RESEARCH ARTICLE

Preparation and characterization of mucoadhesive microcapsules of salbutamol sulfate

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Salbutamol sulfate microcapsules with a coat consisting of sodium alginate and mucoadhesive polymer such as sodium carboxy methyl cellulose (NaCMC), methyl cellulose (MC), carbopol-934, and hydroxy propyl methyl cellulose (HPMC) were prepared by ionotropic gelation technique and were evaluated for morphological characters, drug content, loading efficiency, drug–polymer interactions, swelling ratio, mucoadhesive properties, and *in vitro* release. The resulting microcapsules were discrete, spherical, and free-flowing, and microencapsulation efficiency was 51.28–96.70%. The microcapsules prepared with alginate alone (A4) have exhibited good mucoadhesive property in the *in vitro* washoff test. The swelling ratio of microcapsules was enhanced with increased alginate concentration. Salbutamol sulfate release from these mucoadhesive microcapsules was slow and extended over a period of 8 h and depends upon the concentration of the alginate. The drug release from alginate-HPMC/carbopol microcapsules followed diffusion-controlled first-order kinetics. The release rate of alginate-HPMC microcapsules (A4H) was higher than other formulations and comparable with commercially available controlled-release capsules. Microcapsules with alginate alone (A4) followed diffusion mechanism. In conclusion, alginate-HPMC/carbopol mucoadhesive microcapsules could be promising vehicle for oral controlled release of salbutamol sulfate.

Key words: *Alginate microcapsules, in vitro release, ionotropic gelation, mucoadhesive property, salbutamol sulphate, swelling*

INTRODUCTION

Multiple unit dosage forms such as microspheres or beads have gained popularity as oral drug delivery systems because of more uniform distribution of the drug in the gastrointestinal tract, more uniform drug absorption, reduced local irritation, and elimination of unwanted intestinal retention of polymeric material, when compared to nondisintegrating single-unit dosage form.[1,2]

Microencapsulation by various polymers and their applications are well known.[3,4] Microencapsulation and resulting microcapsules have gained good acceptance as a process to achieve controlled-release drug targeting. Mucoadhesion is a topic of current interest in the design of drug delivery systems to prolong the residence time of the dosage form at the site of application or absorption and to facilitate intimate contact of the dosage form with the underlying absorption surface to improve and enhance the bioavailability of the drug.[5-7] The objective of this study was to develop, characterize, and evaluate mucoadhesive microcapsules of salbutamol sulfate using various mucoadhesive polymers. Salbutamol sulfate is a β-2 adrenergic agent with more bronchodilatory effect and is useful in the treatment of asthma. Asthmatic patients require continuous drug therapy for a long period. It can be achieved through controlled-release systems. The microcapsules are effective carriers to maintain prolonged effective concentration of a drug salbutamol sulfate (biological half-life 2.00±0.49 h). In the treatment of asthma, the equivalent of the 6-16 mg of salbutamol is used in daily divided doses (Martindale). Salbutamol is a direct-acting sympathomimetic agent with predominantly β-adrenergic activity.

In the present work, we prepared controlled-release microcapsules of salbutamol sulfate by ionotropic gelation technique, and their morphological characters, drug content, mucoadhesive property, and drug release properties were evaluated.

MATERIALS AND METHODS

Salbutamol sulfate I.P. was procured as a gift sample from Cipla Ltd., Bangalore, carbopol-934 P from Goodrich USP, methyl cellulose (methoxy 28.3%, 65 capsules of 0.5% wt/vol) purchased from S.D. Fine chem. Ltd., Mumbai, hydroxy propyl methyl cellulose (HPMC)

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(E15) and sodium alginate from Ontop Pharmaceutical Ltd., Bangalore. All other chemicals used were of analytical grade.

Preparation of microcapsules

Microcapsules were prepared by using sodium alginate in combination with four mucoadhesive polymers namely NaCMC, MC, carbopol, and HPMC as coat materials. An orifice ionic gelation process[8,9] has been used to prepare large-sized alginate beads.

