12 h while cumulative % drug release from N5 formulation is 63.87% at the end of 12 h. The drug release from N formulations is lesser than F formulations. This is because with increase in Cross linking agent concentration more drug gets entrapped in the microspheres and the microspheres formed are more intact when compared to F formulations and drug release slowly. More sustained release in N formulations than F formulations.

Table 2: Composition and Physical characteristics of Microspheres

Formulation	Drug: polymer	Cross linking agent, ml	Particle size(µm)	% yield	Entrapment efficiency
F1	1:1	0.5	100±6.73	56	33.12
F2	1:2	0.5	121±5.42	68	46.54
F3	1:3	0.5	214±7.25	78	59.64
F4	1:4	0.5	313±4.18	84	67.8
F5	1:5	0.5	360±6.28	86	52.90
N1	1:1	0.8	105±5.24	62	38.23
N2	1:2	0.8	118±6.05	71	54.48
N3	1:3	0.8	220±5.97	79	68.89
N4	1:4	0.8	314±7.65	84	76.73
N5	1:5	0.8	370±4.31	90	65.28

It is observed that increasing the polymer ratio the mean particle size was increased. When the drug polymer ratio was increased from 1:1 to 1:5, larger particles were formed, because the viscosity of the emulsion medium was increased with increasing amount of polymer. Due to increase in viscosity, larger emulsion droplets were formed which were difficult to break and hence they are precipitated as such leading to increase in mean particle size. As the polymer concentration increases the percentage yield and entrapment efficiency also increases. The volume of Light liquid paraffin was selected (100, 200 ml) based on the characteristics of microspheres, with less volume irregular microspheres were obtained. As the volume of external phase was increased to 300 ml spherical particles were obtained. Similarly at different speed of 1000 rpm and 1500 rpm irregular particles was obtained due to less shearing force and at the speed of 2000 rpm spherical particles were obtained because of high shearing force. SEM of microspheres at magnification 75X, 100X were presented in the Fig.3, which shows that the microspheres were almost spherical in nature with smooth surface morphology.

The in vitro drug release profiles and kinetic data for all batches are shown in Table 3-5. The mechanism of release was determined by finding the R^2 value for each kinetic model viz. Zero-order, First-order, Higuchi, and Korsmeyer-Peppas corresponding to the release data of formulations. From the Table 5 it was cleared that all the formulations have shown zero order kinetics, from the 'n' values of Korsemeyer-Peppas model it was found to be that the EP microspheres have indicated diffusion Controlled release and as the n value is above 0.5, it indicates Non- Fickian of drug release through EP microspheres.

