THE EFFECTS OF FINE LACTOSE AS A THIRD COMPONENT ON AEROSOLIZATION OF CEFOTAXIME SODIUM FROM DRY POWDER FORMULATIONS

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ABSTRACT

Dry powder inhaler (DPI) formulations usually contain micronized drug particles and lactose as a carrier. Fine lactose could be used as a ternary component to improve drug delivery from DPIs. The aim of this study was to investigate the deposition profile of a model drug, cefotaxime sodium (CS), using coarse and fine carriers after aerosolization at 60 l/min via a spinhaler® into a twin stage liquid impinger (TSI). Two micronization methods. jet milling and spray drying were used to micronize the active drug and carrier. The particle size of CS and lactose were characterized by laser diffraction, and the morphology of formulations was examined by scanning electron microscopy. X-ray diffraction of jet milled lactose showed crystalline nature, but spray dried lactose exhibited an amorphous state. The results showed the existence of fine lactose in formulations significantly ($p<0.05$) influenced the deposition profiles of aerosolized jet milled CS. However, no significant ($p>0.05$) difference was observed between the effect of jet milled and spray dried lactose. On the other hand selection of micronization technique to reduce particle size of CS, was very effective on deposition profile. The highest influence of fine lactose was obtained by formulation containing jet milled CS in ratio of drug/carrier 1/1 and 10% of fine lactose as third component.

Keywords: Cefotaxime sodium, Dry powder inhaler, Spray drying, Jet milling, Fine lactose, particle engineering.

INTRODUCTION

Aerosolized administration of drugs to the lung has been employed for many years to treat localized disease states within the bronchi (1). Metered dose inhalers (MDIs) are the most commonly used inhalers for pulmonary drug delivery (2), which have some drawbacks. For example they require difficult hand-lung coordination by the patients and in normal circumstances, and only 10-15% of the dose reaches the lung (3). Dry powder inhalers (DPIs) are widely accepted as an alternative to MDIs (4). They allow generation of aerosols without the use of propellants and in contrast of MDIs the efficiency of inhalation is independent of the coordination of inhalation and actuation (5). DPIs consist of micronized drug with an aerodynamic diameter between 1 and 5 μm for deep lung deposition (6). To obtain a powder in the required size distribution, several techniques can be applied. These techniques include standard methods such as milling (7, 8), and more advanced techniques such as spray drying (9,10), spray freez drying (11), super critical fluid extraction (12), and crystallization (13).

DPI formulation is prepared from a micronized drug powder by itself or in combination with carrier particles (14). Carrier particle size is an important formulation parameter in the design of dry powder inhalers (15, 16). α-Lactose monohydrate has been employed most frequently as the carrier and it is usually designed to have a size between 63 and 90 μm for this purpose (17). A practical strategy to improve the drug delivery efficiency of DPIs may be the use of a ternary ordered mixture where the third component comprises fine particles of the carrier (18). Fine particles of ternary materials such as magnesium stearate, L-leucine and micronized glucose in DPI formulations have increased the fine particle fraction (FPF) of several drugs. (19,20). The use of fine carrier particles to improve drug delivery is likely to be preferential to the use of ternary materials, since the latter will require toxicological

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testing. Thus ternary mixtures composed of coarse lactose, fine lactose and the drug may be more efficient for the delivery of the drug to the lower airway than the binary mixtures containing only coarse lactose and drug (21). The magnitude of particle interactions between drugs and carriers will be important to the dispersion process (22).

The aim of this study was to investigate the effect of fine lactose as a third component on aerosolization of cefotaxime sodium (CS). CS represents a very good candidate for the empirical therapy of lower respiratory tract infections (23). The antimicrobial spectrum of CS includes most of the clinically relevant Gram-negative and Gram-positive bacterial organisms (24). In this study, various formulations were prepared with different weight fractions. Fine CS and fine lactose were produced by jet milling and spray drying methods and the effect of processes were evaluated by using Twin Stage Inhaler (TSI) and Spinhaler® as a device.

MATERIALS AND METHODS

CS was purchased from Hanemi, Korea. Spinhaler® (Fisons, UK) was purchased from Iranian market. Pharmatose® 80 M was supplied by DMV International, Netherlands, and hard gelatin capsule shells (size 2) by Cipla, India. Methanol (HPLC grade), ether, potassium dihydrogen phosphate and disodium hydrogen phosphate were purchased form Merck, Germany.

Jet milling

CS was micronized using an air-jet mill (JSM-80, Esco-Labor AG, Riehen, Switzerland) operating at air and inject air pressures of 6 bar. Fine particles of CS (CS JM) were collected after one passage through the instrument and were packed into tightly closed amber bottles and stored in a desicator over silica gel.

α-Lactose monohydrate (Pharmatose® 80 M) was micronized using the same apparatus operating at air and inject air pressures of 7 bar. Jet milled lactose (JL) was collected after five passages through the instrument and was packed into tightly closed amber bottles and stored in a desicator over silica gel.

