

New polymeric carriers for controlled drug delivery following inhalation or injection

Jie Fu^a, Jennifer Fiegel^a, Eric Krauland^b, Justin Hanes^{a,b,*}

^aDepartment of Chemical Engineering, The Johns Hopkins University, 3400 N. Charles Street, 221 MD Hall, Baltimore, MD 21218, USA

^bDepartment of Biomedical Engineering, The Johns Hopkins University, 3400 N. Charles Street, 221 MD Hall, Baltimore, MD 21218, USA

Received 14 January 2002; accepted 1 May 2002

Abstract

Inhalation is gaining increasing acceptance as a convenient, reproducible, and non-invasive method of drug delivery to the lung tissue and/or the systemic circulation. However, sustained drug release following inhalation remains elusive, due in part to the lack of appropriate materials designed specifically for use in the lungs to control the release of bioactive compounds. To address this problem, we have synthesized a new family of ether-anhydride copolymers composed entirely of FDA-approved monomers, including polyethylene glycol (PEG). Sebacic acid, a hydrophobic monomer, was copolymerized with PEG in order to produce water-insoluble polymers capable of providing continuous drug release kinetics following immersion in an aqueous environment. Various amounts of PEG (5–50% by mass) were incorporated into the backbone of the new polymers to allow tuning of particle surface properties for potentially enhanced aerosolization efficiency and to decrease particle clearance rates by phagocytosis in the deep lung. The preparation of large porous particles with these new polymers was systematically approached, utilizing central composite design, to develop improved particle physical properties for deep lung delivery. Microparticles containing model drugs were made with sizes suitable for deposition in various regions of the lung following inhalation as a dry powder. Due to such properties as surface erosion (leading to continuous drug release profiles), erosion times ranging from hours to days (allowing control over drug delivery duration), and ability to incorporate up to 50% PEG in their backbone, these new systems may also find application as “stealth” carriers for therapeutic compounds following intravenous injection. © 2002 Elsevier Science Ltd. All rights reserved.

Keywords: Pulmonary drug delivery; