

RESEARCH ARTICLE

FORMULATION & CHARACTERISATION OF CHITOSAN BASED MICROSPHERES OF SALBUTAMOL SULPHATE DRY POWDER INHALER FORMULATION

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ABSTRACT

The dry powder inhalers are gaining increased attention for treating pulmonary diseases due to its unique advantages over MDIs. Salbutamol sulphate, one of the bronchodilator was selected to prepare its DPI formulation. In the present study, chitosan microspheres of salbutamol sulphate were prepared by spray drying method and evaluated in-vitro for surface morphology, particle size, drug entrapment, flow properties & % drug release. The effect of crosslinking agent (citric acid) on characteristics of microspheres was evaluated. Spray dried lactose (Inhalac@250) was used as a carrier for the study. The crosslinking agent was found to decrease the entrapment efficiency & drug release from microspheres. Out of five formulations F1 was found to be optimum showing 81.42% entrapment, 91.96% drug release in 2 hrs.

Key words: Dry powder inhaler, Spray drying, Salbutamol sulphate, Chitosan, Microspheres, Citric acid.

INTRODUCTION

The drug delivery to the respiratory tract has become an important and effective method for treatment of pulmonary diseases such as chronic obstructive pulmonary diseases including asthma, bronchitis, emphysema, lung abscess etc.¹ Pulmonary drug delivery methods have traditionally focused on one of two strategies: (i) drug suspension dissolution in liquid aerosol drops and (ii) mixtures of dry drug particulates with dry carrier particles typically composed of sugars.² Dry powder inhalers (DPIs) are the devices through which a dry powder formulation of an active drug is delivered for local or systemic effect via the pulmonary route. DPIs have number of advantages over metered dose inhalers such as direct delivery of drug into the deep lungs, propellant free, less bulky and have fewer handling errors.³ The delivery of micron sized dry powder particulates to the respiratory tract using dry powder inhalers (DPI), has become commonplace in the prophylactic treatment of asthma and other bronchial related diseases.

In case of dry powder inhaler, in order to reach the lower respiratory tract and optimize systemic drug absorption, dry powder aerosols need to present aerodynamic diameters between 1–5 μm .⁴ Recently, natural chitosan material has attracted great attention in pharmaceutical and biomedical fields because of its advantageous biological properties, such as biodegradability, biocompatibility, and nontoxicity. Chitosan is a cationic polysaccharide obtained by partial deacetylation of chitin, the major component of crustacean shells.⁵ Chitosan microspheres/microparticles can be prepared by number of techniques such as solvent evaporation, emulsification/ internal gelation, coacervation and spray drying.⁶

Spray drying technique is widely used in the pharmaceutical industries because of its numerous advantages over other methods. The advantage of spray drying technique for application to microencapsulation is that it is reproducible, rapid and easy to scale up. Spray drying technique can be used to produce dry powders,

granules or agglomerates from drug-excipient solutions and suspensions. The particle size of the microparticles prepared by spray drying technique ranged from microns to several tens of microns and had a relatively narrow distribution.⁷

Salbutamol sulphate was selected as a model drug for dry powder formulations in treatment of respiratory diseases. Salbutamol sulphate, is a β_2 -adrenoreceptor agonist and currently one of the most prescribed bronchodilators for the treatment of bronchial asthma.⁸

Thus the aim of the present research was to prepare, chitosan microspheres containing salbutamol by a spray drying method and the effects of concentration of citric acid on microsphere properties

MATERIALS & METHODS

Materials:

Salbutamol sulphate was obtained as a gift sample from Rankem, Mumbai (India), Chitosan was kind donation by Srividya Enterprises, Ratnagiri, India. Citric acid was obtained from Loba Chemicals, India. Spray dried lactose (Inhalac@250) was donated by Anshul India Pvt Ltd, Chennai, India as a gift sample. All other chemicals and solvents used were of analytical grade.