Spray drying

The spray drying of CS was targeted at producing a narrow particle size range. Ten grams of CS was dissolved in 100 ml of distilled water using a magnetic stirrer. The resultant solutions were spray dried using a Büchi B-191 mini spray dryer (BÜCHI Labortechnik AG, Flawil, Switzerland) at an inlet temperature of 100 ^{o}C, outlet temperature of 87–89 ^{o}C, aspiration setting of 70% and spray flow of 600 Nl/h. Immediately after termination of the process, the particles of CS (CS SD) were collected and packed into tightly closed amber bottles and desiccated over silica gel.

The same procedure was used for 40 grams of Pharmatose® 80 M in 100 ml of distilled water. The spray dried particles (SL) were packed into tightly closed amber bottles and desiccated over silica gel.

Sieve fractionation

Pharmatose® 80 M was sieved manually between 90 and 63µm for 10 min. The fraction collected over the 63µm sieve was retained in tightly closed amber bottles and stored in a desicator over silica gel.

Particle size determination

Particle size distributions were measured by laser diffraction (Malvern Mastersizer X, Malvern, UK) using a 100 mm lens at an obscuration between 0.19 and 0.21.

CS samples were prepared by suspending the particles in ether by the aid of sonication in a water bath for 3 min. Fine lactose samples were prepared in butanol by the aid of sonication in a water bath for 3 min. Each sample was measured in triplicate.

Scanning electron microscopy

The morphology of particles was examined by SEM (CamScan MV 2300, England). Prior to scanning, the samples were coated with a thin layer of gold, using a direct current sputter technique (Bio-rad e5200, England).

X-ray diffraction (XRD)

Powder X-ray diffraction paterns were measured using a Philips X-ray diffractometer (Philips, Xpert – Pro, Netherland) with a Cu Kα source operating at a tube load of 40 kV and 30 mA. Each sample was assessed between 5 and 35^{0} (2θ) with a step size of 0.02^{0}.

Determination of water content

The water contents of samples were analayzed according to the Karl Fisher moisture method of the European Pharmacopoeia 2000 (method A). The measurements were performed with a Toledo® DL38 KF Titrator (Mettler Ltd, Switzerland).

Infrared spectroscopy

Infrared spectra of samples were obtained with a Nicolet spectrophotometer (Magna 550, Nicolet Instrument Corporation, USA) in the 4000–6000 cm^{-1} region using compressing KBr disc technique. The spectra were obtained by averaging 64 scans at a resolution of 4 cm^{-1}.

Density measurement

A helium pycnometer (Quantachrome Instruments, USA) was used to determine true densities of the

powders. Approximately 1 g of each powder sample was used after calibration of the instrument using standard stainless steel spheres supplied by the manufacturer. The mean values of triplicate determinations are reported.

Preparation of formulations

The formulations were prepared by mixing CS with coarse and fine lactose in different ratios (Table1) in a turbula mixer (Dorsa Novin Afzar, Iran) for 30 min. Fine lactose was pre-blended with coarse lactose for 15 min before addition of CS to optimize the efficiency of mixing process. The mixtures were filled in hard gelatin capsules (size 2) manually equivalent to 40mg ± 1% CS.

HPLC analysis of CS

CS was assayed by HPLC (Waters, Millipore, USA) employing a 15 cm × 3.9 mm C-18 Nova-pack column according to British Pharmacopoeia 2004 with some modifications (25). The mobile phase was prepared as follows: 3.5 g of potassium dihydrogen phosphate and 11.6 g of disodium hydrogen phosphate were disolved in 1000 ml of water at pH 7.0, the resulting mixture was filtered through a 0.22 µm membrane filter, and after addition 180 ml of methanol, it was degassed prior to use. The column effluent was monitored at 235 nm and flow rate was 1 ml/min. The system was calibrated using standard solutions of CS over the range of 0.25–16 µg/ml ($R^2 = 0.999$).

Content uniformity method

The potency of CS from ten capsules of each formulation was determined separately and their coefficients of variance (CV) were calculated.

In vitro deposition

The in vitro aerosol behavior of the formulations was investigated in terms of fine particle fraction (FPF). The FPF values were obtained using Spinhaler® connected to TSI (Apparatus A, European Pharmacopoeia 2000, Copley, Nottingham, UK). Powders were dispersed at steady flow rate of 60 l/min. HPLC mobile phase was introduced to upper stage (stage 1; 7 ml) and lower stage (stage 2; 30 ml) of the TSI. Once the assembly had been checked and found to be airtight and vertical, a Spinhaler® had been inserted into the rubber mouthpiece attached to the throat of the impinger. One capsule was placed in the inhaler and the vacuum pump was switched on. The pump was operated for 5 sec so that a steady flow rate of 60 l/min was achieved, and the dose was released. The pump was operated for another 5 sec at the established flow rate following the release of the dose and it was then switched off. All formulations were aerosolized for this period to compare their deposition data under the same condition. Each deposition experiment involved aerosolization of one capsule. The inhaler body, capsule shells of stages 1 and 2 were separately washed with mobile phase. Concentration of CS in each sample was analyzed by the HPLC method.

