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Aerosolized aqueous suspensions of poly(L-lactic acid) microspheres

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Summary

A method is described for the preparation of microspheres of poly(L-lactic acid) possessing suitable characteristics for aerosolization. Microscopic analysis of the sample revealed a median projected area diameter of 4.2 μm, with a geometric standard deviation of 1.7. Electrical resistance particle size analysis showed that approx. 1% of the population had a size > 12.1 μm, and only 16.6% exceeded 5.5 μm. Scanning electron micrographs show well formed, round and smooth microspheres. Jet nebulization of an aqueous suspension produced a mass output of 44.4% of the microspheres over a period of 30 min. Aerodynamic particle size measurement showed that 51.2%, by number (approx. 16.9%, by volume) of the aerosol generated was < 2.1 μm in diameter. Greater than 80%, by volume, of the aerosol was smaller than 5.8 μm in aerodynamic diameter.

Introduction

Nebulizers are frequently used to produce aerosols for the treatment of lung diseases (Lancet, 1984; Crompton, 1985; Hickey, 1992). Most recently they have been effective in the treatment of *Pneumocystis carinii* pneumonia (Debs et al., 1987). The advantage of nebulizer therapy is that the droplets generated have appropriate size for lower lung deposition, and have been more successful in this regard than metered dose (MDI) or dry powder (DPI) inhalers (Cochrane et al., 1985). Human subject studies utilizing radiolabelled aerosols formulated as suspensions and emitted from MDIs and DPIs show low dose delivery of drugs to the lung (Newman and Pavia, 1985). In contrast, the efficiency of jet nebulizers in delivering a therapeutic dose with minimal oropharyngeal loss has been reported (Clay et al., 1983; Matthys and Kohler, 1985).

The possibility of reducing or eliminating the use of chlorofluorocarbon (CFC) propellants in MDIs provides an opportunity for the development of alternative methods of drug delivery to the respiratory tract. Dry powder inhalers have been developed as a possible alternative to the use of CFCs in MDIs. These devices have the potential disadvantage that they are dependent on the patient's breath for both powder disper-

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sion and aerosolization. Variation in patient breathing pattern, as occurs in respiratory diseases, may result in dosing irregularities from the DPI. In addition, powder particles are subject to aggregation, resulting in a greater tendency to deposit in the mouth and upper respiratory tract. Nebulizers disperse droplets in an airstream for passive inhalation by the patient. Generation of suspensions of particles by nebulization may be an effective alternative to metered dose and dry powder inhalers in aerosol therapy.

Drug delivery to the lung for local action has been demonstrated to be beneficial in the treatment of reversible and non-reversible airway diseases. This approach has several advantages over alternative routes of administration (Paterson et al., 1979). The large surface area and highly vascular nature of the lower lung would potentially facilitate the rapid absorption of drugs (Enna, 1973; Burton, 1974; Brown, 1983; Brown and Schanker, 1983). The pulmonary region may, therefore, be useful as a route for systemic administration, especially for drugs that cannot be delivered via the oral route because of poor absorption, degradation or significant first pass metabolism. The controlled delivery of therapeutic agents to the lung, including products of biotechnology may be used to achieve systemic activity. To develop the lung as a route of administration requires an approach to increasing the residence time of structurally intact, pharmacologically active compounds at the site of action or absorption.

The development of poly(lactic acid) and other biodegradable and biocompatible compounds as prosthetic and suture materials is well documented (Kulkarni et al., 1966; Frazza and Schmidt, 1971; Cutwright et al., 1974). Their application in controlled drug release for implantation and intramuscular injection is increasingly being realized (Woodland et al., 1973; Wakiyama et al., 1982; Beck et al., 1983; Wise et al. 1990). These materials have rarely been utilized for the controlled release of drugs for administration to the respiratory tract for either local or systemic effect (Gupta and Hickey, 1991; Hickey, 1993; Lai et al., 1993). Many studies in this area have used liposomes (Juliano and McCullough, 1980; Farr et al., 1985; Mufson, 1986; Woolfrey et al., 1988). These investigations have demonstrated that liposomes may effectively modulate the systemic absorption of encapsulated compounds following pulmonary instillation. Volunteer studies have shown that nebulized aqueous dispersions of dipalmitoylphosphatidylcholine penetrate the peripheral regions of the lung where they appear to be slowly cleared (Farr et al., 1985). Sustained bronchodilation in guinea pigs has been achieved following nebulized administration of drug loaded liposomes (Mufson, 1986). Since liposome vesicles are known to be unstable, microspheres formed from a more stable material such as poly(lactic acid) may be a feasible, and viable alternative.

