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Effect of carrier morphology and surface characteristics on the development of respirable PLGA microcapsules for sustained-release pulmonary delivery of insulin

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ABSTRACT

The effect of morphology and surface characteristics of carriers were investigated for development of dry powder inhaler (DPI) formulation of insulin-loaded poly (D,L-lactic-co-glycolic acid) microcapsules for sustained-release pulmonary delivery of insulin. Microcapsule/carrier powder mixtures were prepared consisting of insulin-loaded PLGA microcapsules and sorbitol or mannitol as the carriers with various particle surface morphologies prepared by spray-drying and freeze-drying techniques. Powders were assessed by particle size analyzer, scanning electron microscopy, surface area analyzer, atomic force microscopy, helium pycnometer, X-ray diffraction, differential scanning calorimetery, bulk and tapped densitometers. Aerosol dispersion of microcapsules was examined by a twin impinger using Spinhaler® device. The flowability results showed that the lowest ($27.51 \pm 2.24\%$) and the highest ($48.53 \pm 3.36\%$) Carr's indices were obtained for the samples containing sieved mannitol and spray-dried mannitol, respectively. The *in vitro* inhalation properties of the powder mixture prepared using various carrier shape and surface morphology were different, suggesting that the separation of microcapsules from carrier was a determining step to improve inhalation properties of DPIs. The results showed that the highest fine particle fraction ($18.3 \pm 1.65\%$) and fine particle dose (62.22 ± 3.74 μg) were obtained for the microcapsules formulated with sieved mannitol and these values were the lowest when the sieved mannitol was replaced by sieved sorbitol ($10.5 \pm 0.86\%$ and 35.70 ± 2.51 μg). It was concluded that optimization of surface roughness is a critical parameter in development of DPIs. It also suggested that the elongation of carrier particles may play an important role in determining the aerosolization properties of the microcapsules. It seems that decrease in crystalline content of carriers may contribute to a decreased in fine particle fraction delivered.

1. Introduction

The large alveolar surface area renders the respiratory tract a suitable site for drug absorption due to the thinness of the epithelial barrier, extensive vascularization and relatively low proteolytic activity compared to other administration routes, and the absence of the first-pass effect (Courrier et al., 2002). However, much less attentions has been given to the possibility of using the lungs as a site for controlled drug release and prolong absorption, polymer particles are often needed to achieve these types of advanced, controlled-release drug delivery (Edwards et al., 1997). Poly (D,L-lactic-co-glycolic acid) PLGA, a biodegradable polymer, is well known for its safety in biomedical preparations and it has been approved for human use by the US food and drug administration (FDA) (Langer, 2000). Pressurized metered-dose inhalers (MDI), nebulizers, and dry powder inhalers (DPI) are the three main delivery systems used for aerosol inhalation in humans (Timsina et al., 1994). Among these, DPI appears to be the most promising for future use (Todo et al., 2001). They are propellant-free, portable, easy to operate and low-cost devices with improved stability of the formulation as a result of the dry state (Carpenter et al., 1997; Prime et al., 1997). DPI is generally formulated as a powder mixture

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of coarse carrier particles and micronized drug particles with aerodynamic particle diameters of 1–6 μm (Iida et al., 2003a). Carrier particles are used to improve drug particle flowability, thus improving dosing accuracy and minimizing the dose variability observed with drug formulations alone while making them easier to handle during manufacturing operations (Timsina et al., 1994; Schiavone et al., 2004).

With the use of carrier particles, drug particles are emitted from capsules and devices more readily, hence, the inhalation efficiency increases (Iida et al., 2001). Consequently, the carrier forms an important component of the formulation and any change in the physicochemical properties of the carrier particles has the potential to alter the drug deposition profile (Zeng et al., 2000b). Therefore, the design of the carrier particle is important for the development of dry powder inhalations. Currently, lactose is the only excipient used in DPIs marketed in the United States. The reasons for this are as much historical as they are physicochemical/pharmaceutical in nature (Telko and Hickey, 2005). Because of clinical considerations lactose or other sugars cannot be used for drug delivery to diabetic patients. Moreover, for some drugs, e.g., formoterol, or for specific applications, e.g., peptide or protein drugs, lactose monohydrate may not be the carrier of choice due to its reducing sugar function that may interact with functional groups of the drug or the protein, respectively (Patton and Platz, 1992). In addition, lactose monohydrate is produced from bovine or with bovine-driven additives so that the transmissible spongiform encephalopathy (TSE) is still an issue for this compound (EC Statement, 2002). Lactose intolerance is a problem that necessitates the patient to use lactose-free formulations (Glasnapp, 1998). It is therefore logical to look for substitute carriers that still possess the positive aspects but overcome the above mentioned drawbacks of lactose monohydrate. It was the aim of this study to explore potential candidate as alternative carriers in dry powder inhalation products. Mannitol has been shown to be potential alternatives to lactose, and it is expected that it will finally find its way into approved excipient for use in DPIs (Telko and Hickey, 2005). Mannitol does not have a reducing sugar function and is less hygroscopic (Saint-Lorant et al., 2007). Sorbitol, although a stereo-isomer of mannitol, has different physical properties compared with mannitol. It could be concluded that mannitol or sorbitol could be employed in place of lactose as possible coarse carriers for inhaled drugs (Tee et al., 2000). The *in vitro* inhalation properties of DPI are reported to be related to the surface properties of the carrier particles (Heng et al., 2000; Zeng et al., 2000a; Iida et al., 2003a,b). Surface morphology has been demonstrated to directly influence the contact area between drug particle and carrier, leading to variations in interparticulate adhesion. Several studies have reported that variations in contact area, as a result of differing surface structure, could potentially compromise the aerosolization performance of the drug particles (Zeng et al., 2000a; Young et al., 2002; Flament et al., 2004). Some surface modifications of carrier particles have been reported to improve inhalation performance of DPI (Kawashima et al., 1998; Chan et al., 2003). Therefore in the present study an attempt was made to investigate the effect of carriers with different particle shapes and surface morphology on the efficiency of insulin microcapsules in DPI formulations. The present study was focused on roughness because this parameter is supposed to have a strong influence on adhesion (Lucas et al., 1998) and consequently on the aerosolization of the drug (Flament et al., 2004). Mannitol and sorbitol with various particle surface and morphologies were prepared and their physicochemical properties were analyzed by scanning electron microscopy (SEM), differential scanning calorimetry (DSC), X-ray diffractometry (XRD), BET nitrogen adsorption method, atomic force microscopy (AFM), helium pycnometry, and laser diffraction. Finally, interactive mixtures of the carrier with the insulin-loaded PLGA microcapsules were manufactured and tested for their aerosolization behavior.

