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Effect of surface treatment on the respirable fractions of PLGA microspheres formulated for dry powder inhalers[1]

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Abstract

An investigation of the degree of aggregation of dry powders with different surface charge and moisture contents was carried out using poly(D,L-lactide-co-glycolide) (PLGA) microspheres. Microspheres were prepared in the respirable size range (3–7 μm) with an estimated zeta potential (ZP) of -30.6 mV. Suspensions of polylysine (PL) and polyglutamic acid (PGA) in isopropanol were used to treat the microspheres to obtain ZPs of ± 60 mV, respectively. Treatment with isopropanol (IPA) alone yielded a ZP of -36.7 mV, which was similar to the untreated microspheres -30.6 mV. Respirable fractions (RFs) were determined by cascade impaction using a prototype DPI. The IPA-treated formulation was the most deaggregated of the studied samples as indicated by a significantly higher RF (12.9) compared to the PL and PGA formulations (3.29 and 2.42, respectively) and the untreated PLGA microspheres (3.84). Microspheres with moisture contents in the range of 0.40–2.47% were studied and a moisture content of 0.66% resulted in the highest relative deaggregation of the PLGA microspheres. Measurement of the surface charge and mass median aerodynamic diameter in the dry state by electrical-single particle aerodynamic relaxation time analysis (E-SPART) suggested that the higher RF for the IPA formulation may be due to its highly unipolar nature, i.e. a net charge of $+56.3$ μC/g, as compared to net charges in the range of 34.3–38.4 μC/g for the other microspheres. Formulation of microspheres for use in DPIs must proceed on a case by case basis as certain approaches can enhance aggregation. © 1997 Elsevier Science B.V.

Keywords: Dry powder inhaler; Poly(D,L-lactide-co-glycolide) microspheres; PLGA; Surface charge; Aggregation of powder; Moisture content; Zeta potential; Aerosols

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1. Introduction

Therapeutic levels of drugs delivered to the respiratory tract (RT) are not maintained for a sufficient duration due to rapid absorption (Clark et al., 1985). It would be beneficial to control or prolong the residence time of the small fraction (less than 20%) of drug that does reach the desired sites in the RT. Biodegradable microspheres may have an application in inhalation therapy by providing sustained release of drug and improving patient compliance. Sustained release would be advantageous in the treatment of asthma and pulmonary tissue diseases such as cancer, fibrosis and emphysema (Kanke et al., 1983; 1986). Isoproterenol microspheres have been shown to be substantially more effective in preventing serotonin-induced broncho-constriction in the airway smooth muscles of rats than the non-encapsulated drug (Lai et al., 1993).

Controlled aerosolized drug delivery has been mostly limited to metered dose inhalers (MDIs). Two major problems with MDIs are the coordination of the dose with inspiration and the depletion of the ozone layer, the latter caused by the chlorofluorocarbon propellants. Coordination of delivery with inhalation is usually not a problem with dry powder inhalers (DPIs), since most DPIs are activated by the breathing of the patient and hence the breathing is synchronized with the drug entry into the RT.

Although dry powders can be micronized to respirable diameters (less than 5 μm), the size reduction also adversely affects the deaggregation and flow characteristics of the powder. Most DPI formulations use drug in the micronized form along with diluents. Hydrophobic poly(D,L-lactide-co-glycolide) (PLGA) microspheres offer an alternative form that is less susceptible to the effects of hygroscopic growth and may reduce the intolerance of diluent materials by patients. The PLGA microspheres are especially suitable for delivering potent drugs since they act as a bulking agent and biodegrade at predictable rates (DeLuca et al., 1993).

Microspheres can be prepared with various morphological characteristics such as size, shape, and porosity by varying the process parameters (DeLuca et al., 1993). The success of a DPI formulation will be dependent on the ability of the microspheres to remain deaggregated and free-flowing. Aggregation of particles in the DPIs or upon their administration can influence the respirable fraction of the delivered dose. The molecular, electric, coulombic and capillary forces responsible for powder aggregation are influenced by particle size, surface morphology, surface charge, and moisture composition (Zimon, 1982; Newman, 1967; Atkins et al., 1992).