Methods:

Preparation of spray dried microspheres:

To 100 ml of 0.5 % acetic acid solution, weighed quantity of chitosan (0.5 % w/v) was added with continuous stirring on a magnetic stirrer at 100 rpm for about 1 hr for complete dissolution of chitosan. When the clear solution was formed, Salbutamol sulphate was added. After that 5 ml of citric acid solution as cross linking agent in the concentrations (0 % w/v, 0.4 % w/v, 0.8 % w/v, 1.2 % w/v, and 1.6 % w/v) were added to give 5 formulations. The formulations were spray dried by Labultima spray dryer (LU-222) with standard 0.7 mm nozzle. The spray drying

conditions such as inlet temperature, outlet temperature, pump rate, pressure and aspirator setting were set as 150⁰ - 170⁰ C, 140⁰C-150⁰C, 3 ml/min, 2 kg/cm² and 65nm³/hr. respectively. DPI formulations of microspheres were prepared with carrier. The drug loaded microspheres were mixed with lactose carrier (Inhalac®250) in mass ratio of 1:66.5 using a polybag for 5 min; further the mixture was passed through 100# and stored in a glass vial in a desiccator.^{7,9}

Evaluation of Salbutamol-chitosan microspheres:

Morphological characterization of microspheres:

Surface morphology of spray dried chitosan microspheres was studied by using scanning electron microscope (Jeol JSM 6360, Japan). Spray dried powders were mounted onto separate, adhesive coated aluminium stubs. Excess powder was removed by tapping the stubs sharply and then gently blowing a jet of particle free compressed gas across each. The SEM was operated at high vacuum with accelerate voltage of 10 kv.¹⁰

Particle Size Analysis:

The particle size was determined by Motic microscope. The photograph of spray dried chitosan microsphere mounted on the slide was taken. Using the motic image plus software particle size of 100 particles was measured.¹¹

X-Ray Diffraction study:

The physical state of the pure drug (Salbutamol sulphate) and in the spray dried chitosan microspheres were assessed by XRD studies. X ray diffraction patterns of pure drug, chitosan microspheres loaded with drug were obtained using XRD Diffractometer Philips PW 1830 instrument operated at 40 kV and a current of 30 mA with Cu K α radiation within the range (2 θ): 0⁰ -50⁰¹²

Differential Scanning Calorimetry:

The characteristics of the pure drug and microspheres were determined using differential scanning calorimetry (DSC60, Shimadzu). The scanning rate was 10°C/min, and the scanning temperature range was in between 30°C and 300°C¹³

Entrapment Efficiency:

For the assessment of entrapment of the drug into the microspheres, following method was used. Weighed quantity of microspheres was added to phosphate buffer solution (pH-7.4) and kept for 24 to extract the drug from the microspheres. After that the solution was filtered and assayed by UV-Visible spectrophotometer at 276 nm. The actual amount of drug loaded is relative to theoretical amount in microspheres was calculated in percentage and expressed as entrapment efficiency.¹⁴

Encapsulation efficiency= [Encapsulated drug / total amount of drug] x 100.

Flow properties of powder:

The good flow property of drug – carrier mixture is very important for administration of DPI. For that, the blend of microspheres with spray dried lactose (Inhalac®250) was subjected to the flow properties of powder tests including angle of repose, bulk density, tapped density, Carr's index, Hausner ratio.¹⁵

Drug content of dry powder formulation of Salbutamol sulphate-Chitosan microspheres:

Accurately weighed 100 mg blend of microsphere and carrier were dissolved in 50 ml phosphate buffer solution (pH 7.4) by stirring on magnetic stirrer for 1 hr. This solution was further kept for 24 hrs. & stirred, filtered, diluted suitably & analysed by Shimadzu 1800 UV-Spectrophotometer at 276 nm.