2. Materials and methods

PLGA 50:50 (RG504H, lot # 1020751) was supplied by Boehringer Ingelheim, Germany. This polymer has an uncapped free carboxylic acid group in the terminal end. Molecular weight (*MW*) was determined by gel permeation chromatography (GPC: Agillent 1100, Agillent Technologies, USA) using standard polystyrene. PLGA with a *MW* of 36,000 was used in the following experiments. Recombinant human insulin (27.5 U/mg on dried bases by HPLC) was kindly supplied by Exir Pharmaceutical Co. Iran. Viscous paraffin oil and Span 80 (Merck, Germany) and acetonitrile and hexane (both HPLC grade, Merck, Germany) were used as received. Trifluoroacetic acid (TFA) (Fluka, Switzerland) was used in HPLC analysis. Mannitol and sorbitol (Fluka, Switzerland) were used as the carrier in DPI formulations. Hard gelatin capsule shells (Size No. 2) were purchased from Cipla, India. All other chemicals and reagents were of analytical grade, purchased from commercial vendors and used as received.

2.1. Preparation of microcapsules

Insulin-loaded microcapsules were prepared in respirable sizes as described previously (Emami et al., 2009a,b). Briefly, insulin-loaded microcapsules were prepared at the theoretical loading of 6.25% (6.25 mg of insulin per 100 mg of polymer). Insulin was dissolved in 0.01 N hydrochloric acid to give a concentration of 10 mg/ml. The protein solution was incorporated in PLGA solution in acetonitrile at V/V ratio of (1:5). This solution was then dispersed in 30 ml mineral oil in the presence of 3% Span 80 and stirred for 2 h at 5000 rpm with an impeller type stirrer (Tohid Sanat Sepahan, Model TSS55, Isfahan, Iran) to ensure complete evaporation of inner phase. Microcapsules were collected by centrifugation (Sigma 3K30, Germany) at 20,000 rpm for 30 min at 10 °C and washed four times with n-hexane to complete removal of mineral oil. Particles were filtered, vacuum dried, and stored under refrigeration in a desiccator until used.

2.2. Preparation of carriers

2.2.1. Spray-drying method

The mannitol solution (18% (w/v) in distilled water) was spray-dried using a Buchi B-191 mini spray dryer (BUCHI Labortechnik AG, Flawil, Switzerland) at an inlet temperature of 155 °C, outlet temperature of 85 °C, aspiration setting of 85% and spray flow of 400 Nl/h. Immediately after the finishing the process, spray-dried mannitol (SDM) particles were packed into tightly closed amber bottles and desiccated over silica gel.

2.2.2. Freeze-drying method

The mannitol solution (18% (w/v) in distilled water) was freeze-dried overnight (Lyotrap Plus, LTE Scientific Ltd., UK). After freeze-drying process, the particles were sieved manually through the 45 μm sieve. The fraction of freeze-dried mannitol (FDM) collected under the sieve were retained in tightly closed amber bottles and stored in a desiccator over silica gel.

2.3. Sieve fractionation

Commercially available mannitol and sorbitol were sieved manually through the 45 μm sieve for 10 min. The fraction of sieved mannitol (SM) and sorbitol (SS) collected under the sieve were

retained in tightly closed amber bottles and stored in a desiccator over silica gel.

2.4. Particle size determination

Particle size distributions based on volume were measured by laser diffraction (Malvern Mastersizer X, Malvern, UK) using a 100 mm lens at an obscuration between 0.19 and 0.21. Samples of mannitol and sorbitol were prepared by suspending the particles in chloroform with the aid of sonication in a water bath for 2 min. The suspension of insulin-loaded PLGA microcapsules was prepared in water containing 1% Tween 80 after sonication for 2 min. Measurements were repeated 5–10 min apart to ensure that no dissolution or agglomeration of the powders occurred. Each sample was measured in triplicate. The size distribution was expressed by the volume median diameter (VMD) and span. Span is a measure of the width of the size distribution.

Span $= \frac{D(v,90)-D(v,10)}{D(v,50)}$ where $D(v, 90)$, $D(v, 10)$ and $D(v, 50)$ are the equivalent volume diameters at 90, 10 and 50% cumulative volume, respectively.

2.5. Density measurement

A helium pycnometer (Quantachrome Instruments, Boynton Beach, FL, 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 value of triplicate determinations is reported.

2.6. Scanning electron microscopy

The shape and surface morphology of the particles were studied by a scanning electron microscope (TEscan, VEGA II XMU, Czech Republic). Prior to scanning, the samples were coated with a thin layer of gold, using a direct current sputter technique (EMITECH K450X, England). The surface topographies of the carriers and the attachment of microparticles to the carrier surface were assessed qualitatively from the photomicrographs taken.

2.7. Atomic force microscopy

Atomic force microscopy (AFM) was used for more accurate surface analyses of microcapsules. The surface topography and roughness measurements of carriers were investigated using a Dualscope controller, AFM and scanner (DME, Denmark) in non-contact Mode operation (AC-AFM). Tetrahedral-tipped silicon cantilevers (MicroMash, USA) with a nominal tip radius of curvature <10 nm, force constant 42 N/m and a resonant frequency 200–400 kHz were utilized for imaging. Scan ranges were 10 μm × 10 μm, 5 μm × 5 μm, and 1 μm × 1 μm. The roughness descriptors R_a (rugosity, i.e. the arithmetic mean of the departures of the roughness profile from the mean line) and R_q (root mean square deviation of the asperity height distribution) were measured and reported.

2.8. X-ray diffraction (XRD)

In order to confirm that the processing method did not result in crystallographic modifications, XRD of the sieved, spray-dried and freeze-dried mannitol and sieved sorbitol were obtained using X-ray diffractometer (x-Pert, Philips, UK) with a Cu tube anode. The X-ray diffractogram was scanned with the diffraction angle increasing from 5° to 60°, 2θ angle. XRD patterns were compared with XRD patterns of standards polymorphs of mannitol and sorbitol set in default in equipment software database.

2.9. Differential scanning calorimetry (DSC)

A differential scanning calorimeter (DSC822^e, Mettler Toledo, Switzerland) was used to measure enthalpy and melting point of all carriers (8 mg) used in the study. The equipment was calibrated using indium and zinc. Samples were heated ranging 5–250 °C at a scanning rate of 5 °C/min in aluminium pans (40 μl) under nitrogen gas. The melting points and enthalpies of fusion were calculated using the Mettler STAR^e version 8.01 software.

2.10. Surface area analysis

The specific surface area of carriers was measured by the BET adsorption method with nitrogen gas using a Quantachrome surface area analyzer (NOVA 2000 e, Quantachrome, USA), calculating the surface area by BET multipoint measurement.

2.11. Particle shape

The shape of carrier particles was determined using SEM images and evaluated by an image analyzer software (Scion Image, version Alpha 4.0.3.2, Scion Corporation, Maryland, USA). The morphology was quantified by two descriptors, derived from the length (L), width (W), perimeter (P) and area (A) of the projected image of a particle, namely elongation ratio (aspect ratio) (L/W) and shape factor (circularity) ($4\pi \times A/P^2$). A spherical particle will have a shape factor of 1, whilst non-spherical particles will have a shape factor value between 0 and 1. The more irregular shape shows the smaller the shape factor. If shape factor approaches unity, there is increased particle sphericity (Zeng et al., 2000a; Chan et al., 2003; Iida et al., 2004).

