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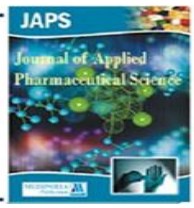

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Formulation and evaluation of Salbutamol sulphate microspheres by solvent evaporation method

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ABSTRACT

Salbutamol sulphate (SS) loaded microspheres were prepared by solvent evaporation method with combination of hydroxy propyl methyl cellulose and Carbopol polymers in various proportions. A total of eleven formulations were prepared i.e. E1, E2, E3, E4, E5, E6, E7, E8, E9, E10 and E11.The particle size of all the formulations were ranged between 110±0.02 and 183±0.02µm. The entrapment efficiency was ranged between 68.3±0.01 and 94.9±0.02%. Based on above parameters four formulations were selected i.e. E5, E8, E9 and E10 for further studies like micromeretic properties, swelling index and in-vitro release profile. It was confirmed with the results of micromeretic property that all the selected formulations showed good flow property. Release data were analyzed based on Highuchi kinetics and Korsmeyer-Peppas equation and all the selected formulations showed good fit to Highuchi model. Stability studies showed almost negligible changes in particle size, entrapment efficiency and drug release throughout the study period.

Key words: Salbutamol sulphate, swelling index, carr's index, microspheres, Higuchi kinetics, stability study.

INTRODUCTION

Asthama is one of the leading diseases in the world and needs some serious attention. It can lead to various complications like bronchospasm, respiratory failure, chronic obstructive pulmonary disease (COPD) etc. Salbutamol sulphate (SS) is a selective β_2-adrenergic agonist which acts on β_2- adrenoreceptor for the treatment of mild to severe asthama. It is a hydrophilic drug which is easily absorbed from GI tract (Dandagi *et al*, 2009) with pka value of 9.2 and the drug undergoes first pass metabolism. It is contraindicated to hypertensive patients, over dosage may cause skeletal muscle irritability and peripheral vasodilation (Hardman et al, 2001; Sweetman and Martindale, 1991; Walter, 1994). The plasma half life of SS is 4-6 hrs and required multiple dosing for a prolonged period of time which may lead to patient incompliance (Murtaza et al, 2009). Moreover as the drug undergoes first pass metabolism so it is rarely given by oral route. These problems can be rectified by using a controlled drug delivery system like microspheres which can deliver the drug for a sustained period of time via the oral route.

Microspheres provide sustained release over a prolonged period of time and better bioavailability than conventional dosage forms which reduces dosing frequency, side effects and thereby increases patient compliance. The smaller size and spherical shape of microspheres increases the surface area which increases the bioavailability of the dosage form. It also has advantage over the microparticles and nano particles as they tend to accumulate at the site of action but microspheres due to its smaller size (i.e. micron size) and spherical shape can be injected and hence shows better bio-availability (Sam Mathew et al, 2008). Microspheres are

defined as spherical microscopic particles having a size range of 1-1000µm (Ketie sarslidze et al, 2010).We can also define it as a monolithic sphere or therapeutic agent distributed throughout the matrix (Karmakar and Faysal, 1999).

One of the very common and suitable method to prepare these polymeric microspheres is solvent evaporation method because it facilitates sustained release of a drug which has many clinical advantages as well as it provides compatibility to use more than one novel polymers like hydroxyl propyl methyl cellulose, Carbopol as encapsulation matrix (Kannan et al, 2009).

MATERIALS AND METHOD

Materials

Salbutamol Sulphate (SS) was obtained as a gift sample from Dr. Reddy's laboratories, India. The polymers hydroxyl propyl methyl cellulose (HPMC) and Carbopol 934p (Cp) were procured from Sigma Chemicals, USA. Dichloromethane, methanol, Sodium Lauryl Sulfate (SLS) was obtained as gift samples from Fine chemicals, Mumbai India. Sodium hydroxide, Potassium dihydrogen phosphate was purchased from Merck (India) Ltd. All other reagents were used of analytical grade.

Formulation of SS Microspheres

SS microspheres were prepared using HPMC, Carbopol and distilled water as continuous phase by solvent evaporation technique (Nighute and Bhise 2009). Initially dichloromethane (DCM) and methanol was mixed uniformly at room temperature, then HPMC and Carbopol in various proportions was dissolved in the above solution. To this mixture, a drug solution corresponding to 1000mg was added and mixed thoroughly and injected drop wise in to the continuous phase consisting of 100mL of 0.2% (w/v) SLS (sodium lauryl sulphate) at 250 rpm. The microspheres obtained was washed for 2-3 times with distilled water and dried at room temperature (Nighute and Bhise 2009). Different concentrations and ratios of polymers used in the formulation of microspheres are mentioned in Table 1.