The total amount (mg) of CS recovered from the inhaler, the capsule shells, the upper and lower stages of TSI was calculated per capsule and defined as recovered dose (RD). The amount of drug deposited on the upper and lower stage of TSI, were identified by S_1 and S_2 respectively. FPF was calculated as the ratio of S_2 to RD and expressed as a percentage. The emission was calculated as the percentage of total amount of S_1 and S_2 to RD. Statistical analysis was performed using a one-way analysis of variance (one way ANOVA) with multiple comparison data using a Tukey honest significant difference test (Statistica, StatSoft, Tulsa, USA).

RESULTS AND DISCUSSION

Physical characteristics

Physical characterisation of micronized lactose particles are presented in Table 2. The particle size distribution of JL and SL, with 50% of particles ($d_{50\%}$) were less than 5.1 µm and 4.6 µm respectively. These results suggested volumetric particle size distributions from two techniques were almost similar and they were suitable for use as a fine carrier.

The morphology of sieved lactose (63-90 µm), JL and SL were analyzed by SEM (Fig 1). The sieved lactose and JL exhibited a tomahawk shape, typical of α-lactose monohydrate. SL particles consisted of sphere-like particles with smooth surface.

XRD scans of the samples are illustrated in Fig. 2. JL (Fig 2a) showed some sharp peaks. These data confirmed that JL had definitely crystalline state. The presence of α-lactose monohydrate in JL was supported by a peak at 12.6^0, whereas the broad (halo) scattering pattern for SL (Fig 2b) confirmed that the sample was amorphous.

The infrared spectra of JL and SL are shown in Fig 3. The water absorption band is often very evident in near-infrared region of the spectra (26). The big absorption bands that observed for jet milled powders (Fig 3a) at 5169 cm^{-1} indicated the presence of higher water content, compared to SL (Fig 3 b). The high absorption bands for JL could be attributed to molecules of water in crystal. These results were in agreement with the results

Table 1. The components of DPI formulations.

Formulations	CS[a]		Drug/carrier		amount of JL[d]		amount of SL[e]	
	JM[b]	SD[c]	1/1	2/1	5%	10%	5%	10%
F_1	+	-	+	-	-	-	-	-
F_2	+	-	+	-	+	-	-	-
F_3	+	-	+	-	-	-	+	-
F_4	+	-	+	-	-	+	-	-
F_5	+	-	+	-	-	-	-	+
F_6	+	-	-	+	-	-	-	-
F_7	+	-	-	+	+	-	-	-
F_8	+	-	-	+	-	-	+	-
F_9	+	-	-	+	-	+	-	-
F_{10}	+	-	-	+	-	-	-	+
F_{11}	-	+	+	-	-	-	-	-
F_{12}	-	+	+	-	+	-	-	-
F_{13}	-	+	+	-	-	-	+	-
F_{14}	-	+	+	-	-	+	-	-
F_{15}	-	+	+	-	-	-	-	+
F_{16}	-	+	-	+	-	-	-	-
F_{17}	-	+	-	+	+	-	-	-
F_{18}	-	+	-	+	-	-	+	-
F_{19}	-	+	-	+	-	+	-	-
F_{20}	-	+	-	+	-	-	-	+

a = cefotaxime sodiume, b = jet milled, c = spray dried, d = jet milled lactose; e = spray dried lactose.

Table 2. Particle size distribution, densities, and water content of the JL and SL (mean ± SD, n=3).

Sample	Cumulative percent (undersize)			Water content (%)[a]	True density (g/ml)
	$d_{10\%}$ (μm)	$d_{50\%}$ (μm)	$d_{90\%}$ (μm)		
JL	1.3 (0.1)	5.1 (0.3)	13.9 (1.2)	4.2 (0.1)	1.48 (0.01)
SL	1.9 (0.2)	4.6 (0.5)	15.2 (1.5)	0.9 (0.1)	1.55 (0.01)

a = Determined by karl fisher method.

obtained by the karl fisher technique and were in accordance with previous findings reported for fine lactose that were prepared by jet milling and spray drying techniques (2).

The particle size distribution for CS JM and CS SD showed that 50% of particles ($d_{50\%}$) were less than 2.93 μm and 5.04 μm, respectively. SEM photographs (Fig 4) indicated that morphology of particles of two samples were completely different. From the SEM pictures, it is clear that CS JM (Fig 4a) and CS SD (Fig 4b) were comprised of rough and special microparticles with some concavity, respectively which was consistent with previous finding (27). The unique shape of CS SD could be improved by the aerodynamic properties of the powders.

Content uniformity

The recovery of CS from ten samples of each formulation, in content uniformity test was between 97.33% and 98.11%. All formulations presented a CV less than 3%, therefore mixing of samples seems to be quite satisfactory.

In vitro deposition

Deposition data for CS after aerosolization of the samples at 60 l/min through a spinhaler®, using TSI, are presented in Table 3. These data show that the presnce and amounts of fine lactose, drug/carrier ratio, and also micronization method of CS could influence the deposition profiles, but the method of micronization of lactose had no effect on deposition data in same formulations. Most of formulations containing CS SD produced significantly ($p<0.05$) higher S_1, S_2 FPF and emission of CS than formulations containing CS JM. It could be related to differences of morphology between two kinds of CS microparticles due to micronization methods. Although the CS SD powders had a higher emitted dose, this resulted in substantial deposition in the S1 stage, equivalent to oropharyngeal deposition (potential side effects), with virtually the same mass deposited in the lung.

