Polycaprolactone Microspheres as Carriers for Dry Powder Inhalers: Effect of Surface Coating on Aerosolization of Salbutamol Sulfate

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Abstract

This study reports the factors controlling the aerosolization of Salbutamol Sulfate (SS) from the interactive mixtures of SS and polycaprolactone (PCL) microparticle carriers. The PCL microparticles were fabricated using an emulsion technique with polyvinyl alcohol (PVA) as stabilizer and were characterized by laser diffraction and Scanning Electron Microscopy (SEM). The Fine Particle Fraction (FPF) of SS from powder mixtures was determined by a Twin Stage Impinger (TSI). Unexpectedly no FPF of SS was observed from the PCL microspheres as prepared. The adhesion force between the PCL microspheres and SS were quantified using Atomic Force Microscope (AFM) in air at ambient humidity and found to be 301.4 ± 21.7 nN. X-ray Photoelectron Spectroscopy (XPS) analysis revealed that the hydrophobic surface of the PCL microspheres was covered with residual PVA stabilizer. Masking the PVA with hydrophobic magnesium stearate (MgSt) solutions (1-2 %) significantly increased ($p<0.05$, $n=5$) the FPF of SS (11.4% - 15.4 %), while coating with leucine had a similar effect (FPF = 11.3 ± 1.1%) but was not dependent on coating solution concentration. The force of adhesion by AFM was similarly reduced to 110.9 ± 30.5 nN and 121.8 ± 24.6 nN, ($p<0.05$, $n=5$) for 1% and 2% MgSt solutions respectively and when coated with leucine to 148.1 ± 21.0 nN. The presence of PVA on the surface of PCL microspheres affected the detachment of SS due to the strong adhesion between the two, presumably due to capillary forces acting between them. Coating the microspheres increased the FPF significantly by reducing the drug-carrier adhesion.

Keywords: Pulmonary drug delivery, Biodegradable polymers, Microspheres, Polymeric carrier, Aerosols, Salbutamol Sulfate, Polycaprolactone, Carriers, Adhesion Force, Dry Powder Inhaler

Abbreviations

AFM	Atomic Force Microscopy
CV	Coefficient of Variation
DPI	Dry Powder Inhaler
ED	Emitted Dose
EDX	Energy Dispersive X-ray Analysis
FPF	Fine Particle Fraction
HPLC	High Performance Liquid Chromatography
k	Spring Constant
MgSt	Magnesium stearate
MW	Molecular Weight
PCL	Polycaprolactone
PVA	Polyvinyl Alcohol
RD	Recovered Dose
SEM	Scanning Electron Microscope
SS	Salbutamol Sulfate
S1	Stage one
S2	Stage two
TSI	Twin Stage Impinger

UV	Ultraviolet
VMD	Volume Median Diameter
XPS	X-ray Photoelectron Spectroscopy

1. Introduction

Dry Powder Inhaler (DPI) formulation has become a popular pulmonary delivery system for the management of both local and systemic diseases [1]. The most common approach to carrier based DPI formulations is to mix the active drug particles (<5 μm) with a large carrier to help reduce the high cohesive forces among micron sized drug particles, improve the flowability of powders and allow reproducible dose metering [2-3]. Drug delivery from these powder formulations is influenced by interactions and friction forces that occur between the constituent particles in the formulation. Drug dispersion is affected by the physico-chemical characteristics of the powder particles in the mixture (Hickey et al, 1994). Key factors include particle size and distributions, shape and surface properties such as surface roughness, geometry of contact and adhesional forces (Hickey et al, 1994; Young et al., 2002; Islam et al., 2005; Begat 2005). Any disparities in the physico-chemical or surface properties of powders in DPI formulations change the adhesion/cohesion and friction forces, affecting the drug detachment and subsequent dispersion from the formulation, ultimately affecting the therapeutic performance [4-6].

Lactose is currently the most commonly employed carrier to which drugs or drug agglomerates are attached by means of adhesional forces. During inhalation, it is the patient's inspiratory force that detaches the drug from the carrier surface with the help of mechanical forces by the design of the device. Most commercially available carrier-based dry powder inhalers deliver only about 20–30% of the total dose to the lungs while the rest of the drug remains adhered on the carrier surface and is subsequently swallowed, resulting in poor performance of DPI devices with respect to drug delivery [7]. The reasons for this inefficient delivery have not been fully elucidated however it may be attributed to surface morphology, i.e. irregular shape and rough surfaces of commercially available inhalation grade lactose which affects drug detachment during inspiration. The presence of amorphous regions on the

surface of lactose represents a metastable thermodynamic state with high free energy and if drug particles are adhered onto the high energy sites, a high amount of force is required to detach drug particles from carrier surfaces [8]. Another disadvantage of lactose is the presence of a reducing sugar function making it incompatible with some drugs such as formoterol, budesonide or peptides and proteins [9].

The difficulty associated with the use of lactose as carriers is controlling the size, shape and surface roughness of the particle which, in turn, affects the detachment of drug [5]. Thus, lactose as a carrier has its own limitations. A number of studies have been conducted to improve the delivery of drugs from lactose carriers into the lungs. These studies have focused on improving dispersion of the drugs by optimizing the carrier size [10], smoothing the carrier surface [4], mixing different grades of carriers [11-12], and using lactose carriers with smooth and rough surface morphologies [4,13]. Alternatively, modification of the particle surfaces has been reported to improve the dispersibility of the drug from the carrier surface using different technologies. One of the approaches is to coat the surface of the coarse lactose by blending it with fine lactose, MgSt or leucine [14-18]. Common techniques for surface modification of carrier particles include spray drying [19], encapsulation using supercritical carbon dioxide [20], physical vapour deposition of particles in an aerosol flow reactor [21-22] and dry coating of active drug particles such as mechanofusion [23]. However, drug dispersion from these powders is still not satisfactory.

Other sugars such as glucose, sorbitol and xylitol have also been explored as carriers in DPI formulations but they are hygroscopic and are not able to efficiently generate the desirable fine particle fraction of the drug [24]. Solid lipid microparticles (SLM) have also been investigated as carriers for pulmonary administration. However, these SLMs are preferred for encapsulation of hydrophobic drugs rather than for surface coating and are suitable for sustained release of the drug into the lungs [25-26].

The culmination of the formulation challenges with the use of lactose and its modified counterparts have led to the exploration of new efficient alternative carriers for inhaled therapy. It is well-known that a curved surface with small asperities and low surface energy generally reduces the contact area between adjacent surfaces [27-29]. The surface roughness reduces the contact area, increases the distance of separation and consequently decreases the adhesion between adjacent particles. Hence, such spherical particles may be useful as carriers in DPI formulations. Indeed, spherical spray dried lactose particles had higher deposition of the drug Prankulast hydrate (FPF: 17.8%) compared with non-spherical lactose with irregular surface morphologies (FPF: 3.4% - 14.7%) [13].