The objectives of this study were: formulation and manufacture of poly(L-lactic acid) (PLLA) microspheres possessing appropriate characteristics, of particle size, dispersion and stability, for nebulization; evaluation of the performance of a jet nebulizer in generating suspension aerosols and; assessment of the electrical resistance technique as an auxiliary analytical method for use in conjunction with an inertial impactor.

Materials and Methods

Materials

The following chemicals were employed as received: poly(L-lactide) (PLLA) (Polysciences, Inc., Warrington, PA) with a nominal molecular weight of 100000 g/mol; chloroform, 99.8%, A.C.S. reagent; sodium oleate, 98% pure (Aldrich Chemical Co., Milwaukee, WI); methylene chloride, HPLC-GC.MS grade (Fisher Scientific, Itasca, IL); Isoton II: azide free balanced electrolyte solution (CMS no. 375-212, Coulter Electronics Inc., Hialeah, FL).

Determination of polymer molecular weight

200 mg of PLLA were dissolved in 100 ml of chloroform. Serial dilutions of the stock solution by chloroform were performed to obtain concentrations of 0.1–0.5 mg ml^{-1}. Approx. 15 ml of the dilute solutions were introduced in an Ubbelohde viscometer no. B62 size IC (Industrial Research

Glassware Ltd, Union, NJ), clamped vertically in a temperature controlled waterbath set at 30 ± 0.1°C (Blue M Laboratory Immersion Heater, Fisher Scientific, Itasca, IL). 20 min were allowed for equilibration before the efflux time of the solution was determined.

Differential scanning calorimetry (DSC)

The thermal transitions of the polymer were determined by differential scanning calorimetry (Model 990, Du Pont Instrument Products, Wilmington, DE). Samples were heated at a rate of 20 K min^{-1}. Indium was used to calibrate the instrument.

Preparation of PLLA microspheres

PLLA microspheres were prepared using an emulsion-based process. A solution of PLLA in methylene chloride was dispersed in water, followed by removal of methylene chloride, leaving behind discrete particles. PLLA (500 mg) was dissolved in 10 ml methylene chloride. The solution was added rapidly from a Hamilton 1010W syringe (Hamilton Co, Reno, NE) fitted with a removable stainless-steel needle to 0.1% aqueous solution of sodium oleate (50 ml), contained in a round bottom flask and stirred at 6000 rpm (Laboratory Dispersator, Premier Mill Corp., Reading, PA). The w/o emulsion was then transferred to a 200 ml beaker and gently stirred by a paddle mixer at room temperature for ~ 3 h. The microspheres were isolated by filtration, washed and dried under vacuum at 60°C for > 12 h. The yield was estimated from the masses of polymer employed and microspheres recovered.

Microscopy

The microspheres were observed microscopically to determine their size distribution and their surface morphology. Scanning electron microscopy (SEM) was performed (Model JSM 35C, Jeol U.S.A., Inc., Peabody, MA). The microsphere suspensions were placed on aluminum stubs, dried at room temperature and coated with gold-palladium under argon atmosphere These procedures were performed on samples prior to, and following nebulization in order to follow the change in the size of particles resulting from nebulization. Optical microscopy (Model 110 Microstar, AO Microstar Scientific Instruments, Division of Warner-Lambert, Buffalo, NY), utilizing a calibrated eyepiece graticule, was performed on five fields of view from five samplings (25 fields in total). 600 particles were counted. The projected area diameters of individual particles were estimated.

Nebulization and inertial deposition

A conventional hospital jet nebulizer (Respigard II) system, model no. 124030 Marquest Medical Products, Inc., Englewood, CO) was used to nebulize 10 mg of the microspheres suspended in 4 ml of 0.1% Tween 80. The output of the nebulizer was conducted through a 1 inch i.d. and 6.8 inch long flexible plastic pipe for analysis. The total output was collected for 30 min on an absolute 0.22 μm filter and vacuum dried before weighing. The fractional mass output of polymer was derived from the dry weight of the sampled aerosol and the initial weight in suspension. The output was also collected in an Andersen 1CFM non-viable ambient sampler (Andersen Samplers Inc., Atlanta, GA) via an inlet chamber, a modified round-bottom flask, having a 25 mm i.d. neck (Fults et al., 1991). The aerosol then passed through a preseparator to eight impaction stages with effective aerodynamic diameter cut-off sizes (ECDs) of 9.0, 5.8, 4.7, 3.3, 2.1, 1.1, 0.7 and 0.4 μm for stages 0–7, respectively, when operated at an airflow of 28.3 l min^{-1} (Vaughan, 1989).