The objective of this work was to evaluate microsphere formulations over a range of surface charges and moisture contents for their propensity to aggregate. The microspheres were treated with a fine suspension of polyamino acids (PAAs) in an alcoholic solvent and the surface charge assessed by zeta potential (ZP) analysis and by electrical single particle aerodynamic relaxation time (E-SPART) analysis. The latter technique measures electrostatic charge and aerodynamic size distributions of particles suspended in air (Mazumder et al., 1991). A DPI that enabled easy characterization in vitro and in vivo was selected for assessing respirable fractions using the Andersen cascade impactor.

2. Experimental

2.1. Preparation of PLGA microspheres

Microspheres were prepared by a previously reported method (Sato et al., 1988). The dispersed phase (DP) consisted of PLGA (Resomer® RG 503, 50:50, 34 000 Da, Boehringer Ingelheim, Ingelheim, Germany) in methylene chloride (Fisher Scientific, Fairlawn, NJ) while the continuous phase (CP) was a 0.04% w/v solution of sodium oleate (EM Science, Cherry Hill, NJ) in distilled water. The DP was added to the CP using a Silverson dispersator (Model L4R, Silverson Machines, Chesam, England) under controlled processing conditions. Solvent removal was effected by extraction evaporation and the resulting microspheres were recovered by filtration, washed with distilled water and dried under vacuum.

2.2. Treatment of microspheres

A 0.1% suspension of polyamino acid (PAA, Sigma Chemical, St. Louis, MO) was prepared in 50 ml isopropanol (IPA, Fisher Scientific) with polylysine (PL) and polyglutamic acid (PGA). This was added to a 0.55% PLGA microsphere suspension in 100 ml IPA. The two suspensions were combined and sonicated for 2 min, then agitated for 12 h at room temperature. The suspension was filtered, rinsed with IPA and vacuum dried. The amount of PAA that remained in the powder mixtures was not quantitated by an additive-specific assay, due to lack of a method suitable for the carrier-additive system. Instead, the effect of the treatment on the physico-chemical properties of the microspheres was assessed by measuring the surface charge and aerodynamic diameter. Microspheres were treated with isopropanol in the same manner as the PAAs but without PAA.

To study the effect of moisture microspheres were transferred to glass vials that were stoppered with open-centered screw caps lined with nylon macrofiltration screen (mesh size 30 μm, Spectrum Products, Houston, TX). The vials were placed in a desiccator of 2 l capacity with a reservoir containing a saturated copper sulfate solution that provided 98% relative humidity at 22°C. Samples were withdrawn at pre-determined time points for moisture analysis and for cascade impaction analysis.

2.3. Characterization of microspheres

2.3.1. Particle size analysis

Microspheres were suspended in Milli-Q® water (Millipore) containing 0.01% w/v Tween-80 (Sigma) and the particle size and size distribution analysed by a Malvern® laser light scattering device (Malvern Instruments, Worcestershire, England).

2.3.2. Scanning electron microscopy (SEM)

The untreated microsphere samples were prepared in Tween-80 by suspension as well as in the dry state using the DPI. Samples of the treated microspheres were prepared only in the dry state since the effect of the surface treatments on the powder would be altered by suspension in a liquid medium.

The wet samples were prepared by sonicating the powder in a 0.01% Tween-80 solution for 1–2 min, then filtering on a polycarbonate membrane filter (Poretics, Livermore, CA). The filter was dried at room temperature and secured on an aluminum sample mount (Ted Pella, Redding, CA). The dry samples were prepared by loading the powder in a Pfeiffer DPI (Pfeiffer, Princeton, NJ) and spraying directly on the surface of the polycarbonate filter that was secured on the aluminum sample mount. A conductive gold/palladium coating was applied to the surface of the stubs prior to examination using a Hitachi Model S-800 SEM instrument (Hitachi, Mountain View, CA) at an energy level of 5–10 kV and magnifications of 0.5–8 K.

2.3.3. Moisture content analysis

Moisture content was determined by the Karl Fisher technique (Metrohm Model 701, Metrohm, Herisau, Switzerland) using methoxyethanol (Aldrich, Milwaukee, WI) to extract the water from the microspheres. The titer value was determined by adding a known amount of water to conditioned methanol (Fisher), and blank determinations were performed by injecting a known amount of methoxyethanol into the cell.