Synthetic polymers are an attractive option as alternative materials for use as carriers in DPIs. Controlling the particle size, shape and surface roughness of polymers is much easier [30] as compared to sugars. Polymers have long been used in various drug delivery technologies and have been investigated widely in pulmonary drug delivery to sustain the release of drugs, but they have not been exploited as carriers in DPIs. Hence, it is of interest to research the use of biodegradable polymers with controlled surface functionality as carriers for the pulmonary delivery of drugs from powder formulation. Polycaprolactone (PCL) has been investigated in drug delivery because it is biodegradable and undergoes hydrolysis by degradation of its ester linkages. PCL nanoparticles, used to encapsulate a variety of drugs by nanoprecipitation, solvent displacement and solvent evaporation techniques, have been reviewed (Kumari et al). Drug loaded PCL nanoparticles such as anticancer Tamoxifen[31], Taxol [32], Vinblastine[33] and Docetaxel[34], peptide (insulin)[35], antiretroviral (Saquinavir)[36] and antifungal (Amphotericin B)[37] have been studied for drug delivery into other routes and none of these formulations have been investigated for lung delivery. Recently, Kho *et al* reported nanoparticle aggregates (270nm) of Levofloxacin-loaded polycaprolactone (PCL) prepared by spray drying using mannitol, lactose and leucine as drying adjuvants to maintain the structural integrity of PCL

and re-dispersibility of the agglomerates for lung delivery with prolonged action[38]. The authors demonstrated the development of drug-loaded PCL nanoparticles with desired aerodynamic diameter for direct delivery into lungs; however, the possible degradability or toxicity of PCL in the lungs has not been demonstrated. Furthermore, they have not studied the surface properties of PCL particles as large carriers used in DPI formulation.

In this study, we characterized inherent and surface modified PCL microparticles as carriers for dry powder inhaler formulation. To the authors' knowledge, no studies have been reported on the surface passivation of PCL microparticles using MgSt/leucine solution coating, characterization of PCL particle surface properties and their subsequent application in DPI formulations. Thus, the specific aim of this study is to explore the potential of using PCL microparticles, a biodegradable polymer with spherical shape and reproducible smooth surface, as an alternative carrier to lactose with a view to getting a better understanding of the polymer carrier surfaces for the development of DPI formulations for achieving efficient drug delivery deep into the lungs.

2. Materials and Methods

2.1 Chemicals

Micronized Salbutamol Sulfate (SS) of inhalation grade (Volume Median Diameter [VMD] 5μm) was obtained from GlaxoSmithKline, Australia. Polycaprolactone (MW 80,000), polyvinyl alcohol (87-89% hydrolyzed, MW 85,000-124,000) and L-leucine were from Sigma Aldrich. Lactose monohydrate (Aeroflo-95) was obtained from Meggle Group, Germany and MgSt was obtained from PCCA, Australia. Tween 80 and ammonium acetate were purchased from Ajax Chemicals, Australia. HPLC grade methanol (LiChrosolv®) was supplied by Merck, Germany.

2.2 Preparation of Polycaprolactone microspheres

The microspheres were prepared by oil in water solvent evaporation method [39] by dissolving PCL (300mg) in dichloromethane (3 ml) and then adding this dropwise into a 1% w/v aqueous polyvinyl alcohol (PVA) (40ml) solution. The emulsion was stirred at ?? rpm with an overhead stirrer (IKA® RW 20 digital, Labtek) for 40 minutes under ambient pressure and then stirred for another 20 minutes under reduced pressure on a rotary evaporator (Rotavapor R-210, BUCHI, Switzerland). Finally the microspheres were collected by filtration, washed with deionized water and dried in a vacuum desiccator at room temperature.

2.3 Coating of microspheres

2.3.1 Dry coating

The coating of the PCL microspheres with MgSt or leucine was performed by a validated hand mixing method [40-41]. Mixtures of MgSt (1% and 2%) or leucine (1% and 2%) and PCL microparticles were prepared in 5.0 g batches. The MgSt or leucine powder was placed between two layers of PCL powder in a glass test tube along with three ceramic beads of approximately 10 mm in diameter. The test tube was vigorously shaken by hand for 5 minutes to ensure proper mixing. During this process ceramic beads provided a ball milling effect for breaking up the agglomerates formed during mixing. This same technique was used to prepare mixture of SS (2.5%) with PCL microspheres, coated PCL microspheres or lactose monohydrate (Aeroflo-95). These mixtures were finally used for homogeneity tests and *in vitro* aerosol deposition tests as mentioned in Sections 2.8 and 2.9.

2.3.2 Solution coating

The solution of MgSt was prepared in ethanol (1% and 2% w/v) with the aid of heat and the aqueous solution of leucine was prepared in milliQ water (1% and 2% w/v). Approximately 4.0 g of PCL microspheres were immersed in 10 ml of MgSt or leucine solutions with stirring

for a period of 24 hours. Finally the coated microspheres were collected by filtration and dried in a vacuum desiccator at room temperature.

2.4 Particle size measurement

The particle size of the carrier particles was measured by laser diffraction (Malvern Mastersizer, Malvern Instruments Ltd, UK) using a small volume dispersion unit. The carrier particles (400 mg) were dispersed in 5 mL of water containing Tween-80 with the aid of sonication in a water bath for 5 minutes. This sonicated sample was added dropwise to the sample cell containing 100 mL of distilled water until an obscuration between 15-30% was obtained. The average particle size (VMD) and size distributions were measured from five replicates of each sample.

2.5 Scanning electron microscopy (SEM)

For surface morphological studies of the carrier particles and the drug-carrier mixture, samples were adhered onto aluminium stubs using double-sided carbon sticky tape and sputtered with gold (BIORAD SC-500 Sputter coater). Several photomicrographs of the samples were taken at different magnifications using a SEM (FEI Quanta 200).

2.6 Energy Dispersive X-ray Analysis (EDX)

Elemental composition analysis of the carrier particles mixed with the drug was carried out using Energy Dispersive X-ray Analysis in a SEM (Quanta 3D FIB). Particles were carbon coated with a Cressington Carbon Coater using an electrical current of 100 A for 2 minutes and 10^{-4} mbar pressure. Samples were analyzed for sulfur that was present as the salt of the SS powder and magnesium for MgSt coatings.

2.7 X-Ray Photoelectron Spectroscopy (XPS)

The surface composition of the uncoated and coated PCL microspheres was analyzed by XPS. Samples were mounted onto stainless steel sample holders using double-sided adhesive tape.