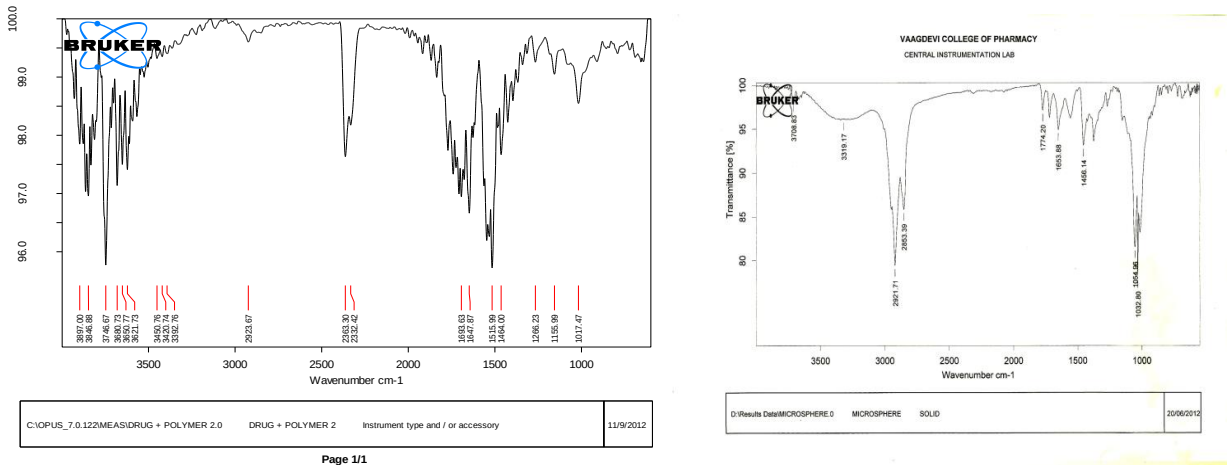

Fig. 1: IR Spectra of drug and polymer Fig.2: IR spectra of microspheres

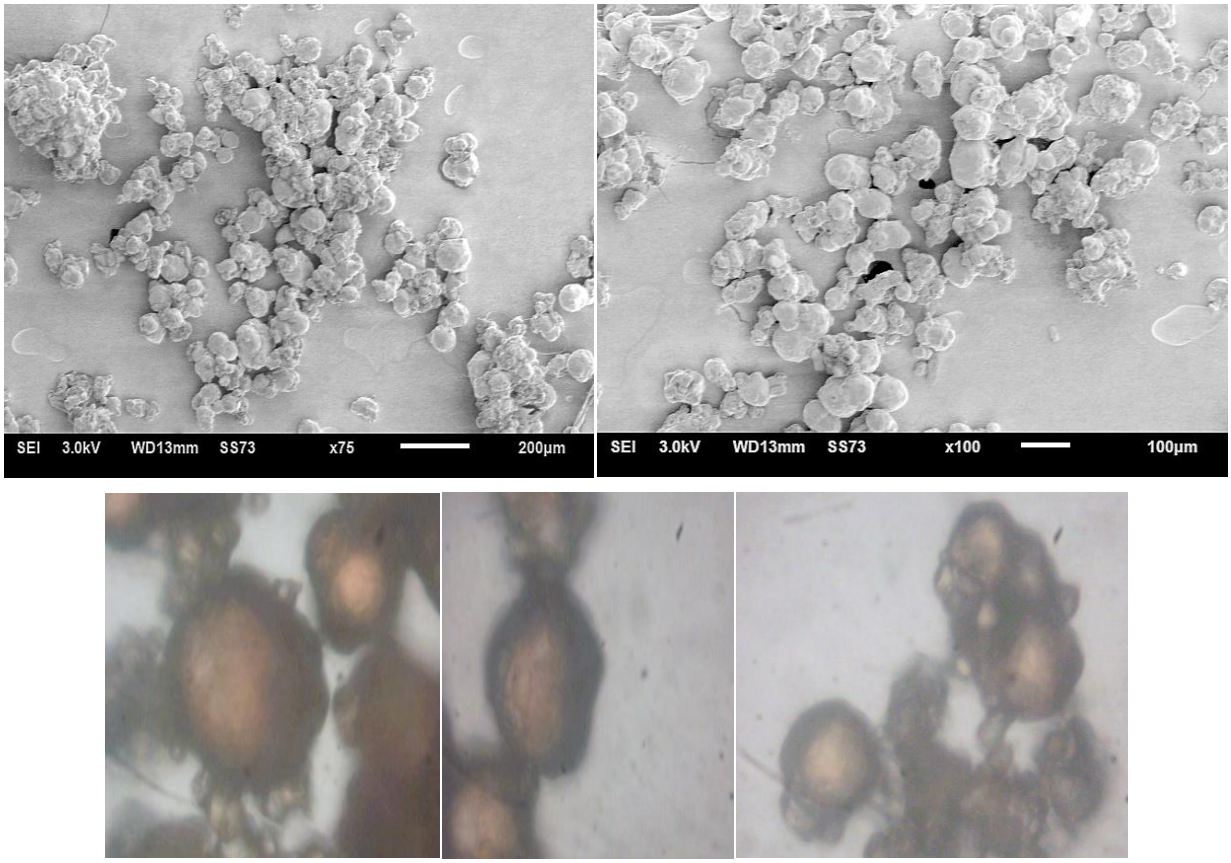

Fig. 3: SEM photographs of EP microspheres was recorded at 75X and 100X magnification to characterize shape and surface properties of the microspheres