2.12. Powder flow properties

The static powder flow was characterized using Carr's compressibility index (CI), determined from the tapped (ρ_{tap}) and bulk densities (ρ_{bulk}):

$$CI = \frac{\rho_{tap} - \rho_{bulk}}{\rho_{tap}} \times 100$$

The tapped and bulk densities were measured in a graduated cylinder (10 ml) using an automatic tapper (Pharma Test tap density tester, PT-TD, Germany). The tapped density was determined after 1000 taps. Lower CI values are indicative of better flow behavior.

Hausner's ratio (HR) was determined from the tapped and bulk density values with the tapping method by the following equation:

$$HR = \frac{\rho_{tap}}{\rho_{bulk}}$$

2.13. Preparation of formulations

Insulin-loaded microcapsules was mixed with SDM, FDM, SM or SS carrier particles in a ratio of 1:1 w/w using a turbula mixer (Dorsa Novin Afzar, Tehran, Iran). Ten samples, each weighing 5 mg were randomly selected to determine the homogeneity of the mixtures. The coefficient of variation <6% in insulin content was used to assess the degree of homogeneity. All formulations were filled in hard gelatin capsules (size 2) manually with 20 mg of powder mixtures. Each 20 mg of insufflated DPI formulation contains 340 μg of insulin. The coefficient variation percentage (CV%) were less than

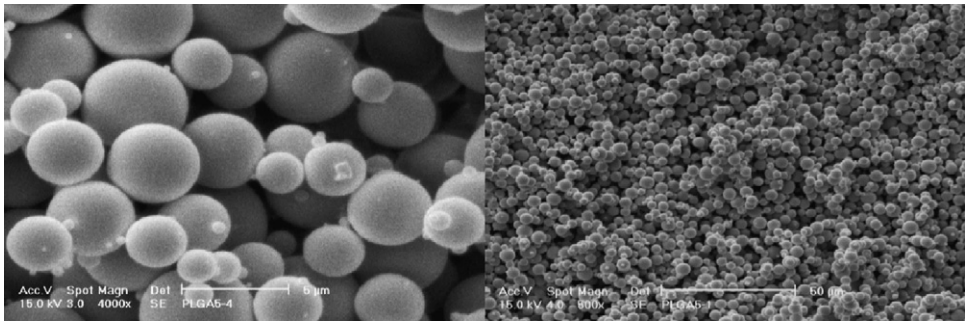

Fig. 1. Scanning electron micrographs of insulin-loaded PLGA microcapsules (left fig., 4000×, bar shows 5 μm; right fig., 500×, bar shows 50 μm).

6% for all blends. The batches that showed CV% above 6% were excluded from the study.

2.14. HPLC analysis of insulin

The amount of insulin analyzed using a reversed-phase HPLC Waters apparatus consisted of a model 600E HPLC pump, 486 tunable absorbance detector and 746 data module (Waters; Milford, MA). Insulin was separated at room temperature on a μ-bondapack C18 column (4.6 mm × 250 mm, 10 μm, 125A°) (Waters, Ireland). The mobile phase was an acetonitrile:water mixture (35:65) containing 0.1% TFA. The flow rate was 1.5 ml/min, sample injection volume was 100 μl and the detecting wavelength was 214 nm. Insulin solutions of known concentrations (1–80 μg/ml) were used to generate calibration curves.

2.15. Drug deposition studies

The dispersion and deposition characteristics of all powder mixtures were assessed by Spinhaler® connected to twin stage impinger (TSI; Apparatus A, European Pharmacopoeia, 2000, Copley, Nottingham, UK). Powders were dispersed at steady flow rate of 60 l/min. Acetonitrile 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 flow rate was achieved using a rotary vein pump and solenoid-valve (Copley Scientific, Nottingham, UK), and calibrated using a reference flow meter. The pump was operated for 5 s so that a steady flow rate of 60 l/min was achieved, and the dose was released. The pump was operated for another 5 s at the established flow rate following the release of the dose and it was then switched off. The capsule shells, inhaler body and impinger throat, stage 1 and stage 2 were separately washed with acetonitrile and the volume of the samples was adjusted with the same solvent. Insulin was collected by centrifugation (Sigma 3K30, Germany) at 14,000 rpm for 30 min at 10 °C and diluted with distilled water containing TFA 0.1% (pH = 2) to dissolve completely. The concentration of insulin in each sample was analyzed by HPLC method. Fine particle dose (FPD) was determined as the amount of drug deposited in stage 2 (d_{ae} < 6.4 μm) and the fine particle fraction (FPF) was expressed as a percentage of the total amount of insulin collected from the stage 2. The emission was defined as the mass of drug delivered from the inhaler (i.e., total amount excluding the inhaler device and capsule), expressed as a percentage of the total amount of insulin collected. Dispersibility was defined as the ratio of FPF per emission. Each value was expressed as the mean ± SD. Statistical analysis was performed using a one-way analysis of variance (one-way ANOVA) with multiple comparisons between deposition data using a Tukey honest significant difference test (SPSS, version 13.0, Chicago, IL, USA). A P value of <0.05 was considered significant.

3. Results

3.1. Insulin-loaded PLGA microcapsules

Insulin-loaded PLGA microcapsules were spherical as shown in Fig. 1. The particles were homogeneous and appeared to have a smooth surface texture without significant irregularities.

They exhibited a VMD of 4.65 with over 90% of particles less than 8 μm with relatively narrow size distribution (Table 1), suggesting that the microcapsules were suitable for use as inhalation aerosol.

3.2. Particle size characteristics of carrier particles

Size characteristics of samples are tabulated in Table 1. The particle size of carriers tried to be less than 45 μm because it

Table 1
Size characteristics of carriers and insulin-loaded microcapsules (mean ± SD, n = 3).

Samples	Cumulative percent (undersize)[a]			VMD[b] (μm)	Span
	$D_{10\%}$ (μm)	$D_{50\%}$ (μm)	$D_{90\%}$ (μm)		
SM[c]	4.50 ± 0.02	20.76 ± 0.36	51.18 ± 2.18	25.37 ± 1.17	2.25 ± 0.07
SDM[d]	2.66 ± 0.64	12.89 ± 2.01	21.67 ± 2.08	14.17 ± 0.64	1.47 ± 0.15
FDM[e]	5.73 ± 0.17	21.58 ± 0.30	57.38 ± 1.11	27.91 ± 0.53	2.39 ± 0.03
SS[f]	5.10 ± 0.11	23.92 ± 0.98	58.88 ± 1.42	29.12 ± 1.27	2.25 ± 0.06
Microcapsules	1.50 ± 0.07	4.04 ± 0.23	8.24 ± 0.22	4.65 ± 0.91	1.67 ± 0.02

[a] Equivalent volume diameters at 10 ($D_{10\%}$), 50 ($D_{50\%}$) and 90% ($D_{90\%}$) cumulative volume.
[b] Volume median diameter.
[c] Sieved mannitol under 45 μm sieve.
[d] Spray-dried mannitol.
[e] Freeze-dried mannitol sieved under 45 μm sieve.
[f] Sieved sorbitol under 45 μm sieve.

Table 2
Shape and surface characteristics of carriers.