XPS analysis was performed with a Kratos Axis Ultra spectrometer (Kratos Analytical, Manchester, UK) equipped with monochromatized aluminium X-ray source (powered at 10mA and 15kV) and an eight-channeltron detector. The analyzed area was 800 x 200 μm. The constant pass energy was set at 160eV for the survey spectrum and 20eV for the multiplex spectrum. The following sequence of spectra was recorded: Survey spectrum, O_{1s}, N_{1s}, C_{1s}, and Mg_{2p} multiplex spectra. The elemental compositions of each material were determined using Casa XPS software Version 2.3.14.

2. 8 Homogeneity tests

The homogeneity of each drug-carrier mixture prepared in Section 2.3.1 was determined by sampling 20 x 20 mg samples from the mixtures and assaying for SS content. The samples were dissolved in 10 ml of water and the amount of SS was quantified based on UV absorbance in triplicates. The UV spectrum of SS in water was analyzed over a wavelength range of 190-400nm by a UV spectrophotometer (U-2800 spectrophotometer, Hitachi) using 10 mm uv-grade silica cells to measure the wavelength of maximum absorbance (λ_{max}). The Beer-Lambert calibration curve was prepared at 276 nm using concentrations ranging from 0-500 μg/ml.

2. 9 *In vitro* aerosol deposition

The *in vitro* aerosol deposition of the powder formulations was determined by a TSI (Copley, UK). Rotahaler® (Glaxo Wellcome) was the DPI device used and the collection liquid used for the study was water. 7 ml of water was placed in stage one (S1) and 30 ml in stage two (S2) of the TSI. The air flow was drawn through the TSI using a vacuum pump (D-63150, Erweka, Germany) and the air flow rate was adjusted to 60±5 L/min at the mouthpiece, prior to each measurement.

The powder formulations were loaded (about 20.0 mg) into hard gelatin capsules (size 3, Fawns and McAllan Pty Ltd.; Australia). The filled capsule was inserted into the Rotahaler which was placed into a moulded mouthpiece attached to the TSI. The Rotahaler was twisted to release the powder into the body of the device and an air volume of 5 L (5 seconds at 60 L/min) was drawn for each measurement. Each section (Inhaler, S1 and S2) was rinsed with water and the liquid was collected and volume was adjusted to 100 mL. The SS content was measured by HPLC analysis. Five replicates of each mixture were performed for TSI measurement.

The recovered dose (RD) was the total amount of drug collected from the inhaler, S1 and S2. The emitted dose (ED) was the fraction of the RD delivered from the inhaler expressed as a percentage:

$$ED = \frac{S1 + S2}{RD} \times 100$$

The fine particle fraction (FPF) was defined as the fraction of the recovered dose deposited in the lower stage of TSI expressed as a percentage of RD.

$$FPF = \frac{S2}{RD} \times 100$$

2.10 HPLC assay

Salbutamol Sulfate was analyzed by HPLC (Agilent HP1100) using a C_{18} column (µBondapak®, 3.9x300mm, Waters) and an UV Diode Array detector (Agilent) at a wavelength of 276 nm. A mixture of methanol and 0.2% w/v ammonium acetate solution (40:60) was used as a mobile phase at a flow rate of 1.0 ml/min by a HPLC pump (Quat pump, Agilent). An injection volume of 100µl was used. The retention time of SS was found

to be 4.3 minutes. An HPLC calibration curve was prepared from the peak area determined by integration over a concentration range of 0-100 μg/ml.

2.11 Adhesion force measurement

The adhesion forces between the microspheres and drug (SS) was determined by Atomic Force Microscopy (AFM) using a colloid probe technique[42-43]. The silicon nitride cantilever (225 ± 10 μm long, 28 ± 7.5 μm wide and 3 ± 1 μm thick) used for the determination of adhesion forces had a single silica particle (sphere of diameter 3.5 ± 0.1 μm) attached on the tip of the cantilever (CP-FM-SiO-B-5, NanoAndMore, Germany). The cantilevers have resonance frequencies ranging from 45-115 kHz and force constants ranging from 0.5-9.5N/m. The spring constant (k) for each cantilever used was determined using the thermal noise method[44-45]. The spring constant was found to vary from 1.12 - 2.55 N/m.

2.11.1 Sample preparation

The polymer microspheres were glued on a clean glass slide with Araldite five-minute epoxy resin glue (Selleys Chemical Company, Australia). A uniform thin smear of the glue was made in the centre of the clean glass slide and the microspheres were sprinkled on the resin glue and allowed to dry for five minutes. The excess particles were removed from the slide by blowing with nitrogen.

2.11.2 Functionalization of silica probe with SS

The silica sphere on the cantilever was functionalized with SS solution to act as SS probe. The drug was coated on the silica sphere of the cantilever with the aid of approach/retract cycle of AFM scanner. This procedure was first developed in our lab and was validated. A small drop of supersaturated solution of SS was placed on a clean glass slide and the slide was placed on the scanner of the AFM. The silica probe to be functionalized was secured on the cantilever holder and positioned exactly above the SS solution. The cantilever was made to

approach to the solution on the surface using the microscope's feedback loop with a controlled motion, was kept immersed in the SS solution for 10 minutes; retracted from the solution after 10 minutes and allowed to dry for a period of 30 minutes. This resulted in the formation of multilayered coating of SS on the silica sphere. This method ensured a SS layer on the spherical silica probe, which is perfect for adhesion force measurement. The SS functionalization method was validated for the time required by the silica spheres to be completely coated by the SS solution. This was achieved by coating the silica spheres with SS solution for periods of 5, 10 or 30 minutes, dried and analyzed by XPS to detect the presence of sulfur on the sphere. The XPS analysis revealed that the spheres coated for the time interval of 10 and 30 minutes had a very strong signal of sulfur and silica was not found in the spectrum indicating that 10 minute time interval was sufficient to coat the spheres with SS. Therefore, the time period of 10 minutes was selected to functionalize the silica probe with SS.

2.11.3 Force Measurement

All force-distance measurements were recorded in air and ambient humidity and were performed using AFM MFP-3D (Asylum Research, Technical Manufacturing Corporation, USA) and IGOR Pro 6.21 software (Wavemetrics, USA). Force volume measurements were performed between the silica sphere coated with SS and randomly chosen spots on the uncoated and coated polymer microspheres. In the force-volume mode, the AFM raster scans the substrate under the colloidal probe to produce a series of force curves, each from a well-defined location in the x and y directions. The results were produced as a force map which showed the variation in the forces of interaction in the defined area. Individual force curves were measured over a 10 μm x10 μm area, at a scan rate of 1Hz and a total of 32x32 (n=1024) force points. The adhesion force determination was performed on 5 different microspheres for each sample (n=5) and a total number of 5120 force curves were measured for each sample.

2.12 Statistical Analysis

Comparison between different groups of FPF and adhesion forces was performed by one-way analysis of variance (ANOVA) to ascertain statistical significance; $p<0.05$ was considered to be statistically significant.