Sodium alginate (500 mg) with salbutamol sulfate (500 mg) core:coat ratio 1:1, 1:2, 1:3, 1:4 and sodium alginate (500 mg) with mucoadhesive polymer (500 mg) were dissolved in purified water (25 ml) for 12 h. The active substance salbutamol sulfate (500 mg) was dispersed in 10 ml distilled water and mixed uniformly with polymer solution. Aqueous polymer dispersion was added dropwise at the rate of 1 ml/min into 100 ml of $CaCl_2$ solution (20% wt/vol) through a syringe with needle number-23 (0.63 × 25 mm). Further, the medium was stirred for 20 min at 200 rpm to complete the curing reaction and to produce spherical, rigid microcapsules. The microcapsules were collected by decantation, and the product thus separated was washed repeatedly with water and dried at 40°C for 12 h and stored in desiccators. The microcapsule compositions are listed in Table 1.

Evaluation of salbutamol sulfate microcapsules

The prepared microcapsules were evaluated for particle size analysis, drug content, loading efficiency, swelling ratio, mucoadhesive properties, *in vitro* release, morphological characters, and drug-polymer interactions.

The microparticles were analyzed for particle size by optical microscopy. The instrument was calibrated, and 100 microparticle sizes were calculated under magnification. For the determination of drug content, 100 mg of salbutamol sulfate microcapsules were powdered, and 50 mg of the powder was transferred to 100-ml volumetric flask, dissolved in water and made the volume to 100 ml. The solution was kept for 1 h with occasional shaking and filtered through Whatman filter paper. The filtrate was collected and diluted with sufficient amount of distilled water, maintaining the concentration of the drug within the standard plot range. The diluted solution was analyzed for the salbutamol sulfate content by UV-spectrophotometer (UV-1700 Shimadzu Corporation, Japan) at 225 nm.

Drug-loading efficiency (DLE) was studied by dissolving microparticles in distilled water for 24 h. The amount of drug loaded was determined by spectrophotometrically at 225 nm. All the experiments were carried out in triplicate.

$$\text{DLE} = \frac{\text{Experimental drug content}}{\text{Theoretical drug content}} \times 100$$

In swelling studies,[10] a known weight (50 mg) of microcapsules was placed in a glass vial containing 10 ml of distilled water at 37±0.5°C in an incubator with occasional shaking. The microcapsules were periodically removed, blotted with filter paper, and their changes in weights were measured during the swelling until equilibrium was attained. Finally, the weight of the swollen microcapsules was recorded after a period of 3 h, and the swelling ratio was then calculated from the formula. The studies were carried out in triplicate.[11] Swelling ratio (%w/w) was determined from the following relationship and plotted against time.

$$\text{Swelling ratio} = \frac{(\text{Wt-Wo})}{(\text{Wo})} \times 100$$

Where Wo and Wt are, respectively, initial weight of the microparticles and weight of the microparticles at time 't'.

In vitro drug-release study was carried out in distilled water using rotating basket method (model TDT6P Electrolab). About 900 ml of the dissolution medium (water) was taken in covered vessel, and the temperature was maintained at 37±0.5°C. The speed of the paddle was set at 75 rpm.

Table 1: Formulae of different microcapsules of salbutamol sulfate

Formula code	Core:coat	Polymer:co polymer	Quantity (mg) Drug + SA + CO	$CaCl_2$ % (wt/vol)
A1	1:1	–	500+500+000	20
A2	1:2	–	333.3+666.6+000	20
A3	1:3	–	250+750+000	20
A4	1:4	–	200+800+000	20
A4C	1:4	1:1	200+400+400	20
A4H	1:4	1:1	200+400+400	20
A4M	1:4	1:1	200+400+400	20
A4S	1:4	1:1	200+400+400	20
A4 F	1:4	–	200+800	30
A4 G	1:4	–	200+800	40
A4 J	1:4	–	200+800	20
A4 K	1:4	–	200+800	20