2.3.4. Determination of surface charge

About 3 mg microspheres were quantitatively transferred to a 50 ml glass beaker, and 25 ml of freshly distilled water added. The suspension was sonicated for 5 s and allowed to equilibrate for 1 h. The electrophoretic mobility was determined using a Zeta-Meter (Model ZM-80, Zeta-Meter, NY) with a GT-2 electrophoresis cell and a molybdenum anode and platinum-iridium cathode. A pre-determined DC voltage was applied across the cell and the velocity of the particles monitored for a minimum of 30 particles to obtain the average tracking time. Electrophoretic mobility and ZP were determined according the instrument manual (Standard operating procedure manual for measurement of zeta potential; Zeta Meter ZM–80).

2.3.5. E-SPART analysis

A previously described method (Mazumder et al., 1991) was used with some modifications. Fig. 1(a) shows the block diagram for the E-SPART analyzer. The E-SPART analyzer consists of (a) a dual-beam, frequency-biased laser Doppler velocimeter (LDV), (b) a relaxation cell, and (c) an electronic signal and data processing system. The aerosol particles passed through the sensing volume in the direction normal to the plane containing the two converging laser beams. The light scattered from aerosol particles in the sensing volume was collected by receiving lenses and focused on a pinhole directly in front of a photomultiplier tube, which in turn produced an electrical signal that was analyzed to determine the particle diameter.

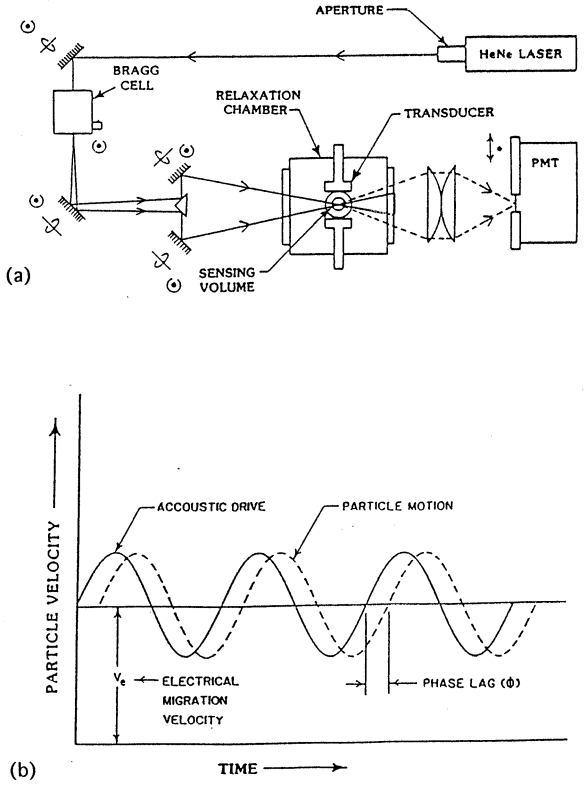

Fig. 1. The E-SPART analyzer. (a) Block diagram; waveform of the motion of a charged particle in the sensing volume (Mazumder et al., 1991)

The particle passing through the sensing volume experiences acoustic excitation causing it to oscillate. A DC electric field is simultaneously superimposed on the acoustic field causing charged particles to move along the direction of the acoustic oscillation, as shown in Fig. 1(b) (Mazumder et al., 1991). The resulting phase lag, ϕ, of the particle motion with respect to the acoustic field driving the particle is determined by the electronic signal and data processor. The aerodynamic diameter, d_a, is derived from the value of ϕ, while the measurements of d_a and the direction and magnitude of the electrical migration velocity provide the polarity and magnitude of the electrical charge of the particle.

2.3.6. Determination of respirable fraction

The Andersen 1.0 CFM Cascade Impactor (Andersen Samplers, Atlanta, GA) equipped with a preseparator and a glass adaptor (throat) was used for the determination of respirable fraction (RF). The RF was the fraction of particles collected on the stages less than 5.8 μm. Glass fiber collection substrates were used instead of the stainless steel collection plates (stages) because of lighter tare weight.

The Pfeiffer DPI which delivers the dose by a spring/air displacement mechanism was selected because it enabled easy in vitro and in vivo characterization of the dry powder formulations, and prototypes were available.