3. Results and Discussion

3.1 Particle sizing and drug dispersion from lactose

Inhalation grade Aeroflo-95 lactose was used as a control because it has a VMD of 112.9 ± 2.5μm which is comparable with the particle size of the PCL carrier that has been fabricated (VMD: 104 ± 0.4μm), although the morphologies of two particles are different. It is worthwhile to mention that the lactose carriers used as control showed a wider particle size distribution. This was due to the presence of a significant amount of associated fine particles of lactose attached on the surface of large carriers; whereas, PCL showed comparatively narrow size distribution (Figure 1). As is illustrated in Figure 1, the large lactose particles have a significant amount of associated fine lactose (less than 10 μm) and the effect of fine particles on the particle size distribution as well as the SS dispersion has not been taken into account in this study. The lactose carrier, SS and the mixture of SS and lactose were characterized by SEM imaging as shown in Figure 2. The fine particles of lactose attached on the surface of large carriers were not easily differentiated from the particles of SS in the mixture of SS and lactose. An EDX analysis of the lactose-SS mixture was carried out to distinguish between the two and confirm the presence of drug adhered onto the carrier surface (Figure 3A). Since the drug is present as a sulfate salt, the detection of the sulfur in the EDX spectrum confirms the presence of the SS on the lactose carriers. This drug-carrier mixture was found to be homogenous (Table 1). When the mixture was subjected to *in vitro* deposition tests using TSI, FPF of 13.5 ± 2.5% was obtained as shown in Table 2.

3.2 Characterization of PCL microspheres

SEM images revealed that the microspheres are spherical and had a rough surface morphology (Figure 4A). These PCL microspheres were dry-mixed with the drug particles and the SEM images confirm that the drug has adhered onto the entire surface of the PCL carrier (Figure 4B). The drug-polymer mixture was found to be homogenous (Table 1). The TSI experiment indicated that the drug had been emitted effectively from the DPI device (% ED: 63.6 ± 7.2) (Table 2) but no FPF of SS was obtained, which means a significant amount of powder was retained in the device and no drug particles were detached from the PCL surface. It seems that the drug was strongly adhered on the surface of the PCL microspheres and could not be detached from the surface by the airflow in the TSI. As evident from the SEM image (Figure 4B), the micronized SS particles formed a close-packed array on the surface of the PCL carrier which is smooth and hence difficult to detach from the surface. Therefore, it was vital to determine the surface composition of the PCL microspheres to understand the reason behind this strong SS-PCL adhesion (301.4 ±21.7 nN). The surface composition was determined using XPS analysis as described in the section 3.3 and data on adhesional force have been demonstrated in section 3.5.

3.3 XPS analysis of PCL microspheres

XPS analysis shows that the spectrum of PCL microspheres was different from PCL powder (Figure 5). A higher C-O signal in Figure 5B suggests that that the initially hydrophobic surface of the PCL microspheres was covered with polyvinyl alcohol which was used as a surfactant in the manufacture of the microspheres. The analysis is complicated by the fact that the PVA used for the microsphere preparation was 87-89% hydrolyzed so it contained 11-13% of residual vinyl acetate groups. However, comparison with the spectrum of the PVA used in the microparticle preparation shows that the surface of the PCL microspheres contains a layer of PVA along with some residual acetate groups. Thus the hydrophobic surface of

PCL is rendered more hydrophilic due to the presence of PVA which might have led to the strong adhesion between the PCL microspheres and the hydrophilic drug particles.

We speculated that this strong adhesion might have occurred due to the formation of a hydrogen bonds between hydroxyl groups of PVA on the surface of PCL (which has a low glass transition temperature of -60^{o}C and is thus rubbery at room temperature) and either hydroxyl groups or nitrogen group of SS. In order to test our hypothesis we analyzed pure PCL, pure SS, PCL microspheres and SS-PCL mixture using infrared spectroscopy. When the spectrum of SS was subtracted from that of the SS-PCL mixture the spectrum obtained was similar to the spectra of PCL microspheres indicating that there was no detectable hydrogen bond formation between SS and PCL. Hence, the more likely reason for this strong adhesion is the formation of capillary bridges between the hygroscopic PVA, which is present on the surface of PCL, and the hydrophilic SS. The hygroscopic PVA might have led to the absorption of moisture from the atmosphere. This atmospheric moisture around the surface contact sites usually exerts strong force between the adjacent solid bodies and this force is much stronger compared to the other surface forces such as electrostatic forces and Van der Waals forces. Therefore, there was no detachment of the drug particles from the PCL surface resulting in no FPF of SS (Table 2).

To overcome the problem of strong adhesion between SS and PVA coated PCL, PCL surface was modified with a suitable excipient to decrease the forces of adhesion with an objective to achieve better detachment of drug particles from the PCL surface. This was achieved by coating the surface of the microspheres with a ternary agent, which exhibits anti-adherent properties. Based on a consideration of the need for excipients that would not only bind to the PVA surface but also produce a hydrophobic layer in order to reduce the bonding to SS, MgSt and leucine were chosen. Both these agents are hydrophobic and may act as lubricants between surfaces thus improving the dispersibility of the powders.

Solution coating and dry mixing were both used for coating the microspheres. The coated polymer microspheres were further subjected to XPS analysis to confirm the presence of the MgSt and leucine on the PCL microspheres. The XPS data (Table 3) confirmed the presence of magnesium and nitrogen elements on the PCL microspheres which indicated that the PCL particles were successfully coated with these two agents. Also this technique was used to determine the thickness of the coating on the surface of the PCL microspheres [46]. If a uniform layer is formed, the thickness of the MgSt or leucine coating obtained from solution may be determined using the following formulae:

$$\frac{d}{\lambda} = -\ln\left(1 - \frac{I}{I_\infty}\right)$$

Where d is the thickness of the layer on the substrate, λ is the photoelectron mean free path in the overlayer, I is the signal intensity of the overlayer (intensity of magnesium or nitrogen obtained from coated PCL microspheres) and I_∞ is the signal intensity from a homogenous infinitely thick sample of the overlayer (intensity of magnesium or nitrogen obtained from pure MgSt and leucine powders respectively).

According to Table 3, I_∞= 2.1 and 11.17 for MgSt and leucine powder samples, respectively. The mean free path (λ) for a magnesium photoelectron was unavailable and Si-2p was taken as a reference element because both photoelectrons have similar kinetic energies. λ was taken to be 40 Å on average for a silicon layer (Si-2p) on polyester or polystyrene material. λ was taken to be 32 Å for nitrogen which is the reference value of N-1s on polyester or polystyrene material [47].Using this equation and the I values in Table 3, the thickness of MgSt coating on the surface of the PCL microspheres was determined to be 10Å and 38Å when deposited from 1% and 2% MgSt solutions, respectively. In contrast, it was found by the same method of analysis that 1% and 2% leucine solution coating provided 5 Å and 8 Å layers on the surface

of PCL microspheres. It should be noted that these calculations assume that a uniform layer has been deposited on the surface, which as noted later is only the case for leucine.