The influences of amounts of fine lactose (5% and 10%) on FPF in formulations containing CS JM and CS SD are shown in Fig. 5 and 6, respectively. It is clear that fine lactose had significant ($p<0.05$) effect on FPF in formulations containing CS JM. In drug/carrier ratio 1/1 of these formulations, by increase of fine lactose (from 0% to 10%), FPF increased (from 22.5% to 28.04%), and in formulations with drug/carrier

Table 3. Deposition data for cefotaxime sodium in different formulations after aerosolization of the samples at 60 l/min through a spinhaler® using TSI (mean ± SD, n=3).

Formulations	S_1[a](mg)	S_2[b](mg)	Emission (%)	FPF[c] (%)
F_1	9.3±0.84	8.91±0.21	46.02±0.50	22.5±0.29
F_2	8.80±0.53	9.55±0.55	46.39±0.89	24.14±0.67
F_3	8.72±0.36	9.49±0.42	46.17±0.89	24.06±0.54
F_4	7.29±0.54	11.03±0.41	46.38±0.53	27.91±0.69
F_5	7.32±0.41	11.09±0.36	46.55±0.58	28.04±0.62
F_6	10.70±0.61	6.89±0.20	44.46±0.45	17.41±0.21
F_7	7.73±0.44	9.15±0.41	42.74±0.75	23.17±0.61
F_8	7.63±0.41	9.19±0.42	42.57±0.54	23.26±0.64
F_9	7.40±0.45	8.31±0.43	39.58±0.92	20.94±0.57
F_{10}	7.39±0.39	8.24±0.32	39.52±0.57	20.83±0.52
F_{11}	22.47±0.57	9.61±0.19	81.09±1.08	24.29±0.33
F_{12}	22.30±0.43	9.71±0.49	80.81±0.92	24.51±0.53
F_{13}	22.47±0.55	9.62±0.51	81.18±0.94	24.34±0.56
F_{14}	22.36±0.43	9.66±0.59	80.98±0.76	24.43±0.58
F_{15}	22.40±0.59	9.71±0.48	81.07±0.97	24.51±0.49
F_{16}	22.98±0.51	12.19±0.24	88.2±0.49	30.57±0.27
F_{17}	23.07±0.69	11.95±0.45	88.64±0.85	30.25±0.52
F_{18}	23.03±0.66	12.02±0.43	88.62±0.84	30.39±0.69
F_{19}	22.65±0.71	10.59±0.41	84.17±0.73	26.82±0.55
F_{20}	22.69±0.74	10.47±0.57	84.06±0.78	26.54±0.57

a = Stage 1 , b = Stage 2 and c = fine particle fraction.

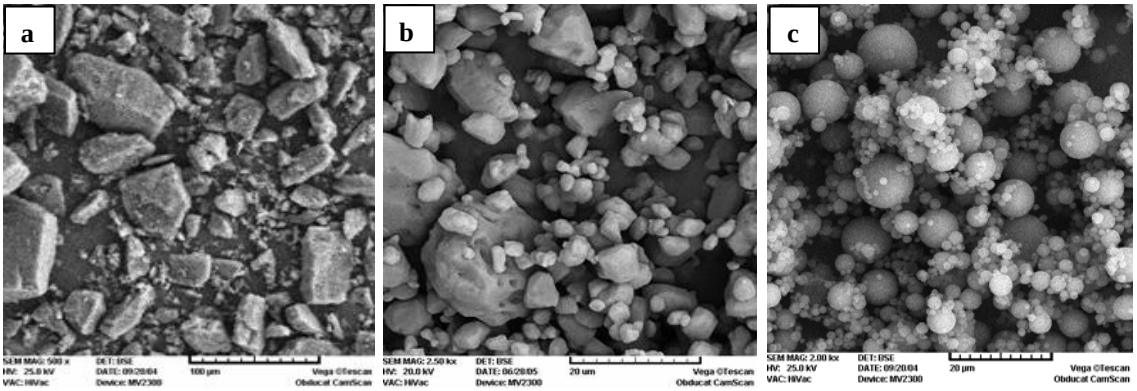

Figure 1. Scanning electron micrographs of (a) sieved lactose (63-90 μm), (b) jet milled lactose and (c) spray dried lactose.

ratio in 2/1, by increase of fine lactose (from 0% to 5%), FPF increased (from 17.41% to 23.26%), but in 10% of fine lactose formulations, FPF decreased (20.83%). Existence of fine lactose in formulations containing CS SD, had no significantly ($p>0.05$) effected on FPF, except for drug/carrier ratio of 2/1. As shown in Fig. 7, CS SD formulations had significantly ($p<0.05$) higher emission than CS JM formulations. These results could be related to differences in flow properties of CS JM and CS SD due to micronization method, and thereby characteristics of CS. It is known that the techniques of micronization may have influence on flow properties (28).

The different tendency of CS JM and CS SD microparticles in interaction with the surface of lactose particles were observed qualitatively by SEM (Fig. 8). In formulations containing CS JM (Fig. 8 a, and b), CS microparticles covered the surface of coarse lactose particle completely. In contrast, CS SD particles exhibited lower tendency to interact with carrier (Fig. 8 c, and d).