Quantitative electrical resistance particle size analysis

Fractions of the PLLA microspheres deposited at each stage and other areas of the separator were collected by carefully washing with single-distilled, Milli-Q water (Milli-Q® Systems, Millipore Corp., Bedford, MA) and transferred into collection vials. The suspensions were dried in vacuo at 60°C for > 12 h. The total number of microspheres at each stage was estimated by an electrical resistance technique (Coulter® Multisizer® II, Coulter Electronics, Ltd, Luton, U.K.). The dried samples were suspended in 1 ml Isoton solution; this suspension was then added to a beaker containing Isoton, to a total volume of 50

ml. Analysis of the particle size distribution was performed assuming a log-normal distribution.

Estimation of aerodynamic diameter

The numbers of particles collected at each stage of the inertial sampler were converted to volumes using the number median diameters estimated by electrical resistance. The estimate of mass median aerodynamic diameter (MMAD) and geometric standard deviation, assuming uniform density, were obtained from a plot of the ECD for the stage and the cumulative percentage undersize, by volume.

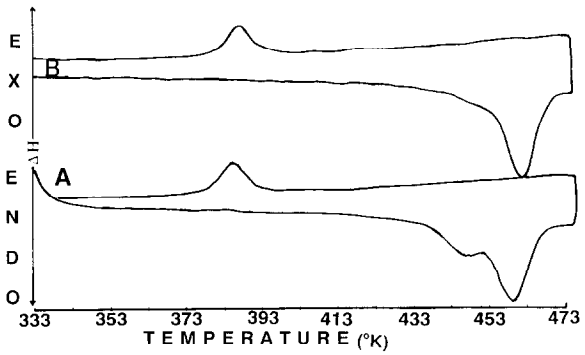

Fig. 2. Thermogram obtained by differential scanning calorimetry of poly(L-lactic acid). (A) First heating followed by cooling; (B) repeated heating and cooling.

Results and Discussion

Determination of polymer molecular weight

The estimation of polymer molecular weight by viscosity measurement is based on the Mark Houwink relationship of $[\eta] = KMa$ (Powel et al., 1966). The value, $[\eta]$, denotes intrinsic viscosity, which is a measure of the polymer molecules' ability to increase the viscosity of the solvent, in the absence of intermolecular influences. Intrinsic viscosity is obtained by extrapolating to infinite concentration, a plot of specific viscosity (η_{sp}/c) vs concentration (c), as shown in Fig. 1. K and a are constants characteristic of the polymer-solvent combination. The two constants for PLLA in chloroform have been established (Brandrup, 1991). The $[\eta]$ of 0.93 dl g^{-1} obtained for PLLA is consistent with the nominal molecular weight of 100000 g/mol given by the manufacturer.

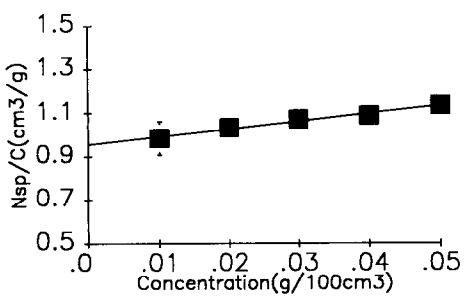

Fig. 1. Plot of specific viscosity (cm^3 g^{-1}) vs concentration (g dl^{-1}) of PLLA solutions in chloroform.

Differential scanning calorimeter (DSC)

The DSC thermographs of PLLA are shown in Fig. 2. The transitions represent the crystallization (T_c) and melting (T_m) temperatures. The stereo-regularity of the polymer is confirmed by the high melting point, 170–180°C (443 and 453 K; Tonneli, 1969). The presence of D units in the polymer chain decreases the melting points of the semi-crystalline polymer (Schulz, 1969). The melting endotherm will not show in stereopolymers, where the proportion of L-lactide units is less than 87.5%, in which case the polymer would be amorphous (Chabot, 1983).