3.4 Characterization of PCL microspheres after surface-coating and drug dispersion

The coated PCL microspheres were dry-mixed with SS and homogenous mixtures were formed (Table 1). The coated PCL microspheres and the SS-PCL mixture were characterized using SEM imaging (Figure 6). It was expected that MgSt solution coating would form a thin layer on the surface of PCL microspheres but in the SEM images Figure 6A and 6C, it was observed that MgSt had deposited in the form of crystals on the surface of the PCL microspheres. This could be due to crystallization of MgSt on evaporation of the ethanol solvent. These SEM images reveal that since the MgSt coat was not formed in a continuous film but was found as discontinuous patches of crystals on the surface of microspheres, then the calculations for the determination of its thickness via XPS analysis are inappropriate for this particular sample. The SEM images further confirm that the drug has adhered onto the surface of the coated PCL carrier but not to the same extent as compared with the uncoated PCL microspheres (Figure 6B, 6D, 6F and 4B). It is noted that in case of coated microspheres the attachment of the SS particles on the carrier is more isolated and the surface corona is rougher as compared to the uncoated PCL carrier.

As may be seen from the SEM images (Figure 6B and 6D), in case of 1% and 2% MgSt coated PCL microspheres, it was difficult to distinguish between the particles of MgSt and SS; therefore, it was necessary to determine elemental composition to confirm the presence of drug and MgSt on the surface of PCL. The presence of elemental magnesium and sulfur in the EDX spectrum confirms that the PCL microspheres have been coated with MgSt and the drug particles are adhered onto it (Figure 2B).

The FPF values obtained from these surface-modified samples are shown in Table 2. The FPF obtained with MgSt and leucine coating was found to be promising. It was found that 1% MgSt powder coating yielded an FPF of 12 ± 1.3%; however no significant improvement in the FPF of the drug was obtained from PCL carriers coated with 1% MgSt solution (FPF: 11.4 ± 0.9%) ($p>0.05$, n=5). It is evident from the SEM images that even in the case of solution coating the MgSt had precipitated in the form of crystals on the surface of the PCL microspheres. 2% MgSt powder coated and solution coated PCL microspheres showed a significant improvement in the FPF of SS (15.8 ± 1.5% and 15.4 ± 1.6%) as compared with 1% MgSt powder coated and solution coated PCL microspheres ($p<0.05$, n=5). However again there was no significant improvement in the FPF when MgSt was used to coat the microspheres from solution compared to powder coating. This is explained since in both cases SEM shows crystals of MgSt were deposited on the PCL surface. SEM shows that the coating was formed as discontinuous patches (Figure 6A and 6C). As the concentration of the MgSt increased there was more crystallization of the MgSt on the PCL surface resulting in increased surface coating of the PCL microspheres with increased FPF of SS.

For the leucine excipient, the dry powder coating yielded a lower FPF (6.0 ± 0.5% and 10.2 ± 1.2% for 1% and 2% concentrations, respectively) compared to the solution coating process. Coating the PCL particles with either 1% or 2% leucine gave the same FPF (11.3 ± 1.1% and 11.3 ± 0.8% respectively) ($p>0.05$, n=5). In contrast for the earlier values quoted for dry powder coating the FPF obtained with 2% leucine was statistically different from that obtained with 1% leucine ($p<0.05$, n=5). In dry powder leucine coating as the concentration of leucine increased on the surface of PCL, it caused improved surface coating of the PCL microspheres and increased the FPF by masking the PVA layer on the surface with leucine. In contrast, the lack of an effect of leucine concentration in the solution coating results could be due to the fact that 1% concentration of leucine was sufficient to cover the PVA layer on

PCL. This caused easy detachment of SS and hence increased FPF. There was no evidence for crystallization of leucine on the surface of the PCL in contrast to the results for MgSt. Consequently, the overlayer calculation is applicable and a thickness of only 5 Å of leucine is required to mask the PVA surface layer on PCL.

3.5 Effect of adhesion forces

The adhesion force between the silica sphere functionalized with SS and PCL microspheres were found to be 301.4 ± 21.7 nN. In contrast, when the adhesion forces were determined between SS and 1% or 2% MgSt coated PCL microspheres, the forces of interaction were found to decrease drastically to 110.9 ± 30.5 nN and 121.8 ± 24.6 nN, respectively ($p<0.05$, $n=5$). Similarly the adhesion forces between SS and 1% or 2% leucine coated PCL microspheres were found to be reduced to 148.1 ± 21.0 nN and 150.2± 18.1 nN, respectively. However there were no significant differences in the adhesion forces between the two concentrations (1% or 2%) in either cases of MgSt or leucine ($p>0.05$, $n=5$) (Figure 7). From the figure, it is clearly revealed that the adhesion forces between the coated PCL and SS have been reduced significantly, which resulted in increasing FPF of SS that has been described previously. Although adhesion forces between 1.0% MgSt coated PCL and SS was lower compared to that of 2.0% MgSt coated PCL carriers, the later carrier provided a significant improvement in the FPF over 1% MgSt coating. This would have occurred due to the fact that when 2% MgSt solution was used, a significant amount of MgSt had crystallized on the surface of the PCL microspheres (as discussed earlier using SEM images), which led to increased coating on the surface resulting in easy detachment of the SS from the surface of microspheres. In addition, MgSt is a highly hydrophobic glidant, which contributed to reduce adhesion between hydrophilic SS and coated carriers, resulting in increasing FPF of SS. In contrast, leucine coated PCL microspheres, although showed to reduce adhesion forces and significantly increased the FPF of SS; there was no difference in the FPF of SS in both

concentrations (1% or 2%) of leucine. This can be emphasized that the solution coating was uniform in case of leucine and 1% concentration was enough to coat the entire surface of PCL microspheres. In addition, leucine is relatively hydrophobic and hence reduced adhesion forces between hydrophilic SS and coated carriers; however, the reduction of adhesion force is less than that of the forces between hydrophilic SS and hydrophobic MgSt coated PCL carriers. In addition, leucine plays an important role as an aerosolization enhancer and thus we believe that the leucine coating contributed to the enhanced aerosolization leading to increase FPF of SS. Thus both the MgSt and the leucine coating may be contributing to the SS detachment by reducing the adhesion forces between SS and PCL particles, as well as increased aerosolization, resulting in increased dispersion of SS. The effect of surface coating on the FPF of SS can be depicted in Figure 8. When the uncoated polymer microspheres were used as a carrier, the drug did not detach from its surface (Figure 8A) due to strong adhesion force between SS and PCL carriers. However upon coating the carrier with the anti-adherent agents like MgSt or leucine, the drug was easily detached from the surface (Figure 8B) due to the reduction of adhesion force and significant improvement in the FPF of drug was obtained.