It seems for the CS JM formulations, two major factors (29) were very important for desirable deposition data: the detachment of drug particles from the large carriers and dispersion of the detached drug particles during aerosolization, and subsequent deposition in the lower airways of lung. For the CS SD formulations only dispersion of drug particles through aerosolization was required.

The role of fine lactose as a third component depends to the method of micronization of CS. In CS JM formulations with drug/carrier ratio of 1/1, increase of fine lactose particles, that has been advanced to explain dispersion involves interaction of fine lactose present in the lactose excipient with the high energy active sites on the

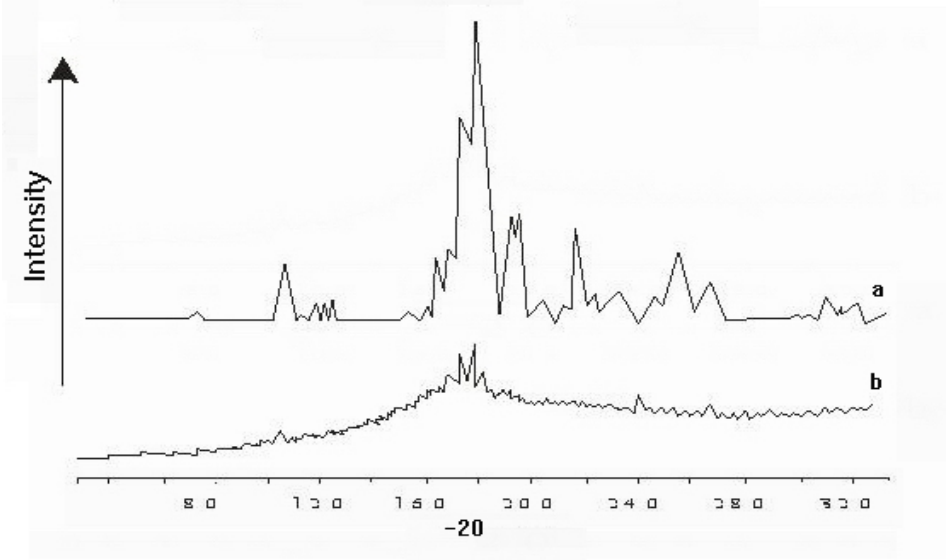

Figure 2. X-ray diffraction patterns of (a) jet milled lactose and (b) spray dried lactose.

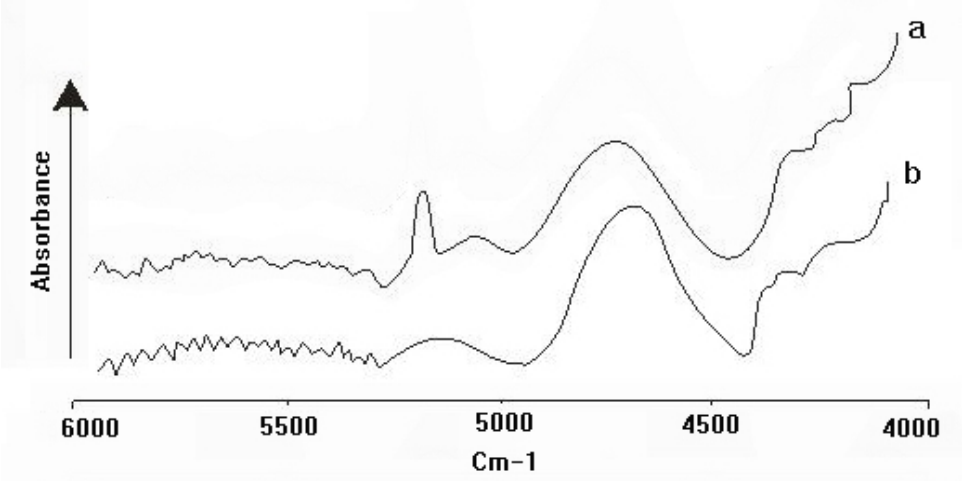

Figure 3. Infrared spectra of (a) jet milled lactose and (b) spray dried lactose at 6000-4000 cm^{-1}.

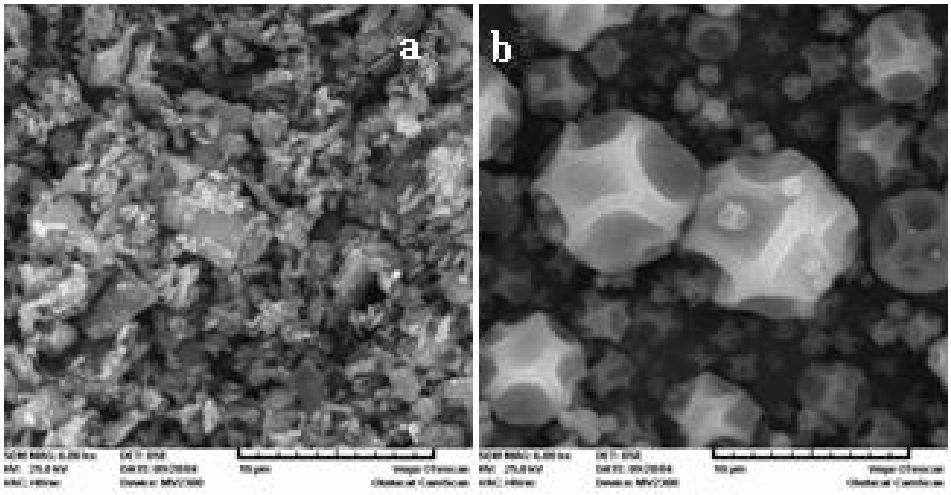

Figure 4. Scanning electron micrographs of (a) jet milled cefotaxime sodium, and (b) spray dried cefotaxime sodium.