Stresses in the polymer develop, as a result of the presence of amorphous and crystalline phases which provide different thermal expansion coefficients, and the existence of tie molecules between crystallites. Stress relaxation has a bearing on the appearance of a glass transition (T_g). It is probable that sample quenching immediately prior to DSC scanning did not provide sufficient time for relaxation of internal stresses, leading to the absence of a T_g in this sample. Storage conditions of the polymer and heating rate also may contribute to the absence of T_g. Values of 55–67°C have been observed with PLLA (Masinde, 1985). Internal structure rearrangement may also explain the difference in baseline at the start of heating and the presence of a shoulder in the melting curve in the first heating regimen.

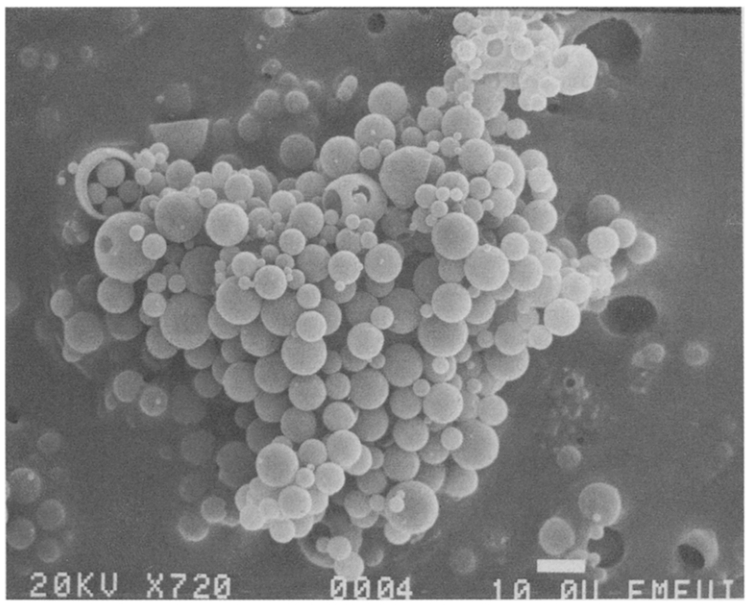

Fig. 3. Scanning electron micrograph of PLLA microspheres before nebulization. Scale bar represents 10 μm.

Preparation of PLLA microspheres

The method used can produce microspheres of the desired size (< 5 μm) efficiently. The rate of stirring, emulsifier type and concentration, and the rate of addition to the aqueous phase, of the lipophilic components are important parameters. Approx. 95% of the polymer was recovered as microspheres.

Microscopy

Fig. 3 shows a scanning electron micrograph of PLLA microspheres following preparation and

TABLE 1

Characteristics of PLLA microspheres before and after nebulization (n = 4)

Sample	ECD (μm)	Median diameter [a] or MMAD[c] (μm)	GSD	RVAL	Percentage Deposition	
					No.	Vol
Before Nebulization	–	4.2 [b]	1.7	0.97	–	–
	–	2.6	2.2	0.99	–	–
Residue	–	2.7	2.3	0.97	–	–
Pre-separator	> 9.0	1.9	2.8	0.98	–	–
Stages 0	9.0	2.0	3.0	0.97	5.1	2.4
1	5.8	2.5	3.2	0.98	7.7	12.4
2	4.7	3.1	2.2	0.99	7.3	22.7
3	3.3	2.9	2.1	0.99	9.6	21.3
4	2.1	2.3	1.6	0.99	19.0	24.1
5	1.1	1.5	1.7	0.94	33.0	11.7
6	0.7	1.4	1.6	0.92	13.5	3.6
7	0.4	1.5	2.1	0.97	4.7	1.6
Aerosol	–	4.6 [c]	2.8	0.95	–	–

[a] Median diameter, by number as estimated from electrical resistance data, or [b] microscopy.
[c] MMAD calculated by volume, assuming uniform density.

prior to dispersion in a suspending medium. The microspheres were spherical in shape and had smooth surfaces. The median diameter of the particle distribution as estimated by optical microscopy was 4.2 μm with a geometric standard deviation of 1.7 as indicated in Table 1.

Nebulization and inertial deposition

The fraction of the suspended particles generated in 30 min was 44.4%, by mass. At the end of the proscribed period, of 30 min, a residue remained in the nebulizer indicating that increasing the generation time may result in greater mass output.