4. Conclusion

The work presented here has highlighted the potential of surface coated biodegradable PCL microparticles as carriers for pulmonary drug delivery system. The surface coating of PCL microparticles carriers have significant impact on the reduction of adhesion forces as well as increase aerosolization of SS from the carrier based DPI formulation. Without surface coating, the drug SS adhering on the PCL carrier has been efficiently emitted from the device but showed no FPF. This was probably caused by the highly adhesive SS-PCL interaction due to the presence of PVA on the PCL surface. Drug particles did not detach from the PCL surface, presumably due to the generation of capillary forces between PVA on the surface of

PCL and SS, resulting in no FPF. We demonstrated that the solution coatings of MgSt and leucine effectively covered the PVA layer on the PCL surface, which enabled lowering of the adhesive forces between drug particles and the surface modified carrier thereby facilitating the easy detachment of the drug particles from the PCL surface and a concomitant increase in drug deposition. These results suggested that the surface coatings of MgSt and leucine on biodegradable PCL carriers have a significant role in improving the performance of DPI formulations.

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Figure 1: Particle size distribution of lactose particles and PCL microspheres

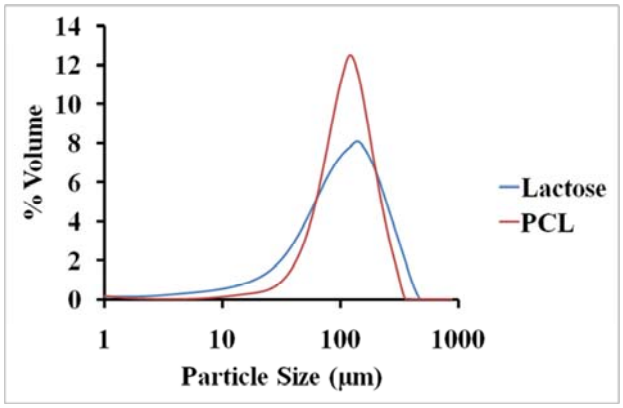

Figure 2: SEM images of (A) Lactose, (B) SS and (C) SS-Lactose mixture

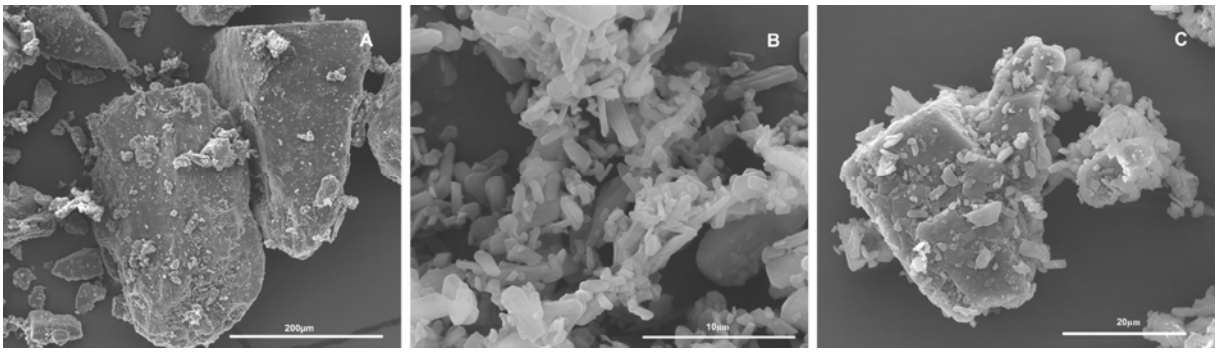

Figure 3: EDX spectrum of (A) SS-Lactose mixture and (B) SS-PCL mixture coated with MgSt

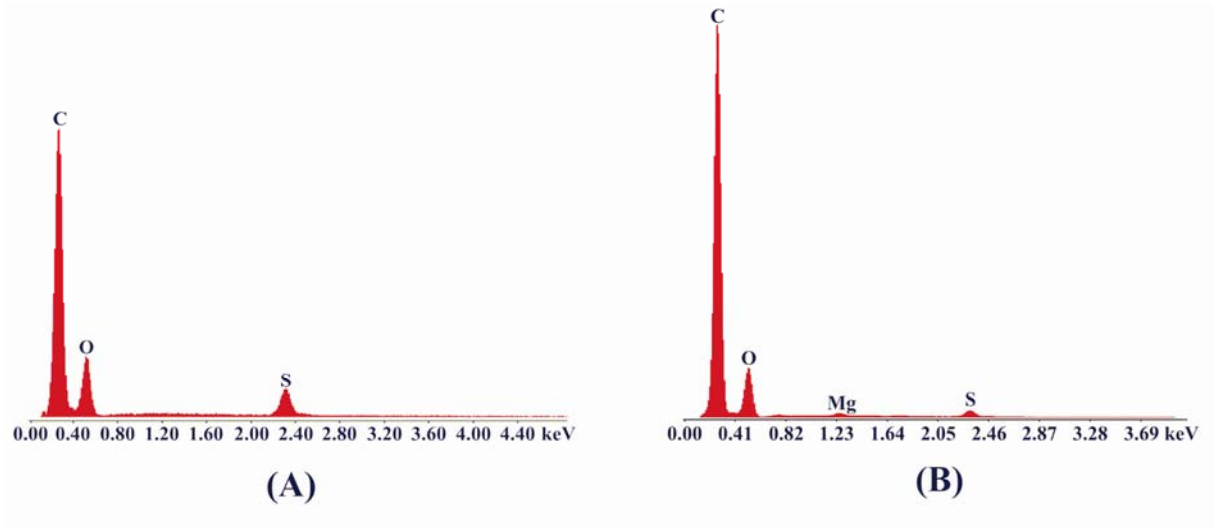

Figure 4: SEM images of (A) PCL microspheres and (B) SS- PCL mixture. Note the high surface coverage of SS on the PCL particle compared to the lactose particle in Figure 1C