In-Vitro Dissolution study:

Dissolution test was performed on a USP Type II tablet dissolution test apparatus (Electrolab) at a stirring speed of 150 rpm. A Himedia dialysis membrane-150 with average flat width (42.44 mm), average diameter (25.4 mm) and capacity approx. (5.07 ml/cm) cut into equal pieces of about 5 cm x 3 cm and pre-treated with phosphate buffer 7.4. Microspheres (50 mg) were accurately weighed out on the pre-treated dialysis membrane and sealed with clips. The pouch thus formed was attached to the paddles of the apparatus using cotton threads over the clips. 900 ml of phosphate-buffer, pH 7.4, was used as dissolution medium, volumes of sample was replaced with equal quantity of fresh dissolution medium to ensure sink condition. Samples were withdrawn for analysis at specified time intervals, and analysed for salbutamol sulphate content by UV spectroscopy (Shimadzu UV-1800, Japan) at λ_{\max} 276 nm.¹⁴

RESULT & DISCUSSION

Morphological characterization of microspheres:

The SEM image of the drug loaded chitosan microspheres is shown below. The irregular shape of microspheres may be due to effect of crosslinking agent.

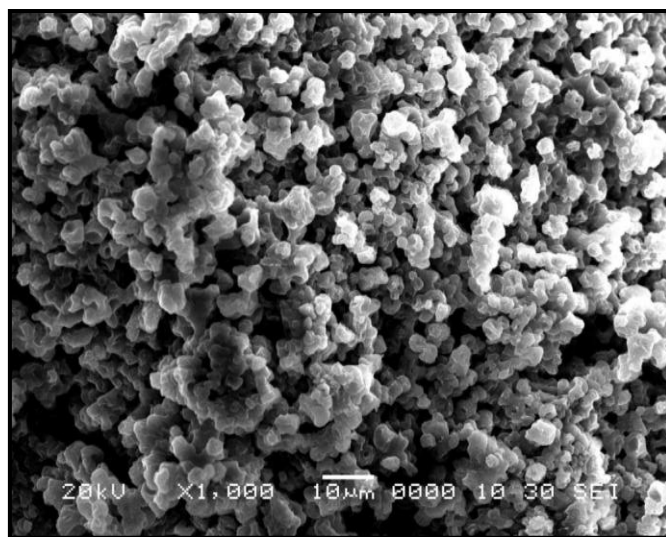


Figure 1: SEM Image of drug loaded chitosan microspheres (1000X) (Formulation F2)

Particle Size Analysis:

The particles produced from the spray drying technique were in size range between 3 – 3.5 μ m. (Table 1) for both batches indicating that there is a very little effect of crosslinking agent on the particle size. The particle size was found within the range required for dry powder inhaler.

Table 1: Particle size, % Entrapment & Drug content of Salbutamol – Chitosan microspheres

Formulation code	Crosslinking agent (% w/v)	Particle size (μm)	% Entrapment	Drug content (mg)
F1	0	3.21	81.42	1.206
F2	0.4	3.31	79.25	1.174
F3	0.8	3.15	78.55	1.163
F4	1.2	3.17	78.21	1.158
F5	1.6	3.25	77.89	1.153

X-Ray Diffraction study:

Fig.2 shows X-ray diffraction patterns of salbutamol sulphate pure, and spray dried drug loaded chitosan microspheres. The peaks on X-ray diffractogram indicate

the crystalline form of Salbutamol sulphate pure form, but these peaks were not observed in chitosan microspheres. The spray dried microspheres showed peaks of salbutamol sulphate having less intensity than pure form indicating entrapment of drug within the polymer.