Carrier type	Elongation ratio[a]	Shape factor[a]	Surface area (m^2/g)[b]	Surface roughness parameters[c]	
				R_a (nm)	R_q (nm)
SDM[d]	1.03 ± 0.03	0.96 ± 0.02	4.20 ± 0.51	103.3 ± 9.7	138.0 ± 22.1
FDM[e]	14.05 ± 3.82	0.21 ± 0.04	24.79 ± 0.86	21.1 ± 6.5	29.9 ± 13.1
SM[f]	2.21 ± 0.58	0.73 ± 0.07	10.10 ± 0.62	144.7 ± 23.4	186.2 ± 26.1
SS[g]	1.86 ± 0.29	0.77 ± 0.08	21.52 ± 0.93	464.0 ± 86.1	569.5 ± 103.1

[a] Data are represented with mean ± SD (n = 30).
[b] Data are represented with mean ± SD (n = 3).
[c] Data are represented with mean ± SD (n = 10).
[d] Spray-dried mannitol.
[e] Freeze-dried mannitol.
[f] Sieved mannitol.
[g] Sieved sorbitol.

was shown that lower carrier particle size resulted in higher FPF of active ingredient in DPI formulations. Gilani et al. (2004) showed that coarse lactose (VMD of 53.5 μm) produced lower delivery efficiency of cromolyn sodium than fine lactose (VMD of 12.3 μm), when were compared at the same weight fraction. FPF of budesunide aerosolized from DPI formulation contained lactose carrier <32 μm was higher than those with carriers in size fractions of 63–90 and 125–180 μm using Easyhaler® and Spinhaler® (Steckel and Muller, 1997). It was shown that the smaller-sized lactose carriers (<30 μm) performed best in the Turbospin, providing the highest emitted dose with the lowest variability (Schiavone et al., 2004).

3.3. Morphology and surface properties of carriers

Different batches of carriers had different surface textures and shapes which were supported by the visual comparison of their scanning electron micrographs (Fig. 2). Differences in the morphological features of the different coarse carriers were also confirmed quantitatively by image analysis process of the SE micrographs by elongation ratio and shape factor (Table 2). Different values of the roundness and elongation ratio were obtained for the four coarse carriers (Table 2). The surface morphology of carriers was shown in a better way by AFM images (Fig. 3).

The surface topographies of carriers were also described quantitatively with surface area and surface roughness in Table 2. SDM showed circle particles (the highest shape factor and lowest elongation ratio) with smooth surface. SM particles appeared to be slightly more elongated with more surface roughness than SDM. While SS showed the most surface roughness and FDM the most elongated and asymmetric particles. SEM and AFM images clearly present and interpret the surface roughness parameters reported in Table 2. FDM had the smoothest surface and SS showed the highest degree of surface roughness. The specific surface area (measured by BET technique) of the materials differed considerably (Table 2). SDM had the lowest specific surface area, whereas SS and interestingly FDM exhibited larger surface areas.

3.4. Powder flow properties

The true, bulk and tapped densities, Carr's index (CI), and Hausner's ratio (HR) of insulin-loaded microcapsules and carrier particles are listed in Table 3. SDM showed the highest CI and the highest HR value demonstrating apparently poor potential flow characteristics for SDM while SS exhibited the least degree of compressibility.

3.5. XRD and DSC studies

XRD and DSC analysis were performed on the carrier particles in order to assess possible relevant modifications of crystallinity. Fig. 4 shows the XRD patterns of carriers. All samples were highly crystalline and consisted of γ-sorbitol (ICDD # 43-1520) for SS and β-D-mannitol (ICDD # 22-1797) for SM. Additional polymorphic form can be defined by XRD for SDM and FDM. In addition to β-D-mannitol, α-D-mannitol (ICDD # 22-1793) appeared at SDM and FDM in the percentages of about 28 and 37%, respectively. it has been shown that during freeze-drying of the solutions of high concentrations of mannitol, generation of the α form is more favorable (Izutsu et al., 1993).

DSC data are shown in Fig. 5. A dehydration peak occurs at approximately around 86 °C and a melt peak at about 98 °C for SS which is in good agreement with previous reports (Bhandari and Roos, 2003; Seo et al., 2005). It was reported that many small pores on the surface of the SS (Figs. 2 and 3), may form during the hydration and dehydration process since sorbitol is highly hygroscopic and easily loses its water of crystallization (Nash, 2000). Mannitol batches (SDM, FDM and SM) had similar DSC profiles. A single sharp endothermic peak was observed around 166 °C in all samples (Fig. 5), which corresponded to the melting of mannitol (Louey et al., 2004). These melting peaks indicated the crystalline nature of all used components as the carriers. The onset and peak temperatures were similar for each sample (SM, SDM and FDM) but enthalpy of fusion (ΔH) was slightly lower for FDM and SDM than SM which was 272.8, 274.3 and 303.4 J/g, respectively (Fig. 5). These results (XRD and DSC) confirmed that an amorphous form and an

Table 3
Flow properties of carriers and insulin-loaded microcapsules (mean ± SD, n = 3).

Samples	True density (g/cm^3)	Bulk density (g/cm^3)	Tapped density (g/cm^3)	Hausner's ratio	Carr's index (%)
SDM[a]	1.48 ± 0.011	0.292 ± 0.011	0.568 ± 0.015	1.947 ± 0.127	48.53 ± 3.36
FDM[b]	1.55 ± 0.002	0.169 ± 0.019	0.269 ± 0.012	1.586 ± 0.187	36.96 ± 2.57
SM[c]	1.53 ± 0.006	0.381 ± 0.013	0.601 ± 0.001	1.577 ± 0.054	36.55 ± 2.19
SS[d]	1.51 ± 0.004	0.287 ± 0.005	0.395 ± 0.007	1.380 ± 0.043	27.51 ± 2.24
Microcapsules	1.33 ± 0.024	0.229 ± 0.032	0.339 ± 0.022	1.478 ± 0.012	32.35 ± 1.03

[a] Spray-dried mannitol.
[b] Freeze-dried mannitol.
[c] Sieved mannitol.
[d] Sieved sorbitol.