Table 1. Composition of various SS microspheres formulations

Formulation code	*HPMC (mg)*	*Carbopol (mg)*	*Salbutamol sulphate (mg)*	*Dicloro methane (mL)*	*Methanol (mL)*	*Sodium lauryl sulphate (mg)*
E1	1000	-	1000	10	10	200
E2	900	100	1000	10	10	200
E3	800	200	1000	10	10	200
E4	700	300	1000	10	10	200
E5	600	400	1000	10	10	200
E6	500	500	1000	10	10	200
E7	400	600	1000	10	10	200
E8	300	700	1000	10	10	200
E9	200	800	1000	10	10	200
E10	100	900	1000	10	10	200
E11	-	1000	1000	10	10	200

PARTICLE SIZE ANALYSIS AND PERCENTAGE YIELD

Particle sizes of the SS microspheres were determined using an optical microscope. Around 250 microspheres were randomized and their diameters were measured (Satit et al, 2005). Percentage yield was calculated by using the following formula (Surini et al, 2009).

$$\text{Percentage yield} = \frac{\text{Actual weight of microspheres}}{\text{Weight of starting materials}} \times 100$$

ENTRAPMENT EFFICIENCY

SS microspheres was dissolved in 10 mL of phosphate buffer (pH 6.8) under occasional shaking for 2-3 hrs. The resultant solution was filtered through 0.46µm filter paper and after suitable dilution, the amount of SS present in the formulation was determined using a UV Visible spectrophotometer at 278nm (Shimadzu 1800, Japan). (Malay and Prakash, 2007)

Drug incorporation efficiency can be given by the following formula

$$\text{Drug incorporation efficiency} = \frac{\text{Actual drug content}}{\text{Theoretical drug content}} \times 100$$

SWELLING INDEX (SI)

The swelling of microspheres were carried out in phosphate buffer (pH 6.8) for 30 hrs. The excess liquid drops adhered to surface were removed by blotting and the swollen microspheres were weighed. The microspheres were then dried in hot air oven at 40^0C for 60 hrs until there was no change in dried mass of sample. The swelling index was calculated from the following equation (Dandagi et al, 2009).

$$\textbf{SI} = \frac{\text{Mass of swollen microspheres- Mass of dry microspheres}}{\text{Mass of dried microspheres}}$$

SCANNING ELECTRON MICROSCOPY (SEM)

All samples were randomly examined for their surface properties by using scanning electron microscopy. The microspheres were placed on one side of an adhesive stub and the stub was then coated with conductive gold-palladium with sputter coater attached to the instrument. The microspheres were then examined under Hitachi S 3000 N SEM at 15 to 20 kV (Parul Trivedi et al, 2008).

MICROMERETIC PROPERTIES

Bulk density

Bulk density was determined by the following formula (Murtaza et al, 2009)

$$\text{Bulk density} = \text{Sample weight/Sample volume}$$

Tapped density

The tapped density was determined by tapping method, in which the cylinder containing known amount (M) of microspheres was subjected to a fixed number of taps (approximately 100) until the bed of microspheres had reached the minimum. The final volume after tapping 'V_o' was recorded and the tapped density was calculated by the following equation:

$$\text{Tapped density } (P_p) = M/V_o$$

Compressibility index (CI), Haussner's ratio and Angle of repose

Carr's index (% compressibility index), Hausner ratio and Angle of repose were determined to predict flowability and these can be determined by following equations.

$$\text{CI} = \frac{\text{(Tapped density- Bulk density)}}{\text{Tapped density}} \times 100$$

$$\text{Haussner's ratio} = \text{Tapped density/Bulk density}$$

Angle of repose is measured by passing the samples through funnel on the horizontal surface. The height (h) and radius (r) of the cone funnel was measured. The angle of repose (θ) was given by the following formula,

$$\text{Angle of repose } (\theta) = \tan^{-1} h/r$$

***In vitro* drug dissolution**

USP 23 Type-2 rotating paddle dissolution test apparatus (Electrolab, EDT-08Lx) was used to study the *in vitro* drug dissolution. 900 mL phosphate buffer (pH 6.8) at 37± 5^0C stirred at 100 rpm was used as the dissolution medium. 100mg equivalent of drug samples of microspheres were placed in the dissolution medium. Samples (1mL) were withdrawn at pre-determined time intervals (1, 2, 3, 4, 5, 8, 10, 12 and 14 hrs) and replaced with equal volumes of dissolution medium. Samples were filtered through 0.46µm filter and appropriately diluted with phosphate buffer (pH 6.8) and analysed UV spectrophotometrically at 278 nm. Drug release mechanism was determined by finding the best fit of the release data to Higuchi and Korsmeyer-Peppas plots (Sam Mathew et al, 2009; Sam Mathew et al, 2007).