The microsphere samples were quantitatively filled into the DPI. A known amount was delivered to the cascade impactor, such that measurable quantities were recovered on the individual stages. Gravimetric analysis was used to obtain a percentage distribution of the microsphere mass in each part. Statistical analyses of differences between treatment sets were performed using the Student's *t*-test.

3. Results

3.1. Characterization of microspheres

3.1.1. Particle size distribution of microspheres

The average particle size by volume for a typi-

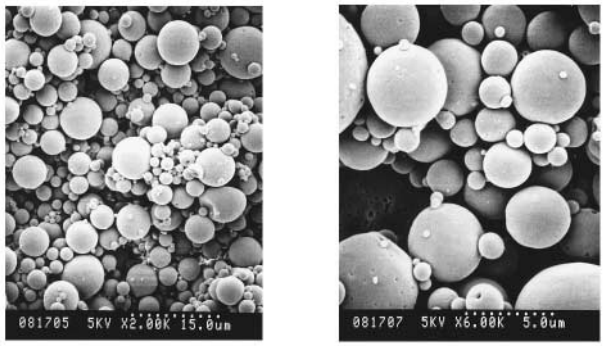

Fig. 2. SEM photomicrographs of untreated PLGA microspheres. The microspheres were collected by filtration of a suspension in 0.01% w/v Tween-80 solution.

cal batch of microspheres was 4.6 μm with 90% less than 5.3 μm. The average size of the microspheres tested was in the range of 3.5–4.6 μm, with 60–75% particle volume below 5 μm.

3.1.2. Surface morphology of microspheres

SEM photomicrographs of a typical batch of PLGA microspheres prior to treatment are shown in Fig. 2. The microspheres were polydisperse (the majority in the 3–7 μm size range) with a smooth surface containing very small pores. The SEMs of untreated and PAA/IPA treated microspheres prepared in the dry state are shown in Fig. 3. The untreated microspheres analysed in the dry state appeared to be much more aggregated than those prepared by suspension in 0.01% Tween-80. In the case of the PL/PLGA and PGA/PLGA microsphere mixtures, the powder appeared to be in a considerably aggregated state with clumps of microspheres, some of which were fairly large (over 100 μm). However, the IPA treated microspheres were mostly deaggregated or in relatively small aggregates compared to the PAA treated and the untreated microspheres.

The PAA/IPA treated PLGA microspheres samples showed very few traces of PAA particles, which appeared to be of much smaller size than their original size. This reduction in size appeared to be due to a partial solubilization of the crystals during the mixing process. Any modification of the surface morphology of the microspheres such as coating/adsorption due to the additives was not discernible from the SEM analysis. However, this does not preclude the possibility of a change in the surface properties of the PAA/IPA treated microspheres as a result of the physico-chemical interaction of the microspheres with these additives. Alteration of the surface state and the net surface charge of a given particle is known to be significantly influenced by the immediate environment as well as past physico-chemical treatment of the particle (Zimon, 1982).

The SEM analysis of the moisture treated samples indicated a trend of increasing aggregation of the microspheres with increasing exposure to high humidity, especially at moisture content values above 1%. Scanning electron microscopy showed enhanced aggregation of untreated microspheres that were incubated for 113 h. However, there were no apparent morphological changes.

3.1.3. Moisture content of microspheres

The moisture content of the untreated microspheres was around 0.4% following 18 h drying in a vacuum oven. When the microspheres were dried for longer durations, the powder was difficult to handle presumably due to generation of high triboelectric charges.

Table 1 lists the moisture contents of the microspheres incubated for different in 98% RH. The microsphere formulations with moisture contents above 2% could be visually seen to be

clumping due to the presence of water in the interparticulate spaces.

3.1.4. Surface charge determined by zeta potential analysis

Table 2 shows that the mean ZP was -30.6 mV for the untreated microspheres and -36.7 mV for the IPA treated microspheres. The ZP values of the PAA/PLGA microsphere mixtures were adequately high (targeted ZP: ± 55 mV).