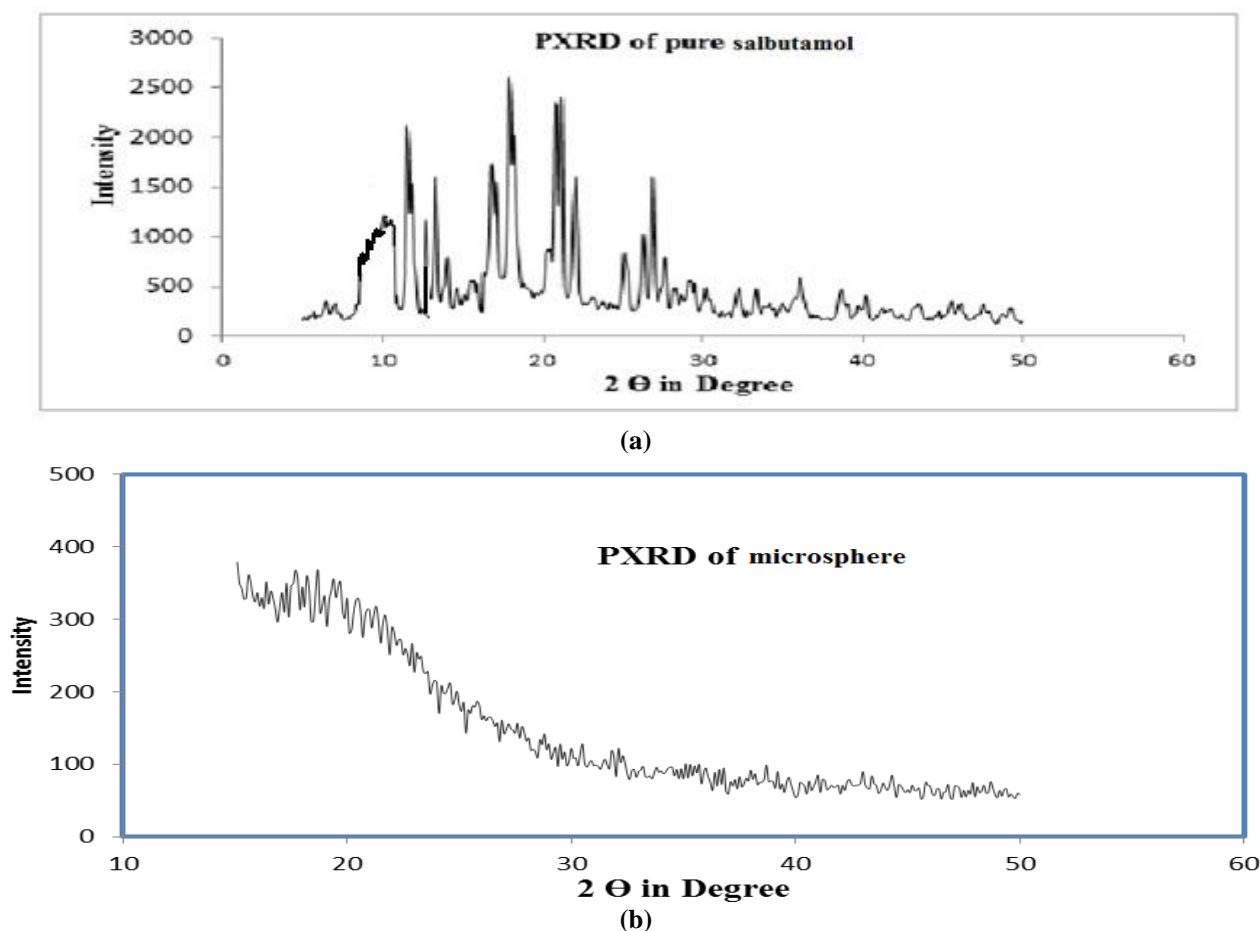


Figure 2: X-Ray Diffractograms of Salbutamol sulphate (a) & drug loaded Chitosan microspheres (b)

Differential Scanning Calorimetric (DSC) Analysis:

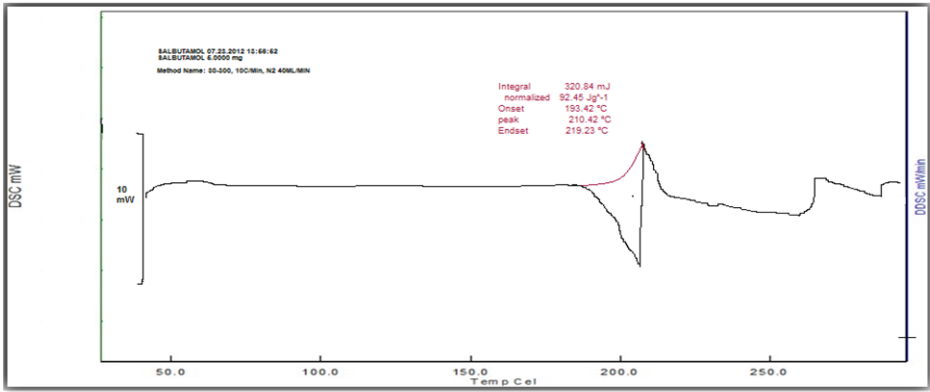
The DSC graphs of salbutamol sulphate (a) and salbutamol sulphate-loaded microspheres (b) are presented in Figure 3. The DSC Thermograph of pure drug shows a sharp endothermic melting peak with the onset of about 210°C reaching maximum at 216°C. The DSC curve of salbutamol sulphate-loaded microspheres shows broad peaks from 190 to 240°C which is due to the physicochemical binding of the drug with the polymer structure. Hence the entrapment of drug in to Chitosan microsphere can be confirmed.

Entrapment Efficiency:

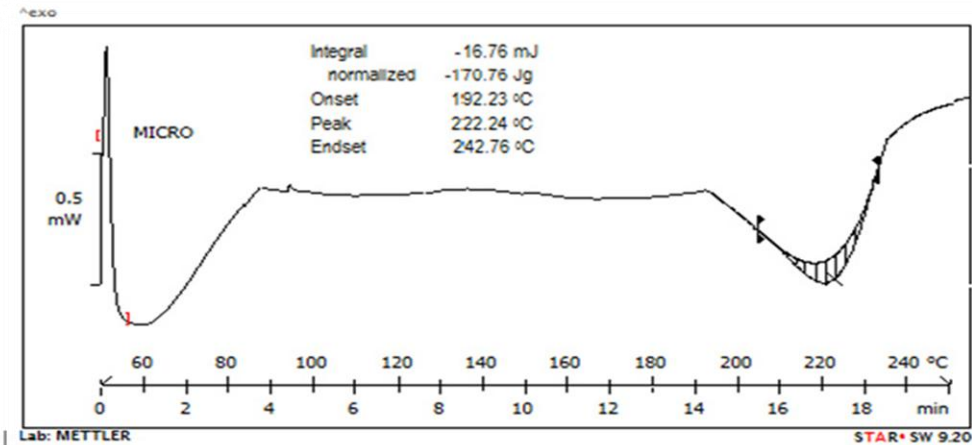
All the batches produced shows entrapment in the range of 77%-81% of drug into the microspheres (Table 1) the percentage of entrapment reduced as the concentration of crosslinking agent increased.

Flow properties of powder:

Table 2 shows the result of angle of repose, bulk density, tapped density, Carr's index, Hausner ratio. Both the batches showed values within the limits required for the excellent flow properties.



(a)



(b)

Figure 3: DSC Thermogram of (a) pure Salbutamol &(b) drug loaded microspheres

Table 2: Flow properties of powder

Parameter	F1	F2	F3	F4	F5
Angle of Repose (°)	26 ⁰ 45 ¹	27 ⁰ 51 ¹	26 ⁰ 45 ¹	26 ⁰ 51 ¹	27 ⁰ 38 ¹
Bulk Density (g/cm ³)	0.7843	0.7692	0.7843	0.7834	0.7843
Tapped Density (g/cm ³)	0.8163	0.8000	0.8334	0.8361	0.8331
Carr's Index	5.9215	4.2310	5.88231	7.69238	5.88451
Hausner Ratio	1.03825	1.04004	1.06521	1.08334	1.05231