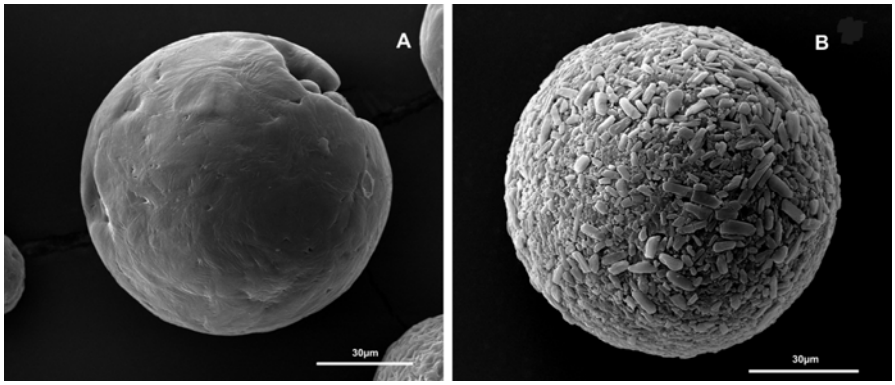

Figure 5: XPS scan of (A) PCL powder and (B) PCL microspheres

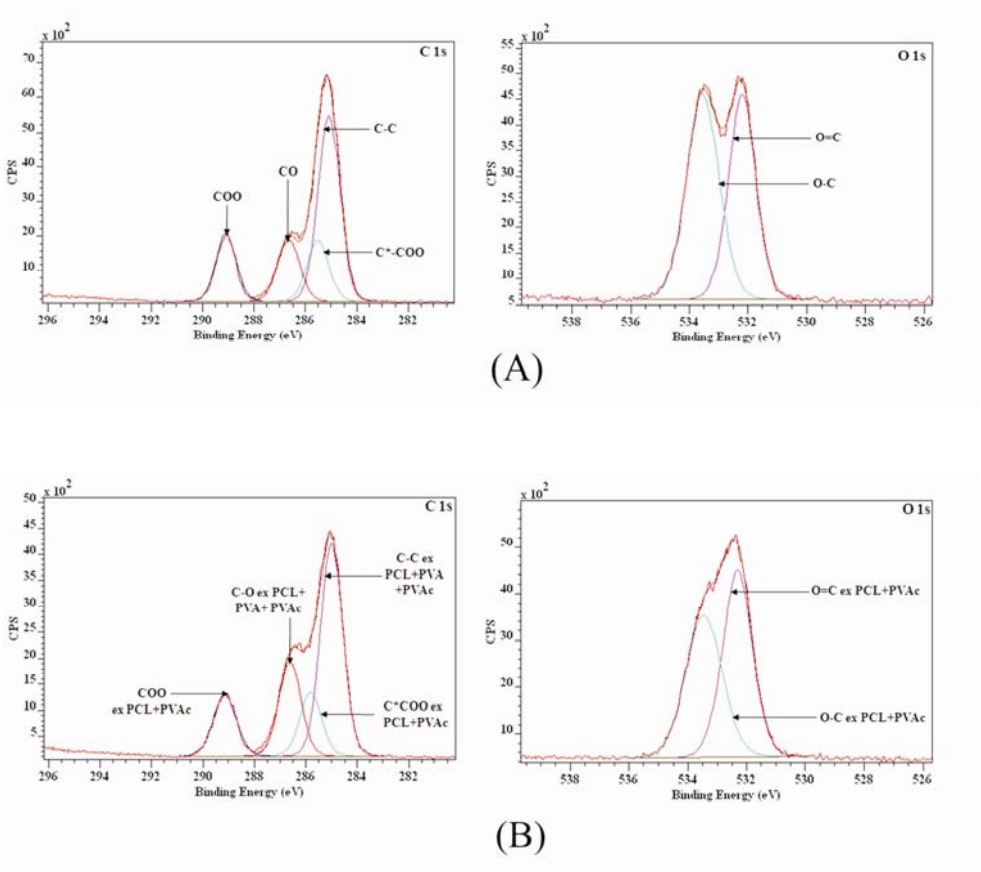

Figure 6: SEM images of (A) PCL coated with 1% MgSt solution (B) Mixture of SS (2.5%w/w) and PCL coated with 1% MgSt solution (C) PCL coated with 2% MgSt solution (D) Mixture of SS (2.5%w/w) and PCL coated with 2% MgSt solution (E) PCL coated with leucine solution and (F) SS-PCL mixture coated with leucine solution

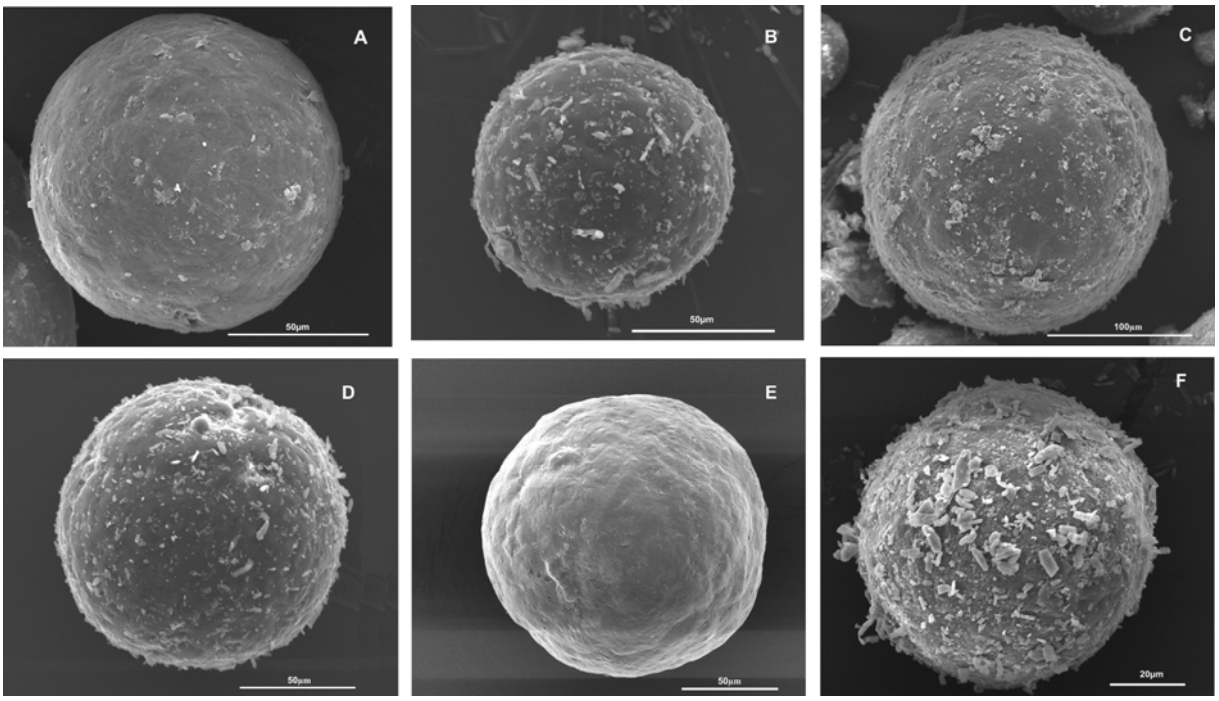

Figure 7: SEM images of (A) PCL coated with 1% MgSt powder (B) Mixture of SS (2.5%w/w) and PCL coated with 1% MgSt powder (C) PCL coated with 2% MgSt powder (D) Mixture of SS (2.5%w/w) and PCL coated with 2% MgSt powder (E) PCL coated with leucine powder and (F) SS-PCL mixture coated with leucine powder