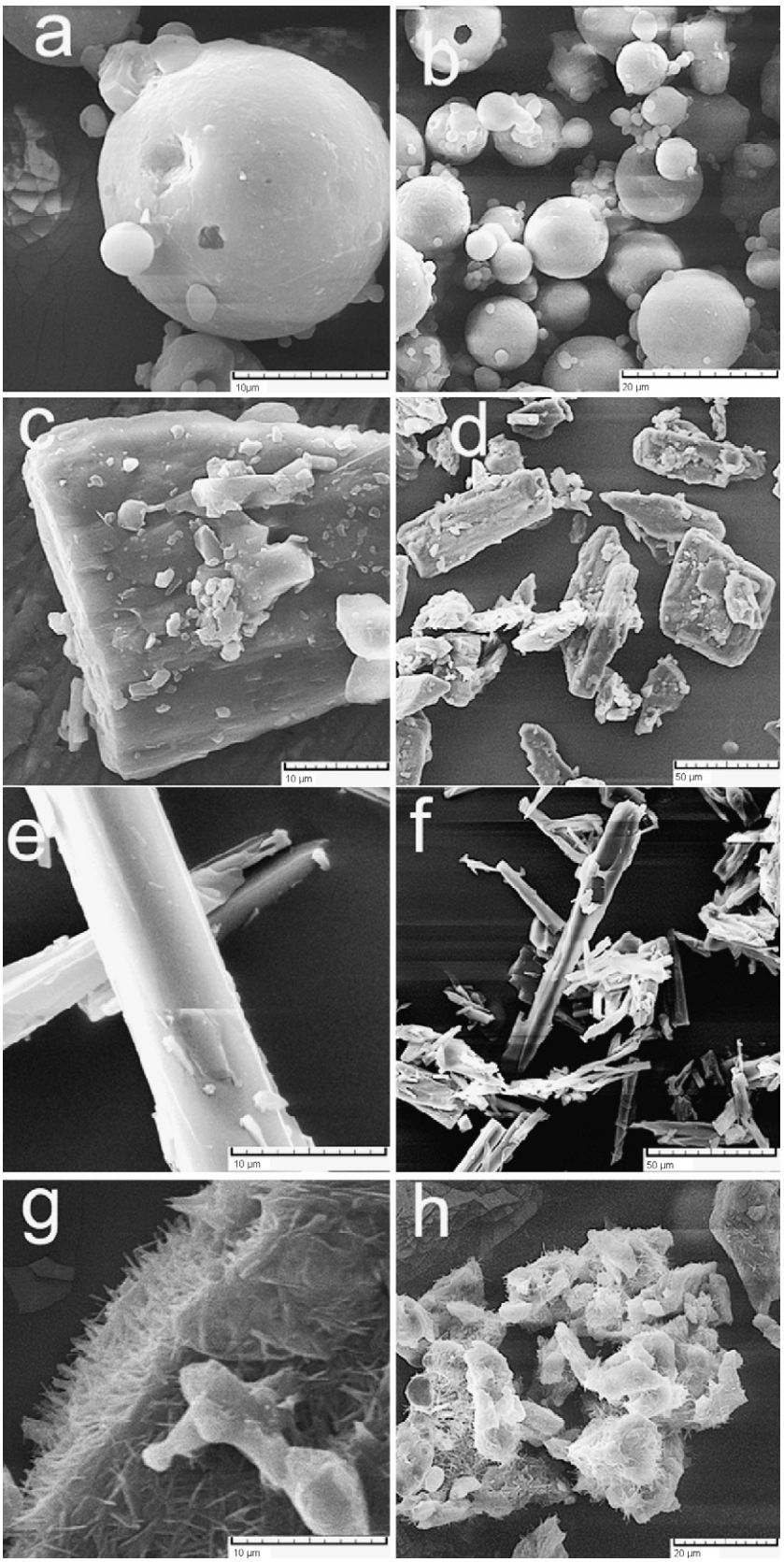

Fig. 2. Scanning electron micrographs of carrier particles; spray-dried mannitol (SDM) (a, b), sieved mannitol (SM) (c, d), freeze-dried mannitol (FDM) (e, f) and sieved sorbitol (SS) (g, h).

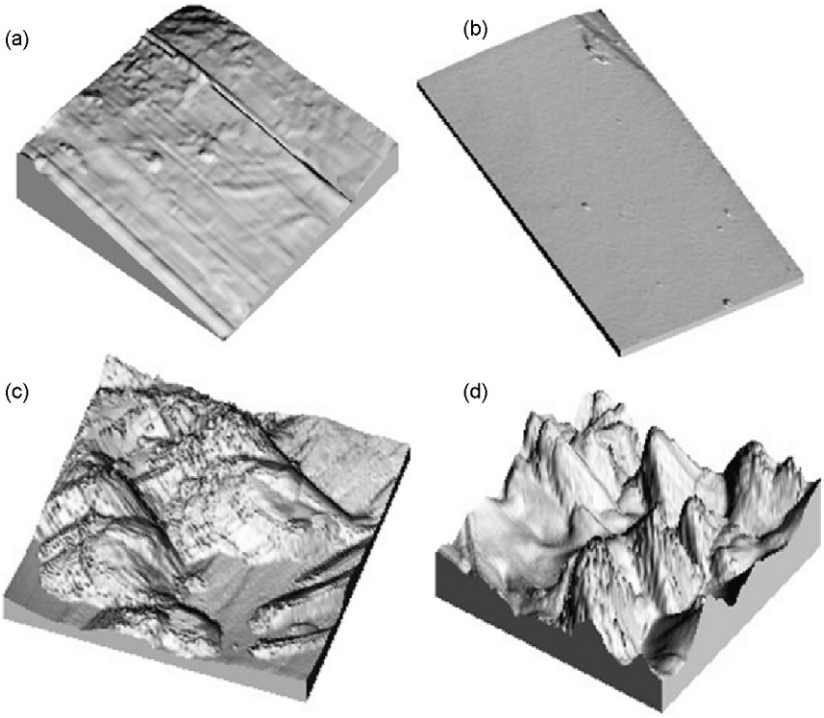

Fig. 3. Atomic force microscopy images of carrier particles; spray-dried mannitol (SDM) (a), freeze-dried mannitol (FDM) (b), sieved mannitol (SM) (c) and sieved sorbitol (SS) (d).

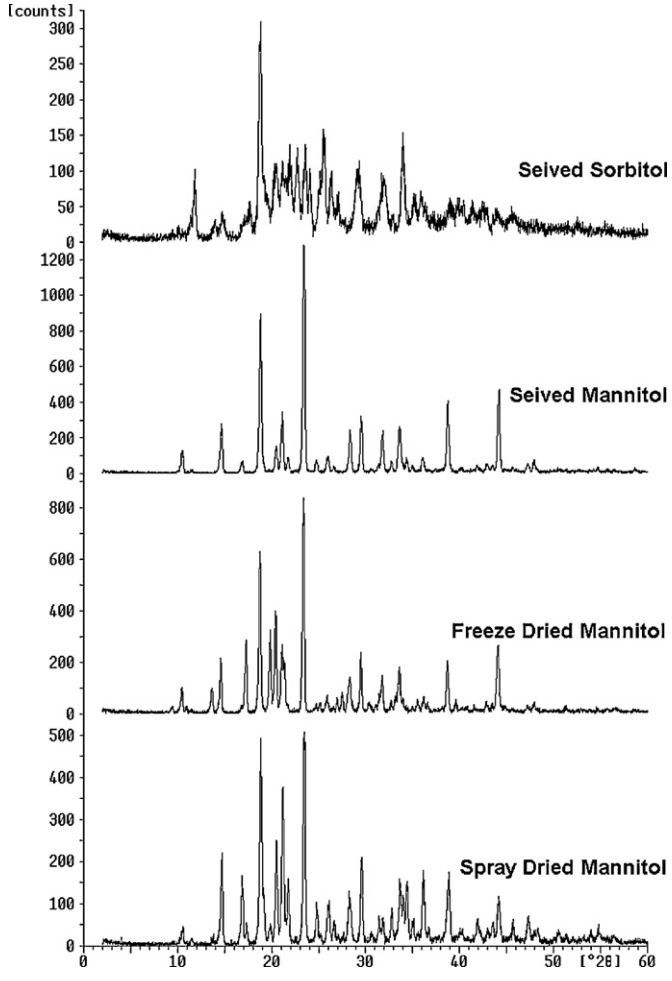

Fig. 4. X-ray powder diffraction (XRPD) patterns of carrier particles.

alpha form were obtained under our freeze-dried and spray-dried conditions.