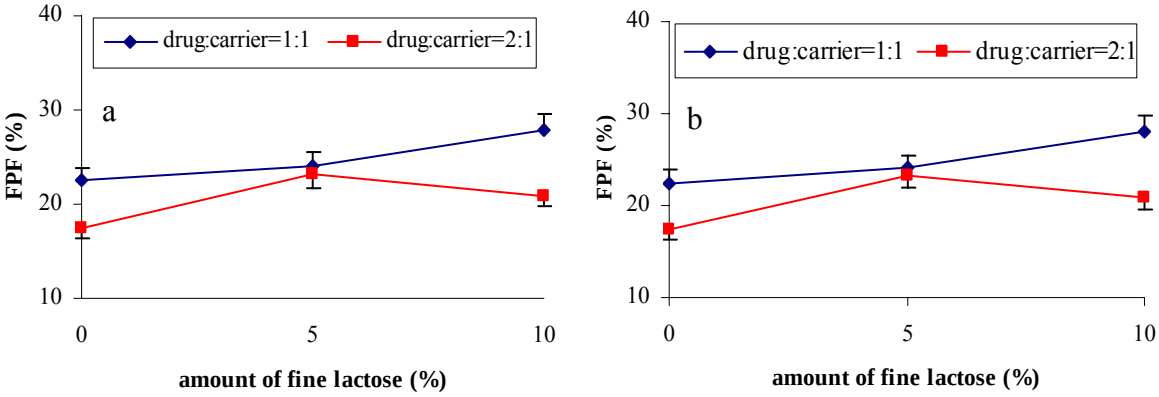

Figure 5. Influences of amounts of fine lactose on fine particle fraction in formulations containing jet milled cefotaxime sodium via Spinhaler® at 60 l/min using TSI (mean ± SD, n=3) (a) jet milled lactose and (b) spray dried lactose.

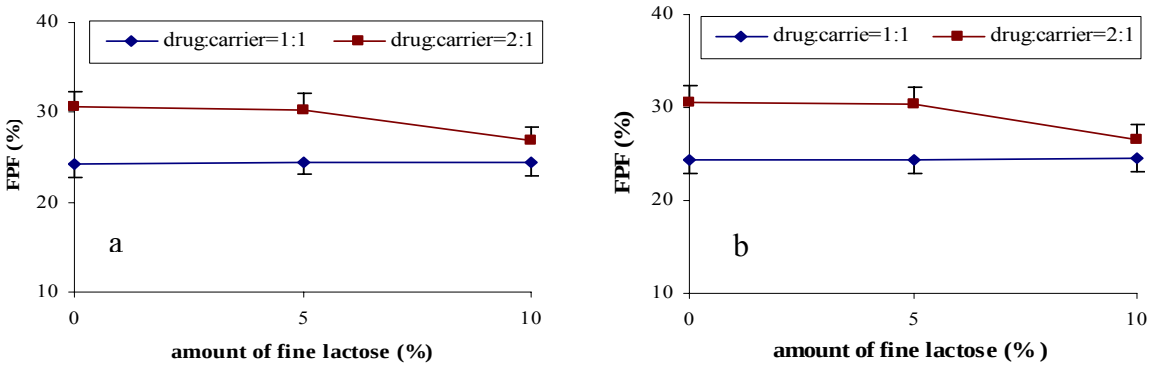

Figure 6. Influences of amounts of fine lactose on fine particle fraction in formulations containing spray dried cefotaxime sodium via Spinhaler® at 60 l/min using TSI (mean ± SD, n=3) (a) jet milled lactose and (b) spray dried lactose.

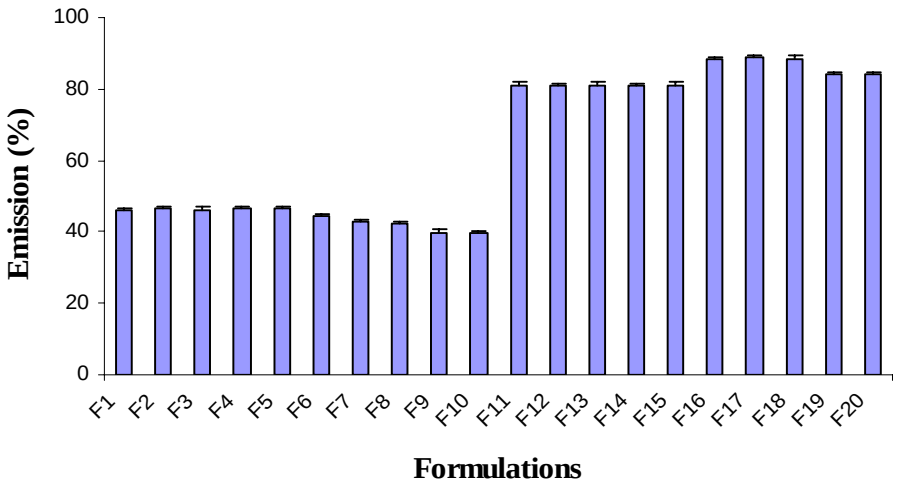

Figure 7. Emission after aerosolisation of all formulations via Spinhaler® at 60 l/min using TSI (mean ± SD, n=3).