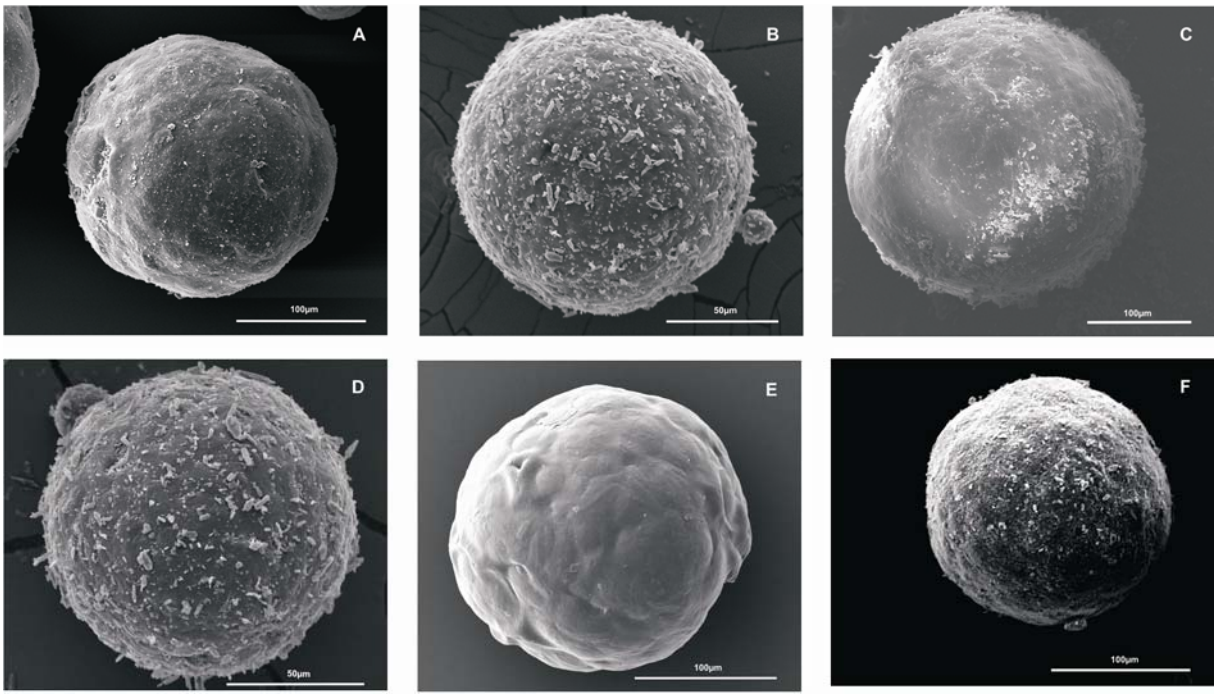

Figure 8: Adhesion forces of PCL microspheres, PCL microspheres coated with MgSt and PCL microspheres coated with leucine (Mean ± S.D., n=5)

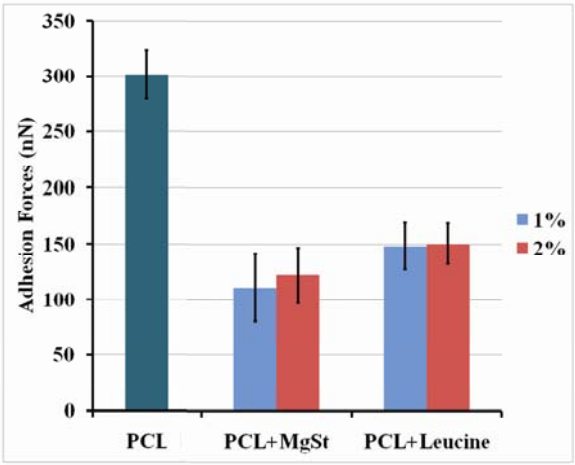

Table 1: Homogeneity tests on SS - Carrier Mixture

Sample	Accuracy (%)	CV (%)
SS and Lactose (Aeroflo-95)	99.8	0.4
SS and PCL	99.8	0.5
SS and PCL coated with 1% MgSt powder	99.4	0.7
SS and PCL coated with 2% MgSt powder	99.6	0.5
SS and PCL coated with 1% MgSt solution	99.3	0.9
SS and PCL coated with 2% MgSt solution	99.9	0.2
SS and PCL coated with 1% leucine powder	99.0	0.5
SS and PCL coated with 2% leucine powder	98.7	0.3
SS and PCL coated with 1% leucine solution	98.8	0.8
SS and PCL coated with 2% leucine solution	99.2	0.8

Table 2: Fine Particle Fraction (FPF) of SS (Mean ± SD, n=5)

Sample	FPF (%)	Emitted Dose (ED) (%)	Recovered Dose (RD) (%)
SS and Lactose (Aeroflo-95)	13.5 ± 2.5	54.5 ± 8.4	98.0 ± 2.0
SS and PCL	-	63.6 ± 7.2	92.4 ± 5.0
SS and PCL coated with 1% MgSt powder	12.0 ± 1.3	60.3 ± 5.0	87.0 ± 5.2
SS and PCL coated with 2% MgSt powder	15.8 ± 1.5	50.3 ± 7.1	96.9 ± 3.9
SS and PCL coated with 1% MgSt solution	11.4 ± 0.9	40.3 ± 2.4	86.2 ± 5.0
SS and PCL coated with 2% MgSt solution	15.4 ± 1.6	64.4 ± 5.6	95.0 ± 5.7
SS and PCL coated with 1% leucine powder	6.0 ± 0.5	67.1 ± 5.4	90.6 ± 5.0
SS and PCL coated with 2% leucine powder	10.2 ± 1.2	65.3± 3.0	97.4 ± 6.2
SS and PCL coated with 1% leucine solution	11.3 ± 1.1	56.1 ± 9.3	85.0 ± 3.5
SS and PCL coated with 2% leucine solution	11.3 ± 0.8	67.0 ± 4.1	84.4 ± 3.3

Table 3: XPS results of PCL microspheres coated with MgSt and Leucine solution

Sample	Atomic Percentage of Elements			
	C	O	Mg	N
MgSt powder	89.10	8.85	2.10	-
PCL microspheres coated with 1% MgSt solution	77.86	21.67	0.47	-
PCL microspheres coated with 2% MgSt solution	86.39	12.3	1.31	-
Leucine powder	68.77	20.06	-	11.17
PCL microspheres coated with 1% leucine solution	72.20	26.25	-	1.55
PCL microspheres coated with 2% leucine solution	70.98	26.57	-	2.45

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