A - Alginate, C - Carbopol, H - HPMC, M - MC, S - NaCMC. F, G - Calcium chloride 30, 40, % wt/vol. J, K - Reaction time 10, 30 min

A sample of microcapsules (≈8 mg of 16/20) was taken in the basket at specific time intervals and was replaced by the same amount of fresh medium. Samples were assayed at 225 nm for salbutamol sulfate using the UV spectrophotometer. All the studies were performed in triplicate.

The mucoadhesive[12] properties of microcapsules were evaluated by an *in vitro* adhesion testing method known as washoff method. Freshly excised piece of intestinal mucosa (2 × 2 cm) from albino rat were mounted on to glass slides (3 × 1 inch) with cyanoacrylate glue. Two glass slides were connected with a suitable support. About 25 microcapsules were spread onto the arm of a USP tablet disintegrating test machine. When the disintegrating test machine was operated, the tissue specimen was given slowly in regular up and down movement in the test fluid (900 ml of distilled water) at 37°C contained in an L vessel of the machine. At the end of 50 min, at the end of 1 h, and at the hourly intervals up to 5 h, the machine was stopped, and numbers of microcapsules still adhering to the tissue was calculated. The studies were carried out in triplicate.

Characterization of salbutamol sulphate microcapsules

FTIR studies

IR spectra of the drug, and powdered tablets were recorded in a Fourier transform infrared spectrophotometer (FTIR 1615, Perkin Elmer, USA) with KBr pellets.

DSC studies

DSC scans of about 10 mg, using an automatic thermal analyzer system were performed with an accurately weighed salbutamol sulfate and formulation (Mettler Toledo, USA). Sealed and perforated aluminium pans were used for all the samples. Temperature calibrations were performed using indium as standard. An empty pan sealed in the same way as the sample was used as a reference. The entire samples were run at a scanning rate of 10°/min from 50-300°.

Morphology observation (Sem analysis)

The particle size, shape, and surface morphology of microcapsules were examined by scanning electron microscopy. Microcapsules were fixed on aluminum studs and coated with gold using a sputter coater SC 502, under vacuum (0.1 mmHg). The microcapsules were then analyzed by using SEM[13] (Model LEICA S-430, London, UK).

RESULTS AND DISCUSSIONS

Microcapsules of salbutamol sulfate with a coat consisting of alginate alone in core:coat ratio 1:1, 1:2, 1:3, 1:4, and alginate with a mucoadhesive polymer (1:1) namely NaCMC, MC, carbopol, or HPMC could be prepared by ionotropic gelation process. Microcapsules with a coat of mucoadhesive polymer alone could not be prepared due to their water soluble nature. Microcapsules were found to be discrete, large, spherical, and free-flowing. The sizes could be separated, and more uniform size range of microcapsules could readily be obtained. The size analysis of different microcapsules should be about 72-86%. Reactions were in the size range of 16 + 20 (1015 μm) and 20+30 (670 μm) mesh size, respectively. The size of microcapsules was decreased with the incorporation of carbopol, HPMC, NaCMC and MC. The size was also reduced with higher levels of calcium chloride and reaction time.

The drug content and microencapsulation efficiency are shown in Table 2. The overall drug content was uniform and reproducible in each batch of microcapsules prepared. The Microencapsulation efficiency was in the range of 51-94%. Microencapsulation efficiency was lower by incorporation of copolymers (1:1) like carbopol, HPMC, MC, and NaCMC. It was also observed that the entrapment efficiency has enhanced slightly with an increase in cross-linking time, whereas an increase in cross-linking concentration did not influence drug-loading process.