3.1.5. Surface charge determined by E-SPART analysis

Table 3 lists the results of the measurement of the electrostatic charge of the PLGA microsphere formulations using the E-SPART analyzer. All the powder formulations were essentially bipolar in their charge distribution (i.e. having both positive and negatively charged particles), with a net positive charge. The IPA treated PLGA microspheres had the most unipolar nature among all the formulations, with a net charge/mass of $+56.3$ μC/g.

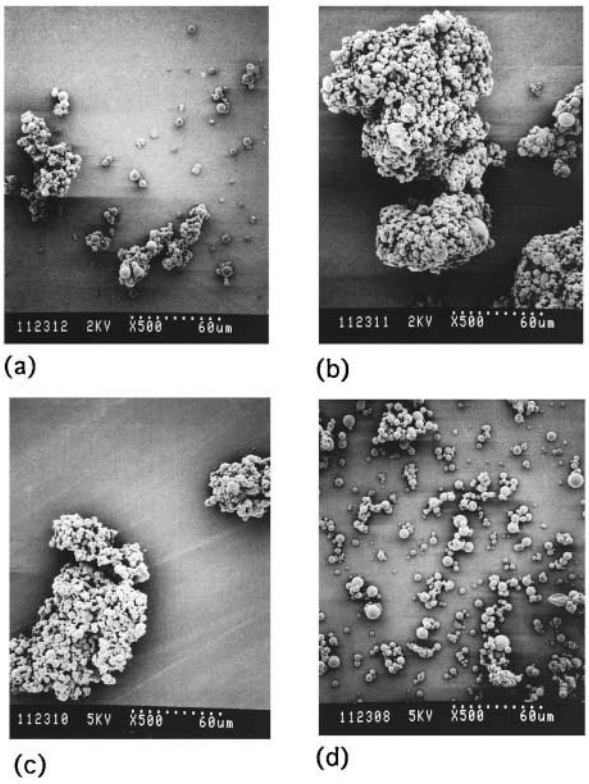

Fig. 3. SEM photomicrographs of PLGA microspheres with various surface treatments, in dry state using a Pfeiffer DPI. (a) Untreated, (b) polylysine-treated, (c) polyglutamic acid-treated, (d) isopropanol-treated.

3.1.6. Respirable fractions of microsphere formulations

The distribution of PLGA microspheres in the various parts of the cascade impactor assembly is tabulated in Table 4. The majority of the untreated powder mass was deposited in the preseparator (67.8%), while an average of about 9.5% of the mass penetrated beyond the first collection stage of the impactor. The low cascade impactor to preseparator distribution (C/P) ratio (0.14) is an indication of aggregated particles. The distribution of PL and PGA treated microspheres is characteristic of aggregated powders, with a small C/P ratio (0.20 and 0.12, respectively). The C/P ratio for the IPA treated microspheres was much higher (0.59), and indicated a relatively deaggregated powder. The IPA treated microsphere sample was discernably finer upon visual inspection, and the aerosol cloud could be seen to move slower down the throat of the cascade impactor assembly.

Fig. 4 shows the distribution of the microspheres that penetrated beyond the first stage of the cascade impactor. About 53% of the microspheres deposited in the non-respirable range (first two stages). The size distribution data indicates a good correlation with the data from the SEM analysis of the microspheres. The clumps of microspheres seen in the SEMs were deposited in the preseparator and the initial stages of the cascade impactor.

The IPA treated PLGA microspheres were the most deaggregated, whereas the PGA/PLGA microspheres had the aerodynamic size distribution with the largest particles. The PL and PGA formulations had significantly higher fractions deposited in the non-respirable stages and significantly lower fractions in the respirable stages, as compared to the IPA formulation ($P < 0.01$).

Table 1
Humidity-treated PLGA microspheres: effect of incubation time on moisture content and distribution in the stages of the cascade impactor assembly following sampling with Pfeiffer DPI

Incubation time (h)	Moisture content (%)	(%) Distribution in			Average % RF ± S.D. (*n*)
		Glass Adaptor[a]	Pre-separator[b]	Cascade impactor[c]	
0	0.40	22.8 ± 2.4	67.8 ± 1.8	9.48 ± 1.2	4.44 ± 0.67 (6)
12	0.66	21.7 ± 2.7	69.6 ± 3.0	8.70 ± 0.4	4.58 ± 0.68 (3)
24	1.01	21.0 ± 2.6	69.7 ± 1.3	9.35 ± 1.3	2.62 ± 0.27 (3)
48	1.88	19.0 ± 1.8	72.5 ± 1.7	8.550.3	2.82 ± 0.49 (3)
65	2.20	13.3 ± 0.7	79.0 ± 0.6	7.67 ± 1.3	2.20 ± 0.76 (2)
113	2.47	11.1 ± 0.9	80.8 ± 1.2	8.03 ± 0.3	2.28 ± 0.36 (2)