3.6. In vitro deposition profiles of insulin-loaded microcapsules

Table 4 shows the emitted dose, dispersibility and FPF (proportion below 6.4 μm) of microcapsules with various dry powder formulations given by Spinhaler®, measured using a twin impinger at a flow rate of 60 l/min for 5 s. DPI formulation of microcapsules with coarse carriers caused an increase in FPF of microcapsules. SDM did not improve FPF of microcapsules significantly (P value > 0.05) compared to microcapsules alone whilst SS not only did not improve FPF of microcapsules but also decreased it. SM and FDM enhanced FPF of microcapsules significantly (P value < 0.05) (Table 4). Interestingly FPF and dispersibility improvement of microcapsules with SM and FDM carriers accompanied with decreasing the emitted dose of microcapsules (Table 4). All formulations gave emitted dose higher than 80%.

4. Discussion

Several attempts have been made to introduce new carriers as alternatives to lactose to be used in DPIs (Tee et al., 2000; Louey et al., 2004; Traini et al., 2006; Saint-Lorant et al., 2007). In the present study various carriers were tried and the results showed that spray-dried mannitol (SDM) did not change microcapsules deposition significantly while sieved sorbitol (SS) decreased it. Comparing the surface roughness (R_a) (Table 2) of carriers and FPF (Table 4) showed that an optimized degree of carrier surface roughness is necessary for suitable and enhanced inhalation efficiency of microcapsules. Previous investigations reported that the carrier surface morphology directly affected the aerosolization efficiency from a DPI (Kawashima et al., 1998; Podczeck, 1998; Larhrib et al., 1999; Zeng et al., 2000a; de Boer et al., 2003b). In general terms, a decrease in roughness is believed to improve aerosolization efficiency of a drug-carrier blend (Ganderton and Kassem, 1992; Kawashima et al., 1998; Zeng et al., 2001). However, it was shown that coarse

Table 4
Deposition profiles of insulin-loaded microcapsules in a TSI after aerosolization from different formulations through a Spinhaler® at a flow rate of 60 l/min (mean ± SD, $n = 3$).

Formulations[a]	FPD[b] (μg)	FPF[c] (%)	Emission (%)	Dispersibility (%)
MCap[d] + SDM	47.26 ± 0.81	13.9 ± 0.24	82.2 ± 1.09	16.9 ± 0.59
MCap + FDM	56.10 ± 3.22	16.5 ± 1.14	90.2 ± 2.62	18.3 ± 1.79
MCap + SM	62.22 ± 3.74	18.3 ± 1.65	91.8 ± 1.86	20.0 ± 0.89
MCap + SS	35.70 ± 2.51	10.5 ± 0.86	93.2 ± 2.50	11.2 ± 1.16
MCap alone[e]	45.56 ± 2.04	13.4 ± 0.74	96.6 ± 1.74	13.9 ± 0.93

[a] DPI formulations containing the blend of insulin-loaded PLGA microcapsules and carrier.
[b] Fine particle dose.
[c] Fine particle fraction.
[d] Insulin-loaded PLGA microcapsules.
[e] Microcapsules were aerosolized alone without carrier.

carrier particles which normally exhibit large surface discontinuities may provide shelter to drug particles from the press-on forces during mixing, as the drug particles tend to assemble in these discontinuities during mixing (Iida et al., 2003b). Therefore, high carrier rugosity does not necessarily have a negative effect on the drug detachment from carrier crystals during inhalation, providing that inertial detachment forces are applied. Chan et al. (2003) also reported that a positive linear trend was established between the roughness of the lactose surface and the FPF and dispersibility of the drug. Therefore, an important balance between the surface morphologies of both the drug and carrier can exist (Young et al., 2002). It was shown by Heng et al. (2000) that an optimum lactose surface roughness (R_a) was required for an increased fine particle fraction of salbutamol sulphate. All these studies demonstrated that the different surface roughness of the carrier led to different adhesion forces between the drug and carrier, which was reflected in the *in vitro* deposition results.

The carrier surface plays an important role in drug-to-carrier interaction (Kawashima et al., 1998; Podczeck, 1998; Zeng et al., 2000a, 2001). These interaction forces have to be strong enough to guarantee good mixture stability during handling and proper drug deaggregation, but weak enough to enable the separation forces during inhalation to detach a substantial fraction of the drug dose from the carrier crystals. This requires that the size distributions of the interaction forces (during mixing) and separation forces (during inhalation) are balanced properly (de Boer et al., 2003a). Comparing Tables 2 and 4 showed that the increase in surface roughness of carriers generally led to increase in emitted dose of DPI formu-

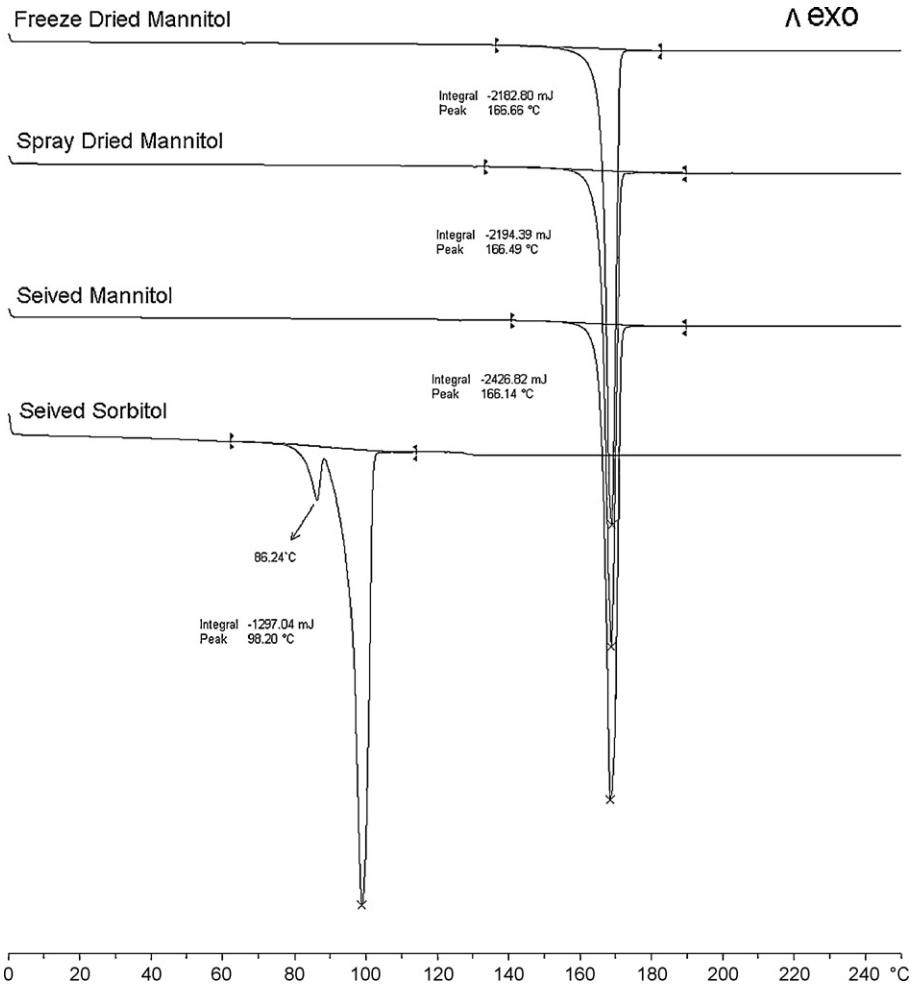

Fig. 5. Differential scanning calorimetry thermograms of carrier particles.