Stability studies

Selected formulations were kept tapped with vials in an incubator maintained at 40±2°C and 75±5% RH for three months (Nighute and Bhise 2009). Changes in the appearance, particle size, drug content and release profile of these stored microspheres were investigated at regular time intervals (1, 2 and 3months).

RESULT AND DISCUSSION

A total of 11 formulations, E1, E2, E3, E4, E5, E6,E7,E8,E9,E10,E11 were prepared using HPMC and Carbopol by solvent evaporation technique using distilled water as continuous phase. Use of water as a solvent was the reason for the long duration of drying time during the formulation step (8 hours).

Particle size analysis, percentage yield, entrapment efficiency and scanning electron microscopy

Physicochemical characteristics of the SS microspheres are shown in Table 2. The particle size of the medicated microspheres ranged between 110±0.02 and 183±0.02µm. It was noticed that the particle size of the microspheres increased with increased concentration of Carbopol and this may be due to high viscosity of Carbopol which increases the droplet size and results in increase in particle size. The percentage yields of the formulations were ranged between 46±0.03 and 96±0.01% and the entrapment efficiency is between 68.3±0.01 and 94.9±0.02%. The entrapment efficiency is increased with lower HPMC concentration and this may be due to the diffusion of drug into the aqueous phase because of decrease in interfacial tension by HPMC between drug and aqueous phase. Of the eleven formulations, based on the entrapment efficiency and percentage yield, 4 formulations (E5, E8, E9 and E10) were selected for further evaluations.

Table 2. Physicochemical characteristics of SS microspheres

Formulation Code	*Particle size(µm) Mean±SD*	*Entrapment efficiency (%)*	*Percentage yield%*
E1	110±0.02	71.70±0.04	46±.003
E2	117±0.04	69.20±0.04	54±.002
E3	135 ±0.01	68.30±0.01	56±.002
E4	123±0.05	73.40±0.03	62±.004
E5	169±0.04	94.49±0.02	86±.002
E6	178±0.03	80.82±0.03	78±.004
E7	183±0.02	86.40±0.05	83±.001
E8	194±0.02	89.91±0.02	75±.001
E9	213±0.02	91.37±0.04	88±.005
E10	217±0.04	93.42±0.03	96±.001
E11	224±0.01	79.70±0.05	67±.003

Swelling index

Swelling behaviour of selected SS microspheres as a function of time is illustrated in Fig. 1.The swelling indices of the microspheres were high (up to 0.989±.001 for E10 at the end of 14 hrs) and varied between the formulations. The swelling indices increased in the following order: E5<E8<E9<E10. It was seen that microspheres with more Carbopol concentration showed more swelling compared to those with HPMC. Differences in swelling of the tested hydrophilic polymers could be explained by the difference in resistance of the matrix network structure to the movement of water molecules.

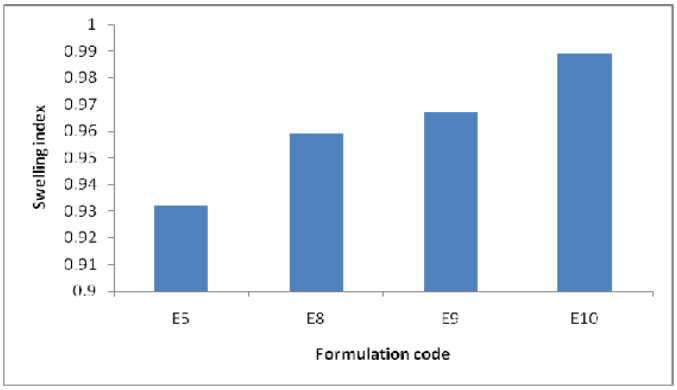

Figure 1. Swelling behaviour of selected SS microspheres

Scanning electron microscopy

SEM of the selected formulations is shown in Fig 2 All the selected microspheres were smooth, almost spherical in shape and non porous. Due to the presence of HPMC some microspheres showed rough surface.

Micromeretic properties

The micromeretic properties of the selected formulation are shown in Table 3. Flow properties of selected microspheres