Table 2: Drug content and encapsulation efficiency of different alginate microcapsules of salbutamol and commercial capsule

Formulation code	Drug content - (mg)		Microencapsulation efficiency (%)	*t* 50% (h)	% Drug released in 8 h (± SD, n = 3)
	Theoretical	Practical			
A1	25	12.82	51.28	1.4	90.91
A2	16.6	7.61	45.84	1.5	89.15
A3	12.5	10.98	87.84	1.75	85.60
A4	10	9.47	94.70	1.8	82.04
A4C	10	7.38	73.80	1.7	89.51
A4H	10	7.19	71.90	1.6	93.87
A4M	10	7.78	77.80	1.95	80.29
A4S	10	7.44	74.40	1.75	86.01
A4F	10	9.65	96.50	1.95	80.25
A4G	10	9.67	96.70	2.1	78.45
A4J	10	9.28	92.80	1.78	86.91
A4K	10	9.66	96.60	2.75	80.61
Ventrolin CR capsule	8	7.95	99.37	1.5	98.68

*Average of 3 determinations

The swelling characteristics are shown in Figures 1 and 2 and related to the mucoadhesion and its environment. The swelling depends on the polymer concentration, ionic strength, as well as the presence of water. The dynamic process of mucoadhesion *in vitro* occurs with optimum water content. Overall hydration results in the formation of a wet, slippery mucilage without adhesion. The swelling ratios of alginate microcapsules were found in range of 3.18-5.54 at the end of 2 h. The result showed that the incorporation of HPMC in alginate microcapsule was enhanced, whereas the ratio reduced when combined with MC.

Salbutamol sulfate release from microcapsules was slow, spread over extended periods of time, and depended on the composition of coat. Microcapsules of alginate-HPMC gave relatively fast release when compared to others. The order of increasing release rate observed with various microcapsules was alginate-MC < alginate- NaCMC < carbopol < alginate-HPMC. It indicates that alginate-HPMC microcapsules gave relatively higher release ($t_{50\%}$, 1.6 h) than alginate-MC ($t_{50\%}$, 1.95 h) microcapsules (13).

The concentrations of calcium chloride have little influence on the release of different concentrations namely 30 and 40% wt/vol solutions. However, minimum concentration of 20% wt/vol solution of calcium chloride was found effective.

The release of drug from microcapsules depends on particle size. The release rates of major fraction (#16/20) and minor fraction (#20/30) were found 82.04 and 90.51% at end of 8 h. The higher drug release was obtained with smaller particle size (#20/30). Prepared microcapsules alginate (A4), alginate-HPMC (A4H), and alginate-carbopol (A4C) were compared with commercial CR capsule. The order of release rates of microcapsules and commercial products was ventrolin > A4H > A4C> A4. Hence, controlled release pattern of alginate-HPMC microcapsules (A4H) was comparable to the commercial CR capsule [Figures 3 and 4].

Thus, large size spherical microcapsules of salbutamol sulfate with a coat consisting of alginate and mucoadhesive polymer (NaCMC), MC, carbopol, or HPMC could be prepared by an orifice ionic gelation process. The microcapsules exhibited good mucoadhesive property in the *in vitro* washoff test [Table 3].

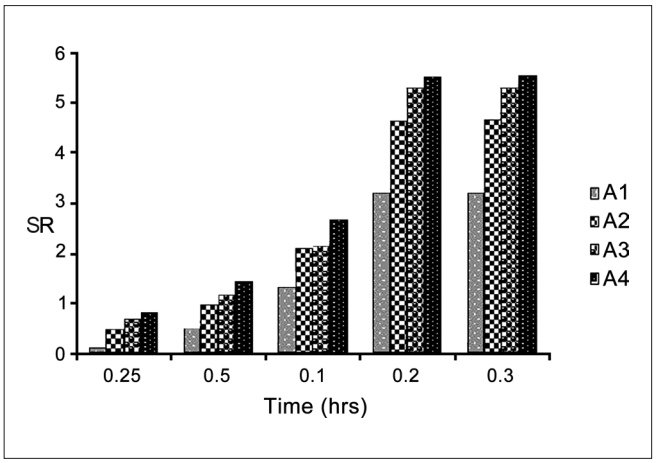

Figure 1: Swelling ratios of microcapsules of salbutamol sulphate. (A1 to A4)