Particle penetration and entrapment in the branching system of the lung involves a number of mechanisms (Gonda, 1992). Several studies have characterized particle deposition in this region (Gonda, 1981; Stahlhofen et al., 1983; Moren, 1987). Deposition of therapeutic aerosols in the respiratory system results predominantly from inertial impaction. Therefore, it is appropriate to adopt an inertial sampling technique, cascade impaction, to segregate particles for size analysis. The particle sizes that are collected on each stage of the cascade impactor may be used to infer the extent of their penetration into the respiratory tract. Several factors, including particle size, shape, density and other physical properties of the particle influence the site of deposition, and the fraction retained in the respiratory system.

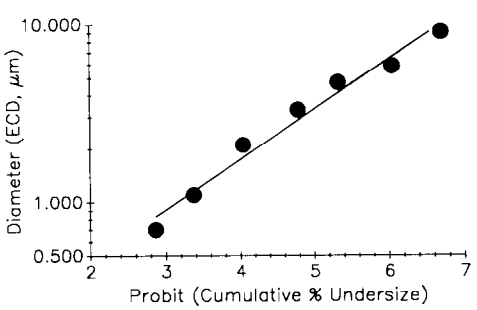

Fig. 4. Particle size distribution of PLLA microspheres after nebulization and sampling by the cascade impactor. The effective cut-off aerodynamic diameter for each of the impactor stages is plotted against the frequency (cumulative percent undersize), linearized on a probit scale.

Aerosol particles or droplets deposited in the lower stages of the impactor, with effective cut-off diameters (ECDs) < 5.8 μm, were designated as the respirable fraction (RF) of the emitted dose. The distribution of particles after nebulization is represented in Fig. 4.

Analysis of the deposits at each stage of the impactor showed that the size progressively decreased with distance from the nebulizer. Fig. 5 is a SEM representation of particle size distribution on stages 3–6. The particle sizes observed were consistent with the ECDs for the stages. The particles appear to have retained their shape and surface morphology, indicating that their superficial structure is unaffected by nebulization. Therefore, the suspended particles may be considered stable under the conditions required to generate the aerosol.

Some particles much larger than the ECD of respective stages were found at all levels of the cascade impactor. Re-entrainment during nebulization (Boulaud et al., 1982), the low density nature of the polymer and the presence of hollow microspheres (some are shown in Fig. 3) would also contribute to this phenomenon.

Quantitative electrical resistance particle size analysis

Table 1 provides a summary of the size parameters of the particles before and after nebulization. Column 2 lists the effective cut-off diameters (μm) of each of the stages of the inertial sampler. These are transcribed from the manufacturer's calibration data (Andersen Samplers Inc., Atlanta, GA). Columns 3 and 4 list the median diameter, by number, and geometric standard deviation, as derived from electrical resistance measurements. Column 5 represents the correlation of the assumed log-normal fit to the experimental particle size data. Column 6 lists the fraction of the total number of particles collected at each stage of the inertial sampling device. The number data were converted to volume using the median diameters at each stage and are shown in column 7. This should be viewed with caution as the broad distribution of the particle size, by number, for each stage renders the conversion to volume an approximation. The median

diameters, by number, estimated for each stage of the inertial impactor may be used to calculate the volume data accurately when the GSDs approach monodispersity. The mass median aerodynamic diameter and geometric standard deviation for the entire aerosol distribution, shown in the last row of Table 1, were derived from plots of the volume data and assume uniform particle density.

The median diameters for the initial particle distribution, in suspension, of 4.2 and 2.6 μm, estimated by optical microscopy and electrical resistance methods, respectively, require explana-