Table 3: *In-vitro* drug release of Salbutamol sulphate-chitosan microspheres formulations

Time (min.)	% Drug release				
	F1	F2	F3	F4	F5
5	28.67±1.44	19.50±1.65	27.86±1.45	16.72±1.71	19.50±1.56
10	48.74±1.26	23.68±1.39	11.14±1.54	19.50±1.33	22.29±1.89
15	43.01±1.33	22.29±1.62	26.47±1.33	15.32±1.38	13.93±1.86
20	58.78±1.46	48.77±1.55	19.50±1.53	29.26±1.56	12.54±2.11
25	88.88±1.47	96.14±1.47	32.04±1.50	48.77±1.35	33.44±1.98
30	58.78±1.43	50.16±1.54	72.45±1.47	47.37±1.29	43.19±1.30
45	74.55±1.25	66.88±1.68	45.98±1.43	57.13±1.23	62.70±1.21
60	73.11±1.41	71.06±1.76	59.91±1.27	64.09±1.78	58.52±2.14
90	68.8±1.35	71.06±1.56	68.27±1.21	58.52±2.17	58.52±1.25
120	91.96±1.49	83.60±2.13	66.88±1.39	58.52±2.12	54.34±0.80

S.D. (n=3)

Drug content:

Table 1 shows the drug content of the formulations. The concentration of crosslinking agent affected the drug content.

In-Vitro Dissolution study:

The drug release of salbutamol sulphate from chitosan microspheres is shown in Table 3 (Fig. 4). From the *in-*

vitro dissolution study it was found that as the concentration of crosslinking agent was increased, the drug release was found to be decreased. This effect might be due to the less swelling ability of the chitosan microspheres due to increase in the crosslinking agent which impart more rigidity to the microspheres.

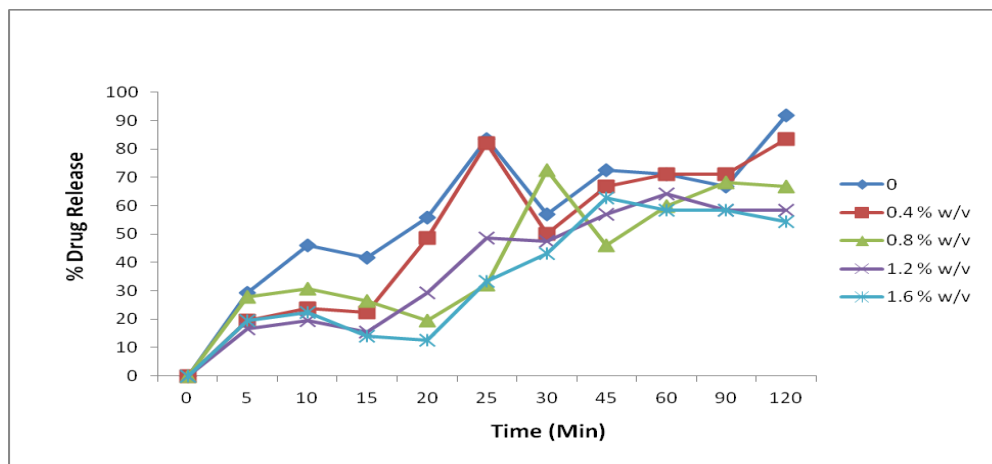


Figure 4: In-Vitro Dissolution Study of Chitosan Microspheres

CONCLUSION

Spray drying is suitable technique for the preparation of microspheres. In this project this method was successfully used to prepare salbutamol sulphate loaded chitosan microspheres in presence & absence of crosslinking agent. Spray dried lactose (Inhalac®250) was used as a carrier in this study. Crosslinking agent citric acid was found to affect the surface properties, drug entrapment & drug

release from microspheres. The drug loaded microsphere – carrier blend showed excellent flow properties. The particle size of all formulations was found to be in the range of 3.21 μ m – 3.25 μ m desired for dry powder inhaler.

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