[a] Mass of microspheres recovered from glass adaptor, expressed as percentage of total mass delivered from the DPI following cascade impaction.
[b] Difference in mass of microspheres obtained as: DPI − (Glass adaptor + Cascade impactor), and expressed as percentage of total mass delivered from the DPI following cascade impaction.
[c] Mass of microspheres penetrating beyond first impaction stage of cascade impactor, expressed as percentage of total mass delivered from the DPI following cascade impaction.

The aerodynamic size distribution data of the PAA/PLGA microsphere mixtures showed good correlation with the results of the SEM analysis. Fig. 3 indicated considerable aggregation of the PL/PLGA and PGA/PLGA microsphere mixtures, with large clusters of particles of up to 100 μm. These aggregates were presumably responsible for the large fractions of these microspheres depositing in the preseparator and the higher stages of the cascade impactor. The IPA treated microspheres (Fig. 3) appeared fairly deaggregated with many separate particles and small clusters of particles with aggregate size of 5–15 μm. This observation is clearly reflected in the larger fraction of the microspheres deposited in the lower stages of the cascade impactor.

The RF values obtained for the different formulations are tabulated in Table 5, along with their charge and the MMADs. The untreated microspheres had an average RF of 3.84. Malvern particle sizing had indicated that about 60–70% of the particle mass of microspheres was below 5 μm. The Malvern sizing was performed following ultrasonic suspension in 0.01% w/v Tween-80 in order to achieve the maximum state of deaggregation of the particles. There was a significant shift of the particle size distribution to larger sizes due to aggregation in the dry state, which is expected for untreated particles in this size range. A significantly higher RF was obtained with the IPA-treated PLGA microspheres suggesting a relationship between the unipolarity of the microspheres and degree of aggregation. The highly unipolar IPA formulation had the smallest

Table 2
Zeta potential of microsphere formulations in distilled water

PLGA formulation	*n*	ZP (mV, ± S.D.)
Untreated	19	−30.6 (± 3.99)
Polylysine	12	+63.0 (± 3.72)
Polyglutamic acid	12	−59.0 (± 8.79)
Isopropanol	12	−36.7 (± 1.34)

Table 3
Electrostatic charge distribution of PLGA microsphere formulations measured by the E-SPART analyzer ($n = 3$)

Microsphere formulation	Charge/mass (μC/g)		
	Negative	Positive	Net
Untreated	−1.36	+40.6	+38.4
PL/PLGA	−1.35	+36.5	+34.3
PGA/PLGA	−1.22	+39.0	+37.8
IPA/PLGA	−2.24	+60.1	+56.3

Table 4
Distribution of PLGA microsphere formulations in the stages of the cascade impactor assembly

PLGA sample (n)	(%) Distribution in			Total mass (mg)
	Glass adaptor[a]	Pre-separator[b]	Cascade impactor[c]	
Untreated ($n=6$)	22.8 ± 2.4	67.8 ± 1.8	9.48 ± 1.2	26.4 ± 2.1
Polylysine ($n=8$)	42.6 ± 5.2	48.0 ± 5.2	9.42 ± 1.3	30.9 ± 5.7
Polyglutamic acid ($n=5$)	30.8 ± 5.2	61.6 ± 5.5	7.64 ± 1.2	29.2 ± 4.0
Isopropanol ($n=3$)	40.2 ± 1.1	37.5 ± 2.4	22.2 ± 1.4	20.2 ± 5.3

[a] Mass of microspheres recovered from glass adaptor, expressed as percentage of total mass delivered from the DPI following cascade impaction.
[b] Difference in mass of microspheres obtained as: DPI − (Glass adaptor + Cascade impactor), and expressed as percentage of total mass delivered from the DPI following cascade impaction.
[c] Mass of microspheres penetrating beyond first impaction stage of cascade impactor, expressed as percentage of total mass delivered from the DPI following cascade impaction.