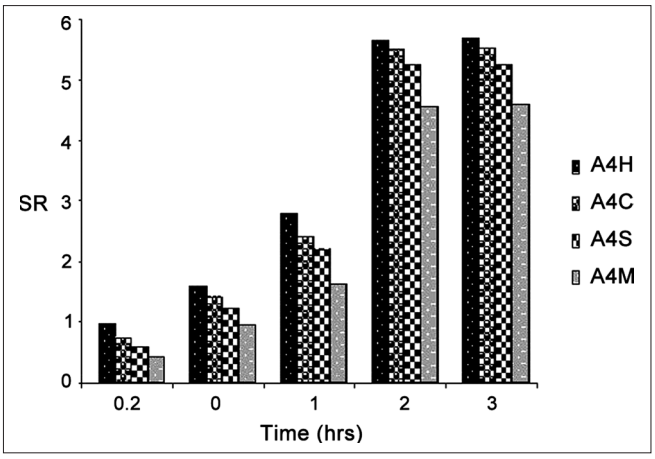

Figure 2: Swelling ratios of microcapsules of salbutamol sulphate. (A4H, A4C, A4S, A4M)

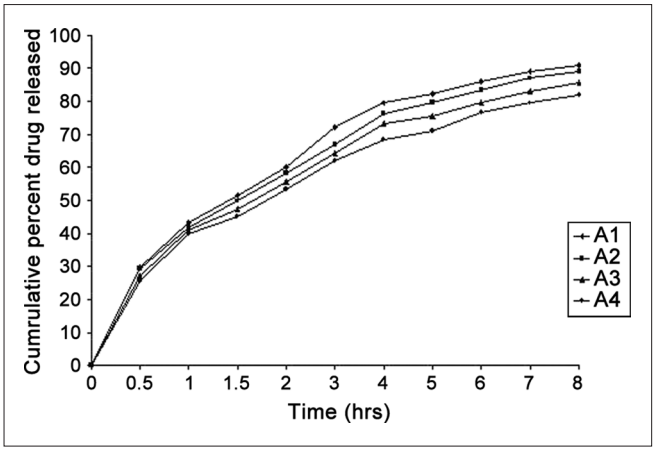

Figure 3: *In vitro* release profile sodium alginate microcapsules. (A1 to A4)

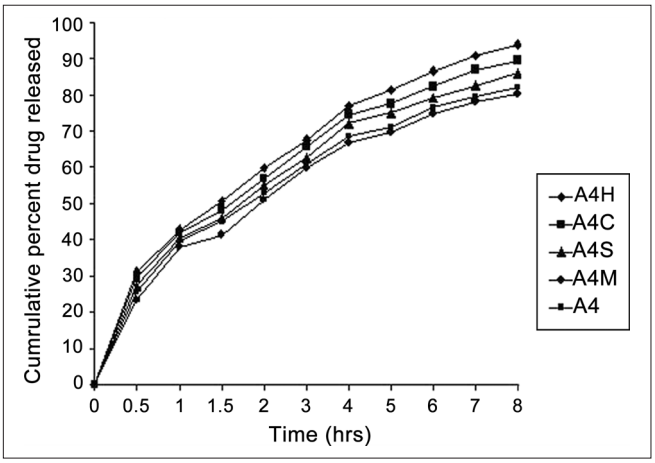

Figure 4: *In vitro* release profile sodium alginate microcapsules. (A4H, A4C, A4S, A4M)

Table 3: *In vitro* mucoadhesion (wash-off) test of alginate and other mucoadhesive microcapsules of salbutamol