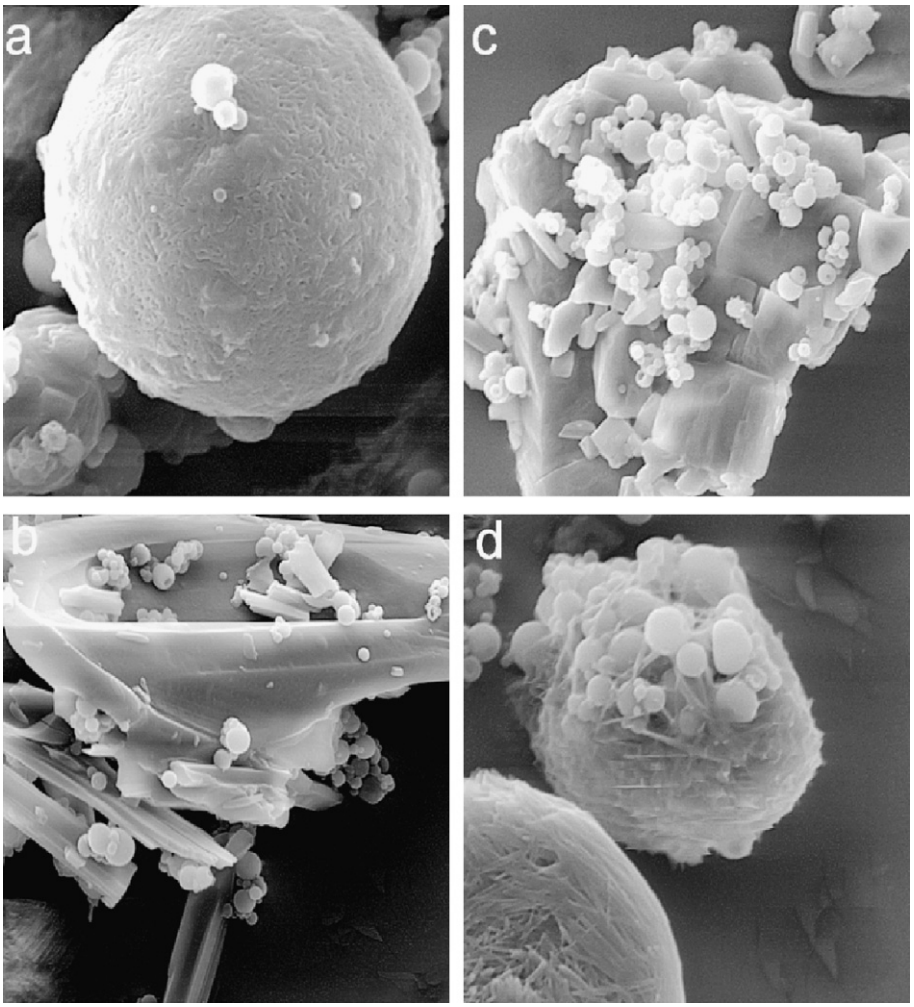

Fig. 6. Scanning electron micrographs of DPI formulations consisting the blend of insulin-loaded PLGA microcapsules and carrier particles; (a) spray-dried mannitol (SDM), (b) freeze-dried mannitol (FDM), (c) sieved mannitol (SM) and (d) sieved sorbitol (SS).

lations. Kawashima et al. (1998) reported that the lactose particles having larger surface area can carry higher amounts of drug particles because of higher capacity of depositing and stronger adhesion with drug particles.

Fig. 6 shows the dry powder inhalation formulations containing the blend of microcapsules and carriers. It can be seen that there are not enough active sites on the surface of SDM and FDM for microcapsules to be deposited on, so carriers cannot disaggregate microcapsules. In the case of sieved sorbitol (SS), microcapsules immersed on the surface of carrier and did not detach easily from its surface after aerosolization. Therefore in spite of better emission of microcapsules from the formulation containing SS, the fine particle fraction (FPF) of the drug decreased (Table 4).

The assumed role of carrier surface on the separation of microcapsules aggregation during preparation of DPI formulations and detachment of microcapsules after aerosolization from the surface of carriers is shown schematically in Fig. 7. Two methods were used to evaluate carrier surface roughness; one involving BET gas permeability technique and the other involving AFM. BET gas permeability technique has been used frequently to calculate surface area of carriers (Kawashima et al., 1998; Young et al., 2002; de Boer et al., 2003b; Nakate et al., 2004; Steckel et al., 2004). As expected, the surface area of SS was larger than SDM and SM whereas the surface area of SDM was smallest amongst all carriers (Table 2). The order of surface roughness of carriers determined by AFM corresponded to that of surface area except in FDM case (Table 2). The reason for the unexpected result can be seen in Fig. 8. The figure shows the presence of loose agglomerates in FDM samples. During BET experiment gas diffused through these lattices and whole of the agglomerate was assumed to be a particle. Therefore, it can be concluded that in some cases like FDM, surface properties measured by BET technique is not representative of real condition. The

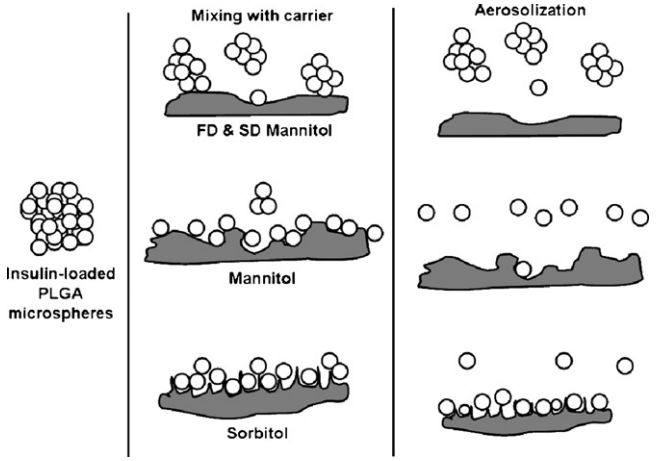

Fig. 7. Schematic representation of the role of carrier surface asperities on the drug entrapment and its fluidization capabilities.