MMAD, while the other relatively less unipolar formulations had larger MMADs. Moreover, the IPA formulation also had the highest RF, while the less unipolar formulations showed lower RFs.

3.1.7. Respirable fractions of humidity-treated microspheres

The distribution of the humidity-treated PLGA microspheres in the various parts of the cascade impactor assembly is shown in Table 1. The fractions deposited in the preseparator were quite high (67.8–80.8%), while those in the adaptor were correspondingly lower (22.8–11.1%). The C/P ratios were quite low and decreased as the moisture content increased (0.14–0.098 for 0.40–2.47% moisture).

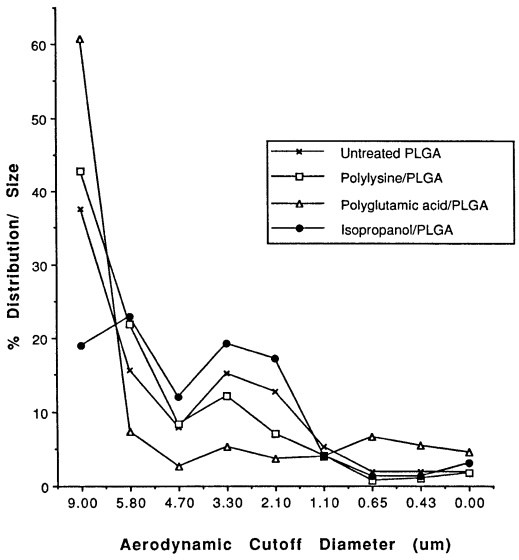

Fig. Particle size distribution of PLGA microsphere formulations entering the cascade impactor following delivery with Pfeiffer DPI.

The microspheres which were not exposed to moisture had a distribution pattern indicating the least aggregation among all the samples. The shifting of the size distribution to larger ranges with increasing moisture content in the sample indicates an increase in the aggregation of the microspheres. The RFs of the humidity-treated microspheres are also shown in Table 1. The RF of the 0.66% moisture sample was not significantly different from the 0 h (0.4% moisture) sample. However, moisture levels above 1% led to a significant lowering of the RF.

4. Discussion

Aggregation of microspheres in the dry state is understandable given the small size of these particles. Deaggregation is expected during dose delivery, due to the dispersion mechanisms of the DPIs as well as the breathing force of the patient. Thus the results of the SEM analysis would not be expected to indicate the state of aggregation during administration. Although the untreated microspheres were in the respirable size range and

Table 5
Respirable fractions and mass median aerodynamic diameters of PLGA microsphere formulations

Microsphere formulation	*n*	Average RF[a] (± S.D.)	MMAD[b] (μm)	Net charge[b] (μC/g)
Untreated PLGA	11	3.84 ± 0.96*	12.2	+38.4
Polylysine/PLGA	8	3.29 ± 0.79	13.0	+34.3
Polyglutamic acid/PLGA	5	2.42 ± 0.33	12.5	+37.8
Isopropanol/PLGA	3	12.9 ± 1.4**	10.9	+56.3

[a] RFs determined by cascade impactor.
[b] Mass median aerodynamic diameters (MMAD) and net charge measured by E-SPART analyzer.
* Significantly higher RF than the PGA formulation ($P = 0.0068$).
** Significantly higher compared to the PL and PGA microsphere mixtures ($F = 107.08$, $P = 0.0001$); also significantly higher than the untreated PLGA microspheres ($P = 0.0001$).

separable following redispersion by ultrasonication, they did not have the physico-chemical characteristics to allow adequate redispersion following aerosolization in dry state. The Pfeiffer DPI by itself did not effect any significant deaggregation of the untreated microspheres at a flow rate of 28.3 l/min.

Most aerosols consist of charged particles, especially when freshly generated. When the particles are bipolar, the collisions between the particles occur more rapidly than with the particles that are uncharged (Task Group on Lung Dynamics, 1966). When the particles are unipolar, the collision rate is even smaller than that for uncharged particles. Thus a particle sample that is highly unipolar will be less aggregated than a microsphere sample that is relatively less unipolar (i.e. more bipolar).