Formulation code	Percent of microcapsules adhering at different times (h)				
	0.5	1	2	3	4
A1	76	48	16	00	00
A2	84	60	24	08	00
A3	88	72	36	20	04
A4	92	80	60	28	08
A4C	76	64	32	16	00
A4H	72	56	28	12	00
A4M	84	72	44	32	12
A4S	80	68	36	24	04

*Average of 3 determinations

The morphological characterization revealed that the alginate microcapsules (A4) were spherical and completely covered with coat material. The surface of the microcapsules was almost smooth, free from pores and deposits. After dissolution, microcapsules retained their shape with deep cracks and few pores [Figure 5]. The SEM photographs of alginate-HPMC microcapsules (A4H) showed that microcapsules were almost spherical and covered with coat composition. The surface was smooth but contained longitudinal depression and fibers deposits.

The compatibility between drug and polymer was confirmed by using FTIR study. Salbutamol sulfate showed [Figure 6] characteristic peaks at KBr (cm^{-1}).

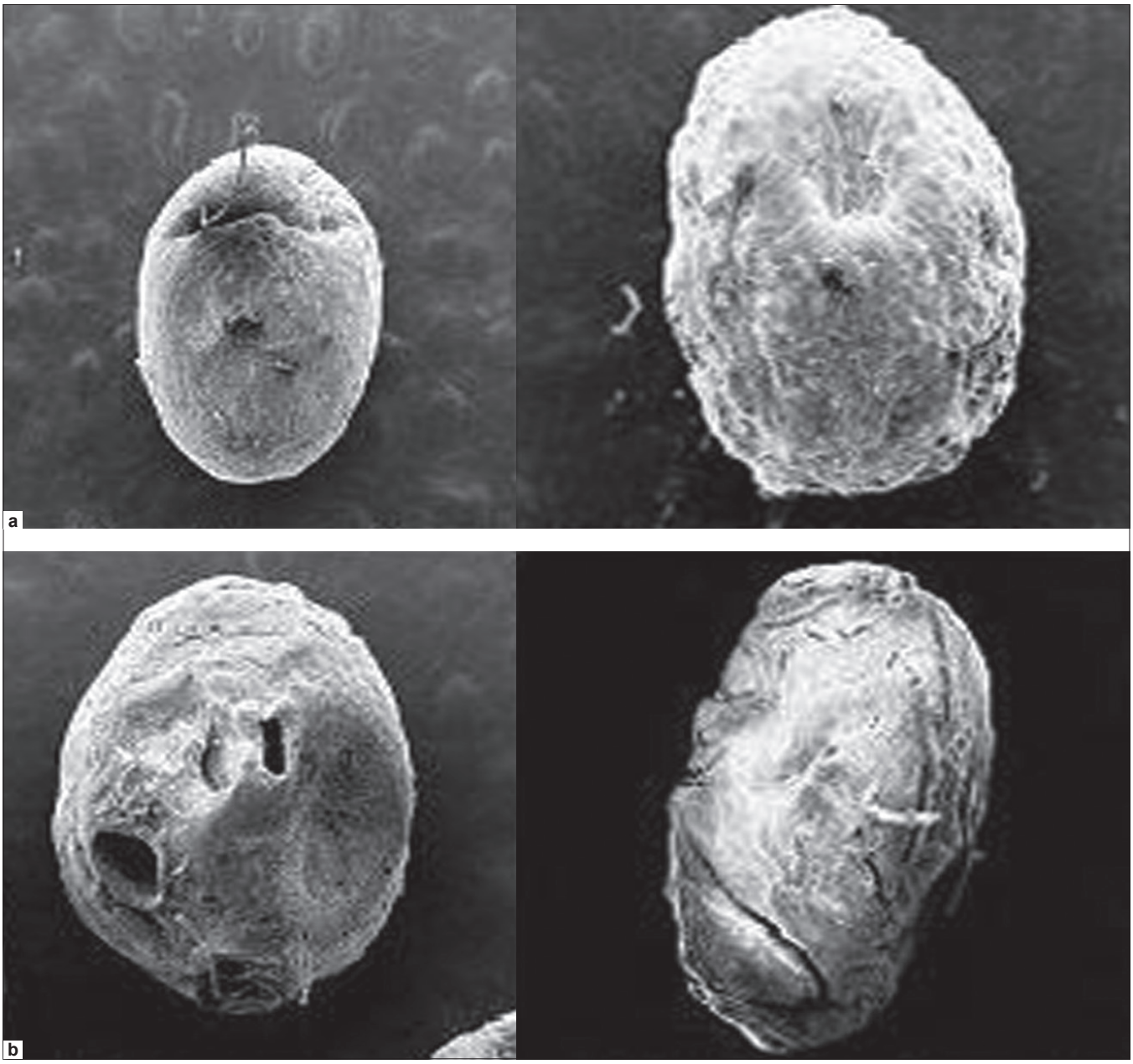

Figure 5: SEM photographs of microcapsules of salbutamol sulphate

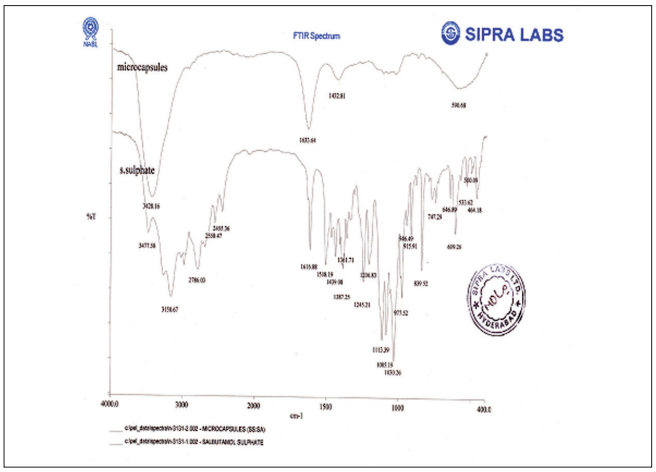

Figure 6: IR spectral overlap of alginate microcapsule (A4) and pure salbutamol

IR spectra of salbutamol sulfate and the formulation of A4 sodium alginate. Pure drug showed characteristic absorption bands at 3478 (OH-hydrogen bonding), 3158 (NH stretching), 3050 (aromatic C-H stretching), 2455-2786 [CH, CH_2, CH_3 etc aliphatic (C-H) stretching], 1616 (C=C aromatic ring stretching), 1439 nm (C-N stretching), and the formulation A4 showed characteristic absorption band at the major peaks due to OH of the pure drug salbutamol sulfate and polymer sodium alginate gives the peaks around 3428 nm in the formulated product (A4). Similarly the peaks at 1633 in the formulated alginate microcapsule (A4) is due to the NH bending and C=C ring stretching of the pure drug salbutamol sulfate. Other peaks did not appear clearly in the product, which may be due to moisture absorption that hinders the groups to resolve and give signals. The IR spectra of pure salbutamol sulfate and formulation reveled that there is no appreciable changes in the position of absorption band. This reveled that there was no chemical interaction between drug and the polymer.

Figure 7 is the DSC thermogram of salbutamol sulfate pure drug with an onset at 192.77°C and maximum occurring at 201.70°C. Figure 5 is the DSC thermogram of alginate microcapsule containing salbutamol sulfate (A4) with an onset at 70.53°C and maximum occurring at 118.74°C. DSC studies revealed the fact that, the formulated alginate microcapsule (A4) has retained the identity of its components pure drug salbutamol sulfate and polymer sodium alginate, as there is no appreciable change in their thermal properties with reference to the enthalpy changes.