Table 3: *In vitro* release data of EP microspheres (0.5ml Glutaraldehyde)

Time (h)	Cumulative % drug release				
	F1	F2	F3	F4	F5
1	16.42±0.63	14.32±0.33	12.29±0.12	11.43±0.31	10.43±0.41
2	29.67±0.56	24.23±0.34	22.65±0.36	20.97±0.75	19.98±0.64
3	32.45±0.48	29.27±0.33	25.46±0.45	23.65±0.34	22.35±0.21
4	36.63±0.56	33.24±0.75	31.48±0.84	29.45±0.25	31.48±0.43
5	43.64±0.58	42.70±0.55	35.27±0.65	38.54±0.64	35.87±0.32
6	50.13±0.50	49.23±0.63	40.98±0.98	39.21±0.65	38.43±0.23
7	56.45±0.50	51.05±0.64	48.46±0.47	45.76±0.75	43.21±0.12
8	64.32±0.53	58.34±0.64	56.7± 0.64	54.32±0.24	53.43±0.18
9	72.12±0.59	66.03±0.70	60.29±0.41	58.76±0.14	57.24±0.75
10	80.35±0.62	75.23±0.94	67.91±0.85	64.89±0.61	60.75±0.45
11	88.82±0.45	85.47±0.69	75.34±0.32	73.98±0.54	71.86±0.36
12	94.92±0.66	88.12±0.79	86.75±0.65	80.95±0.23	78.67±0.24

Table 4: *In vitro* release data of EP microspheres (0.8 ml Glutaraldehyde)

Time (h)	Cumulative % drug release				
	N1	N2	N3	N4	N5
1	13.82±0.45	11.23±0.41	9.45±0.46	8.36±0.21	7.85±0.45
2	25.48±0.56	23.54±0.65	18.26±0.62	15.67±0.33	15.98±0.32
3	26.30±0.34	25.46±0.48	22.46±0.42	18.97±0.48	17.64±0.39
4	30.82±0.39	28.94±0.42	25.98±0.31	22.42±0.55	23.97±0.54
5	36.35±0.45	34.65±0.22	30.87±0.65	28.45±0.43	27.41±0.35
6	41.26±0.13	40.28±0.46	38.53±0.44	34.98±0.34	33.11±0.64
7	45.40±0.32	44.78±0.70	42.78±0.36	40.64±0.46	42.87±0.12
8	51.58±0.41	50.26±0.65	48.97±0.41	44.75±0.47	49.74±0.44
9	56.37±0.25	55.67±0.47	52.65±0.48	48.91±0.59	52.66±0.23
10	64.69±0.42	60.76±0.51	58.98±0.64	52.34±0.23	55.65±0.61
11	68.63±0.38	65.28±0.12	60.56±0.34	58.92±0.33	62.78±0.70
12	70.80±0.46	69.45±0.31	64.37±0.62	60.89±0.18	63.87±0.46

(Standard deviation n=6)

Table 5: Release kinetics of all formulations

Formulation	Zero order	First order	Higuchi Matrix	Korsemeyer peppas	
	r^2 value	r^2 value	r^2 value	r^2 value	n value
F1	0.99	0.85	0.95	0.97	0.68
F2	0.99	0.80	0.93	0.98	0.73
F3	0.99	0.87	0.92	0.98	0.75
F4	0.98	0.87	0.92	0.98	0.77
N1	0.99	0.97	0.96	0.99	0.64
N2	0.98	0.98	0.96	0.98	0.64
N3	0.99	0.99	0.96	0.99	0.76
N4	0.99	0.98	0.94	0.99	0.81
N5	0.98	0.98	0.93	0.98	0.85

CONCLUSION

Formulation and evaluation of Eplerenone loaded chitosan microspheres for sustained release was carried out with good % yield, encapsulation efficiency, particle size distribution, and in vitro release characteristics. The investigation of optimum formulation showed sustained drug release and could therefore produce benefits such as reduction in total dose and frequency of administration.

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