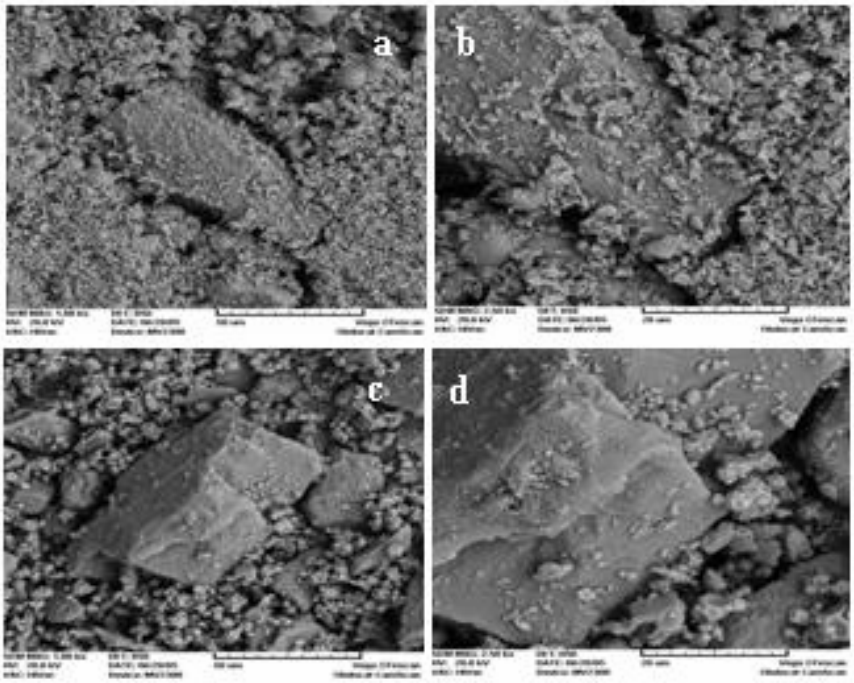

Figure 8. Scanning electron micrographs of formulations (a), (b) jet milled cefotaxime sodium (F_4 formulation), (c),and (d) spray dried cefotaxime sodium (F_{14} formulation).

carrier's surface saturation of these active sites, leaving low-energy passive sites available for drug adhesion. The reduced adhesion between drug and carrier particles increased drug detachment. However, in drug/carrier ratio 2/1 formulations containing CS JM, increase of fine lactose particles, led to saturation of high-energy sites, and remaining of fine lactose particles could compete with active drug particles to occupy low-energy sites of coarse lactose. Then with increase of fine lactose, FPF was decreased. These results were in agreement with the theory of the role of fine lactose in formulations of DPIs (18, 22). On the other hand, presence of the fine lactose in the formulation containing coarse lactose as the carrier was shown to reduce the magnitude of electrostatic charge (30) and could improve deposition data.

In formulations containing CS SD in drug/carrier ratio 1/1, due to low interaction between active drug and coarse lactose, the amount of fine lactose particles could not significantly ($p>0.05$) influence FPF in comparison with the same formulation without fine lactose. While in CS SD formulations with drug/carrier ratio in 2/1, increase of fine lactose particles, could compete in aerosolization with active drugs, and then could cause a decrease in FPF.

CONCLUSSION

Jet milling and spray drying micronization techniques to prepare micronized CS as active drug and fine lactose as third component was applied to investigate deposition profile of CS in dry powder inhaler. Addition of lactose fine particles to the dry powder aerosol formulation appears to reduce the drug-carrier interaction by occupying possible drug binding sites on the larger lactose particles. The major outcome of this research was that fine lactose in appropriate amount improves FPF of CS JM, but in CS SD formulations the effect of fine lactose was not significant ($p>0.05$) and even in higher amount caused a decrease in FPF.

The results of this study also showed that emission of CS formulations was influenced by characteristics of CS due to the method of micronization. While micronization method is of critical importance in particle size reduction of CS, fine lactose prepared by both method of jet milling and spray drying showed insignificant differences in same formulations.