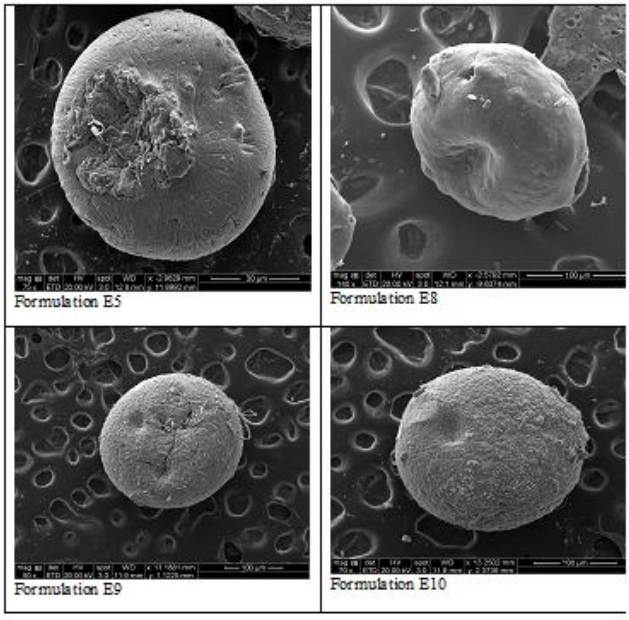

Figure 2. SEM photographs of selected SS microspheres

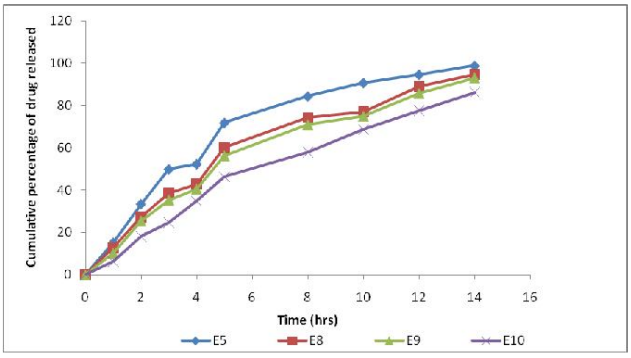

Figure 3. *In vitro* drug release of selected SS microspheres

were evaluated by measuring the angle of repose, haussner's ratio, bulk density, tapped density and compressibility index. All tested microspheres showed excellent flow-ability with angle of reposes being less than 25º. The compressibility of the tested microspheres was less than 9% which indicated good packability of microspheres. It was reported that when compressibility is below 9% and the angle of repose is within 25^0 the powder show good flowability and do not require any glidants to improve the bioavailability. It was reported that powders with a Haussner's ratio lesser than 1.25 is considered to be possessing good flowability. In the present study, all tested microspheres had a haussner's ratio of ≤1.09±0.2 The bulk density of the selected formulations increased with lower HPMC concentration.

Table 3.Micromeretic properties of selected SS microspheres.

Formulation code	*Bulk Density (gm/ml)*	*Tapped density (gm/ml)*	*Compressibility index (%)*	*Haussner's ratio*	*Angle of Repose*
E5	0.420±0.2	0.443±0.2	5.17±0.05	1.05±0.01	14.07
E8	0.478±0.2	0.513±0.3	6.82±0.03	1.07±0.01	16.32
E9	0.492±0.1	0.537±0.3	8.37 ±0.04	1.09±0.02	16.05
E10	0.523±.0.3	0.568±0.1	7.9±0.04	1.1±0.03	23.58

In vitro drug dissolution

In vitro release of SS from different microspheres is shown in Fig. 3. The maximum *in vitro* release was evaluated to be 98.9 ±0.03% over a period of 14^{th} hrs for formulation E5. The drug release is decreased with decreasing concentration of HPMC and it showed controlled release of drug with increasing Carbopol concentration. This may be due to increase in viscosity which will increase the particle size and decrease the surface area. Increase in viscosity may also increase the diffusional path length which might also be the reason for reduction in drug release. All the tested formulations E5, E8, E9 and E10 provided good fit to the Higuchi model. According to this model, the drug releases from these batches may be controlled by diffusion through the micropores.

Stability studies

The results of stability studies indicated that there was no influence on the chemical and physical stability of the formulations during the test period and the results are mentioned in Table 4.

Table 4. Physical stability characteristics of selected formulations

Evaluation parameter	*Formulation Code*	*1st month*	*2nd month*	*3rd month*
Entrapment efficiency (%)*	E5	93.92±0.01	92.87±0.03	92.18±0.02
	E8	88.71±0.01	87.62±0.06	86.92±0.04
	E9	90.42±0.02	90.82±0.06	89.64±0.03
	E10	92.36±0.02	91.46±0.02	91.43±0.01
Drug release*	E5	97.99±0.03	97.64±0.02	97.21±0.02
	E8	94.02±0.04	93.22±0.07	93.11±0.05
	E9	92.09±0.04	91.99±0.07	91.32±0.05
	E10	85.21±0.01	85.02±0.06	85.00±0.04
Particle size (µm)	E5	168±0.12	168±0.10	168±0.01
	E8	193±0.09	193±0.01	192±0.08
	E9	212±0.22	212±0.01	211±0.99
	E10	216±0.14	215±0.91	215±0.42

SD ± n=3

CONCLUSION

The Salbutamol sulphate microspheres were prepared successfully by solvent evaporation technique using combination of novel polymers and the *in vitro* release studies have shown that better release profile with combination of polymers especially with increase in Carbopol concentration. Accelerated stability studies confirmed that the microspheres formed were quite stable and hence concluded that it is a potential drug delivery for Salbutamol sulphate.

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