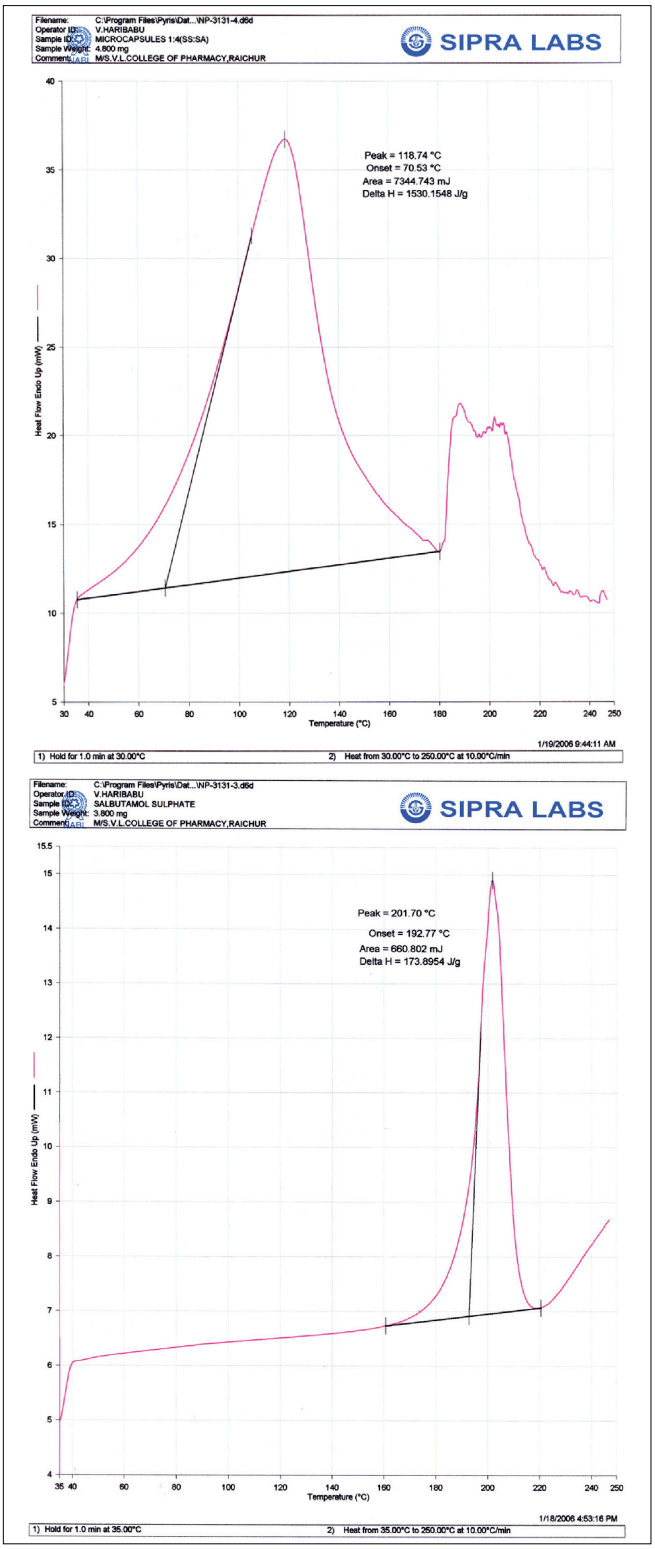

Figure 7: DSC thermogram of alginate microcapsules of salbutamol (A4), and pure salbutamol

CONCLUSION

Results revealed that alginate-HPMC microcapsule gave relatively higher release than alginate-MC. The concentrations of calcium chloride and reaction time exposure have little effects on release rate. Controlled-release pattern of alginate-HPMC microcapsule was comparable with commercial CR capsule. Higher release profiles were obtained with smaller particle size. Microcapsules prepared with alginate-HPMC and alginate-carbopol were found good with respect to release, swelling ratio, mucoadhesion, and morphological characteristics. Hence, these are suitable carriers for oral controlled release of salbutamol.

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