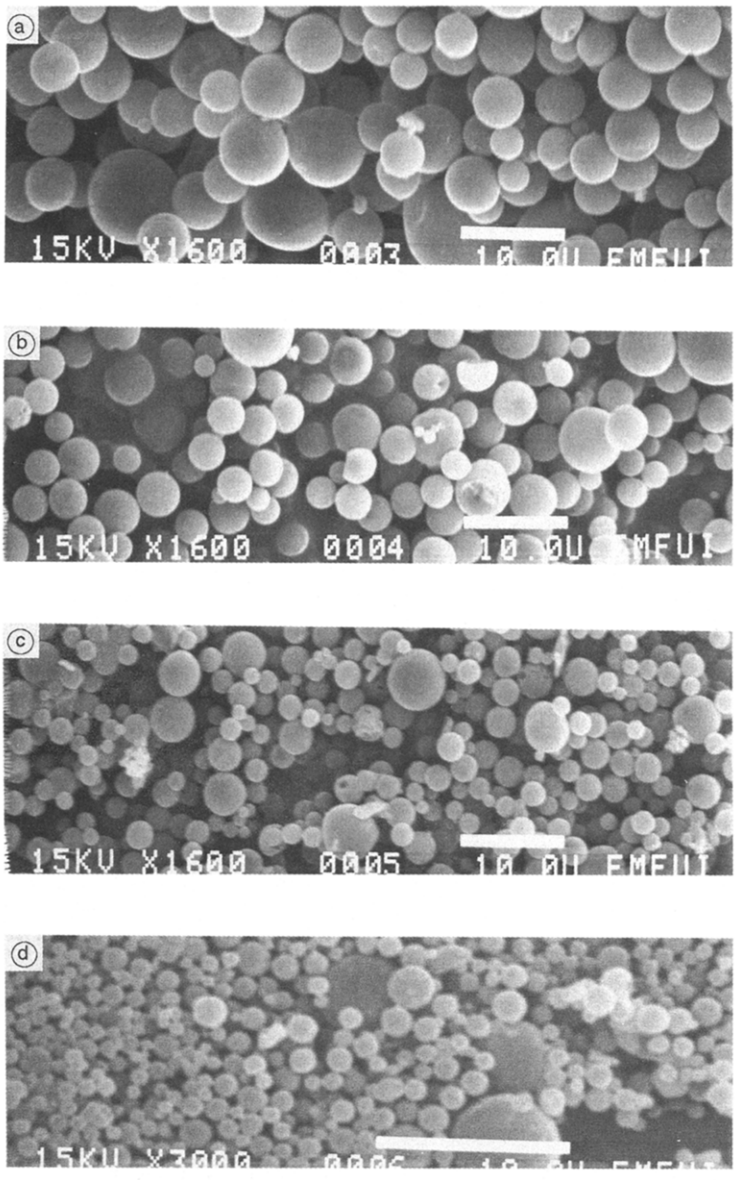

Fig. 5. Scanning electron micrographs of PLLA microspheres deposited after nebulization on stages (a) 3, (b) 4, (c) 5 and (d) 6. Scale bars represent 10 μm.

tion. The difference in the two values obtained may be explained by the difficulty associated with sizing individual small particles at the limit of resolution by light microscopy. In addition, the subjectivity inherent in counting hundreds of particles microscopically may contribute to the disparity in the results obtained by the two methods. The electrical resistance technique measures particle numbers several orders of magnitude greater than conventional microscopy, with a sensitivity to small particles, < 1 μm in diameter. Both estimates suggest sizes suitable for nebulization.

The median diameter (2.6 μm) and distribution (2.2) of particles remaining in the nebulizer following aerosol generation retained the initial particle size (2.7 μm) and distribution (2.3). Therefore, no size dependency of generation of the suspended particles occurred. The RF (< 5.8 μm) was 80–90% (by number and volume) after 30 min of nebulization. Approx. 50%, by number (17%, by volume), of the RF was below 2.1 μm.

Estimation of aerodynamic diameter

Deposition of particles within the inertial impactor depends upon the aqueous droplet size. Large droplets may contain many suspended particles whereas small particles may be generated individually. There is some variation in the size of suspended particles between each stage of the impactor but this is independent of the aerosol aerodynamic size. The variations in particle size and geometric standard deviation on the upper stages appear to be random. Particle size and distribution become smaller as the suspended particles become equivalent to the aqueous droplets. A log-normal fit using the cumulative volume of particles collected by inertial impaction and the ECDs of the stages indicates a mass median aerodynamic diameter of 4.6 μm and GSD of 2.8, as shown in Table 1.

Generally, where chemical analysis is difficult, gravimetric assays are performed. The latter approach is subject to significant error as the mass of particles collected at each stage is much smaller than that of the collection plate. The Coulter counter in combination with a cascade impactor may be an appropriate measurement technique in some of these aerosol investigations.

Conclusion

Poly(L-lactic acid) microspheres were manufactured in a size range suitable for aerosol generation and lung deposition. The shape and the surface morphology of the microspheres were unaffected by nebulization. Aerosols of PLLA microspheres were generated efficiently in terms of mass output and respirable particle size. The cascade impactor/electrical resistance combination facilitated collection of in vitro data describing the particle size characteristics of the aerosols and indicating their suitability for inhalation. This method may be refined and applied to characterization of aerosol particles where chemical analyses are not easily performed, particularly as an alternative to gravimetric methods.

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