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REFERENCES

1. Zeng XM, Martin GP, Marriott C. The controlled delivery of drugs to the lung. Int J Pharm 1995; 124: 149-164.
2. Gilani K, Najafabadi AR, Darabi M, Barghi M, Rafiee-Tehrani M. Influence of formulation variables and inhalation device on the deposition profiles of cromolyn sodium dry powder aerosols. DARU 2004; 12: 123-130.
3. Malcolmson RJ, Embleton, JK. Dry powder formulations for pulmonary delivery. Pharm Sci Technol 1998; 1: 394-398.
4. Gilani K, Najafabadi AR, Barghi M, Rafiee-Tehrani M. Aerosolisation of beclometasone dipropionate using spray dried lactose/polyethylene glycol carriers. Eur J Pharm Biopharm 2004; 58: 595-606.
5. Crompton CG. Problems patients have using pressureized aerosol inhalers. Eur J Respire Dis 1982; 63: 101-110.
6. Byron PR. Some future perspectives for unit dose inhalation aerosols. Drug Dev Ind Pharm 1986; 12: 993-1015.
7. Lizio R, Klenner T, Sarlikiotis AW, Romeis P, Degenhard M, Nolte T, Jahn W, Borchard G, Lehr CM. Systemic delivery of cetrorelix to rats by a new aerosol delivery system. Pharm Res 2001; 18: 771-779.
8. Johnson KA. Preparation of peptide and protein powders for inhalation. Adv Drug Deliv 1997; 26: 3-15.
9. Elversson J, Millqvist-Fureby A, Alderborn G, Elofsson U. Droplet and particle size relationship and shell thickness of inhalable lactose particles during spray drying. J Pharm Sci 2002; 92: 900-910.
10. Stahl K, Claesson M, Lilliehorn P, Linden H, Bäckström K. The effect of process variables on the degradation and physical properties of spray dried insulin intended for inhalation. Int J Pharm 2002; 233: 227-237.
11. Rogers TL, HU J, YU Z, Johnston KP, Williams RO. A novel particle engineering technology: spray-freezing into liquid. Int J Pharm 2002; 242: 93-100.
12. Shekunov BY, Feeley JC< Chow AHL, Tong HHY, York P. Aerosolization behavior of micronized and supercritically processed powders. J Aerosol Sci 2003; 34: 553-568.
13. Rasenack N, Steckel H, Müller BW. Micronization of anti-inflammatory drugs for pulmonary delivery by a controlled crystallization process. J Pharm Sci 2003; 92: 35-44.
14. Ganderton D. The generation of respirable clouds from coarse powder aggregates. J Biopharm Sci 1992; 3: 101-105.
15. French DL, Edward DA, Niven RW. The influence of formulation on emission, deaggregation and deposition of dry powder for inhalation. J Aerosol Sci 1996; 27(5): 769-783.
16. Kassem NM, Ho KKL, Ganderton D. The effect of air flow and carrier size on the characteristics of an inspirable cloud. J Pharm Pharmacol 1989; 41: 14-21.
17. Timsina MP, Martin GP, Marriot C, Ganderton D, Yianneskis M. Drug delivery to the respiratory tract using dry powder inhalers. Int J Pharm 1994; 101: 1-13.
18. Zeng XM, Martin GP, Tee SK, Marriott C. The role of fine particle lactose on the dispertion and deaggregation of sulbutamol sulphate in an air stream in vitro. Int J Pharm 1998; 176: 99-110.
19. Louey MD, Stewart PJ. Particle interactions involved in aerosol dispersion of ternary interactive mixtures. Pharm Res 2002; 19(10): 1524-1531.
20. Staniforth JN. Improvement in dry powder inhaler performance: surface passivation effects. Proc Drug Deliv Lung (London) 1996; 7: 86-89.
21. Zeng XM, Martin GP, Tee SK, Ghoush AA, Marriott C. Effects of particle size and adding sequence of fine lactose on the deposition of salbutamol sulphate from a dry powder formulation. Int J Pharm 1999; 182: 133-144.
22. Zeng XM, Tee SK, Martin GP, Marriott C. Effect of mixing procedure and particle size distribution of carrier particles on the deposition of sulbutamol sulphate fro dry powder inhaler formulations. Proc Drug Deliv Lung (London) 1996; 7: 40-43.
23. Boccazzi A, Tonelli P, Bellosta. C, Careddu P. Clinical and pharmacological evaluation of a modified cefotaxime bid regimen versus traditional tid in pediatric lower respiratory tract infections. Diagn microbial infect 1998; 32: 265-272.
24. Physicians' Desk Reference. Published by Thomson 2004; 58: 733-736.
25. British Pharmacopoeia, Volume I HMS London 2005; p.392-393.
26. Britain HG, Bogdanowich SJ, Bugay DE, DeVincentis J, Lewen G, Newman AW. Physical characterization of pharmaceutical solids. Pharm Res 1991; 8: 963-973.
27. Najafabadi AR, Asgharian R, Tajerzadeh H, Gilani K, Vatanara A, Darabi M. Evaluation of cefotaxime sodium microparticles for respiratory drug delivery. Res Drug Deliv Europe (Paris) 2005; 265-268.
28. Hickey AJ, Concessio NM, Van Oort MM, Platz R.M. Factors influencing the dispersion of dry powders as aerosols. Pharm Technol 1994: 8, 58-64.
29. Newman SP, Clarke SW. Therapeutic aerosol. 1. Physical and practical considerations. Thorax 1983; 38: 881-886.
30. Bennet FC, Carter PA, Rowly G, Dandiker, Y. Modification of electrostatic charge on inhaled carrier lactose particles by addition of fine particles. Drug Dev. Ind Pharm 1999; 25: 99-103.