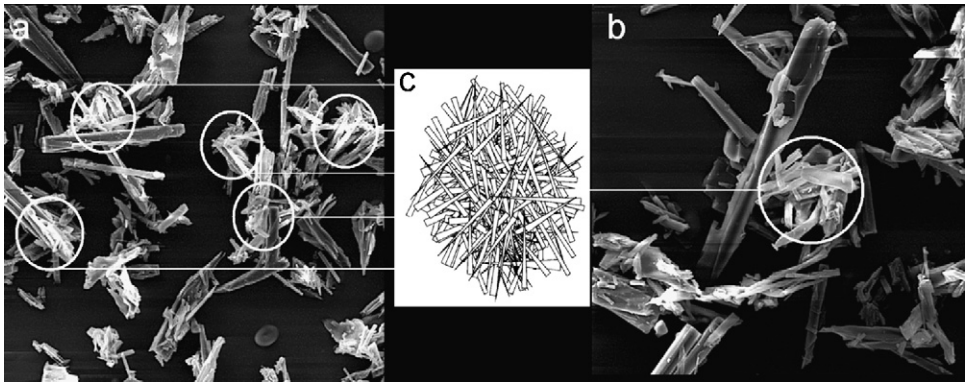

Fig. 8. Scanning electron micrographs of freeze-dried mannitol (FDM) (a, b) and schematic image of formed loose agglomerates of needle shape FDM particles (c).

advantage of AFM is that it provides a direct method of measuring the detailed microscopic roughness of the carrier particle, which is important because drug particles that adhere to and detach from the carrier surface are in the micrometer size range (Young et al., 2002).

There is a direct relationship between emitted dose of powder and FPF of active ingredients in DPI formulations (Nakate et al., 2004). Table 4 shows that there was a good trend between FPF of microcapsules and their emitted dose from DPI formulations of microcapsules with SDM, FDM and SM. However, microcapsules in the blend of SS showed the lowest FPF whereas its emitted dose was the highest among other DPI formulations with carrier particles. Interaction of microcapsules with SS carrier particles due to their surface properties may be the reason for this unexpected low FPF. DPI formulation containing SS aerosolized well from the capsule and inhaler device but microcapsules could not detach from carrier surface easily and keep their way to deeper part of twin impinge (second stage). Table 4 shows that there are not remarkable differences between DPI formulations containing FDM and SM in terms of emitted dose and FPF, however, it has been reported that these minimal differences could be important for powder behavior (Kaye, 1989).

Carr's index has been frequently used in pharmaceutics as a flow index of powders (Iida et al., 2001; Louey et al., 2004). Flow has been shown to be a key property of the DPI formulation as it aids in metering, fluidization, and dispersion (Taylor et al., 2000). Moreover carrier flowability is known to affect the emitted dose of drug from a DPI device and possibly the subsequent delivery of that drug to the lungs (Nakate et al., 2004; Smyth and Hickey, 2005). Comparing Carr's index (Table 2) and emission (Table 4) values showed an inverse relationship between CI and emission of DPI formulations. Microcapsules alone showed a higher CI and also a higher emitted dose than SS, possibly due to its lower true density (1.33 g/cm^3, Table 3). Both formulations containing FDM and SDM had smooth surfaces (Fig. 3 and Table 2) while formulation composed of FDM showed higher FPF and emitted dose than SDM. This was interesting when it was found that SDM had lower true density than FDM (Table 3), resulting in better emitted dose and consequently improved FPF. This aerosolization behavior of FDM can be attributed to its needle shape particle morphology. Spatial hindering effect of rod shape particles can lead their easier aerosolization, and hence higher emission and higher FPF. The results of FPF and emitted dose for different formulations can partially be explained by elongation ratio (ER) and shape factors reported in Table 2. FDM had higher ER and lower shape factor than SDM which indicate more irregular shape. *In vitro* inhalation studies have indicated that elongated, fibrous particles improve lung deposition properties (Chan and Goada, 1989; Fults et al., 1997). Increasing the elongation ratio of the lactose carrier particles also appeared to increase the FPF of salbutamol sulphate (Zeng et al., 2000a). ER and shape factor values were not significantly different ($P > 0.05$) between SM and SS. Comparing the FPF of SM and SS with the same morphological indexes imply the role of carrier surface properties. In summary, the importance of carrier morphology and surface properties in aerosolization behavior of DPI formulations can be well understood by comparison of SDM and FDM with the similar surface roughness and SM and SS with the same morphology characteristics.

Different physical forms of lactose (crystalline or amorphous) may result in changes in drug deposition from dry powder inhalers (Larhrib et al., 1999). Consequently, it is important to know which physical forms of materials may be present as a result of changes in the formulation content and/or the method of processing (Chidavaenzi et al., 2001). DSC and XRD studies showed that all carriers were in crystalline status (Figs. 4 and 5). The amorphous part shows a higher surface adhesion energy compared to crystalline surfaces (Buckton and Darcy, 1999; Young and Price, 2004; Price and Young, 2005). As a result of increase in adhesion energy, poor deaggregation of drug particles is observed (Podczeck et al., 1997). Therefore it can be hypothesized that, the presence of amorphous material may cause problems, for example, due to the fusion of particles, resulting in poor dispersion (Ward and Schultz, 1995; Podczeck et al., 1997; Young and Price, 2004). In addition, the amorphous regions can present difficulties such as decreased chemical stability (Pikal et al., 1978). Partially amorphous or unstable polymorphic forms and changes therein (Harjunen et al., 2002) make the interparticulate contact quite unpredictable and the powder formulation rather unstable (de Boer et al., 2003b). Enthalpy of fusion values of FDM, SDM and SM carriers obtained from DSC experiments were 272.8, 274.3 and 303.4 J/g, respectively (Fig. 5) which indicated that SM had the highest crystalline nature. It was reported that the maximum fine particle dose of terbutaline sulphate is obtained with the crystallized form of mannitol comparing to different polymorphs of mannitol (Saint-Lorant et al., 2007). This is according to our finding that the highest FPF was shown by SM (Table 4). Furthermore, previous reports have suggested increased amorphous content in DPI systems resulted in decreased aerosolization performance (Ward and Schultz, 1995; Young and Price, 2004; Price and Young, 2005). Melting points of α and β forms of mannitol were indistinguishable (166 and 166.5, respectively) in DSC thermograms (Yu et al., 1999), however, XRD patterns showed the presence of α-D-mannitol in the SDM and FDM.

5. Conclusion

This work was carried out to find out which physicochemical properties of the carrier influence the efficacy of a DPI formulation and, if mannitol and sorbitol can be introduced as a carrier to be used for formulation of insulin-loaded PLGA microcapsules in

the form of DPI formulation. FPF, emitted dose and dispersibility of the insulin-loaded PLGA microcapsules were depended on both morphology and surface characteristics of carriers. Optimum carrier surface roughness is necessary for efficient pulmonary delivery of DPIs. Higher degree of smoothness and roughness of carrier both caused decrease in aerosolization behavior of microcapsules. Carrier morphology significantly affects the aerosolization properties of DPI formulations. Needle shape FDM carriers emitted better than circle SDM carriers which consequently led to better FPF. These results suggest that apart from surface properties, the elongation of carrier particles may also play an important role in determining the FPF of the microcapsules. AFM was shown to be more trustable method for evaluation of surface parameters than BET method. Carr's index is a reliable factor to estimate powder emitted dose in DPI formulations and can be used as preliminary tool for prediction of emitted dose. Higher emitted dose does not guarantee better inhalation efficiency. Finally, there appears to be some evidence that the decrease in crystalline content may contribute to a decreased FPF.

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