The aggregation studies do not support a relationship between the ZP of the microspheres and their RF. The significantly higher RF of the IPA formulation as compared to the untreated microspheres cannot be explained based on ZP, since the ZP values for these two formulations were in the same range. Thus, the measurement of surface charge by ZP analysis does not adequately reflect the charge phenomenon in the dry state; moreover, ZP may not be a useful measurement when studying the aggregation of dry powders.

Since the E-SPART technique measures the electrostatic charge of particles in the dry state using the DPI for sampling of the powder, it would presumably encompass all the factors involved in the development of charge for this DPI-microsphere system. A relationship between the RFs of the PLGA microsphere formulations and the surface charge determined by the E-SPART analyzer was apparent, as the IPA formulation with the most unipolar nature had the highest RF.

Although the results of the E-SPART analysis indicate that the differences in aggregation of the microspheres may be related to the electrostatic charge of the particles, it cannot be used to rationalize the RF data by itself. This is so, due to the complicated nature of the factors that influence the magnitude and sign of the electrostatic charge. The electrostatic charge of a dry particle depends on the charging mechanism for the particle in a given situation (Mazumder, M.K., Departmnet of Electronics and Instrumentation, University of Arkansas, Little Rock, AK 72204; personal communication). The type of sampling mechanism involved (DPIs, nebulizers, etc), the treatment of the particles prior to the analysis (trituration, agitation), and the materials of the sampling device that the particles come in contact with (plastic, metal, etc.), could influence the magnitude and sign of the electrostatic charge. Low moisture content in the powder and low humidity conditions generally results in higher estimates of the electrostatic charge.

All particles in nature have a surface layer of contaminants such as moisture and chemical contaminants, which are deposited by processes called chemi-sorption and physi-sorption (Mazumder, M.K; personal communication). It could be speculated that the IPA treatment of the

microspheres may have resulted in a cleaning or decontamination of the surface of the particles, which may have made the particles more susceptible to charging. This phenomenon of modification of surfaces of particles to alter their donor/acceptor properties (i.e. their surface charge states) and consequently, their adhesion tendencies, has been reported in the literature for various materials (Zimon, 1982). Although the IPA was also used in the preparation of the PL and PGA formulations, these formulations were not highly unipolar, which must be attributed to the presence of the polyamino acids that may have altered the charge state of the particles.

The 0.4% moisture remaining in the microspheres following preparation and drying of a batch is distributed throughout the microspheres, with the majority in the bulk of the particles rather than at the surface. The exposure to high humidity probably results in accumulation of the moisture predominantly at the surface of the microspheres. Once the moisture content exceeds a certain limit, capillary forces result from the presence of moisture in the inter-particulate spaces, and this would be expected to lead to aggregation of the microspheres.

The slightly higher RF for the microspheres with 0.66% moisture as compared to the untreated microspheres possibly indicates that the presence of a minimum amount of moisture in the microspheres may be necessary to reduce the aggregation of the powder due to the triboelectric charges. Drying the microspheres to moisture contents much below 0.5% could be accomplished (by drying for longer durations) but would not be practical, since these microsphere formulations would be difficult to handle and manipulate.

5. Conclusions

Microspheres were formulated over a range of surface charge and moisture in order to facilitate an appropriate evaluation of their tendency to aggregate in the dry state. The higher unipolarity of microspheres following treatment with isopropanol resulted in the greatest deaggregation. Analytical techniques performed in the dry state were more effective in determining the tendency of powder to aggregate following delivery by a DPI, as compared to those involving a liquid medium. The optimum moisture level for the microspheres was determined to be about 0.66%. Higher levels caused aggregation due to presence of moisture in interparticulate spaces, while lower levels made the microspheres difficult to handle due to generation of high triboelectric charges.

Currently available DPI formulations depend heavily on the ability of patients to use their breathing force and delivery mechanisms of devices to cause redispersion of the powder. The results reported here suggest that there is considerable benefit to be gained from the study of the properties of primary particles used in dry aerosol formulations. It may be more feasible to improve RFs of dry powders by starting with primary drug/carrier particles that are more conducive to redispersion, rather than attempting to improve the redispersion mechanisms of DPIs, which are limited by patient compliance.

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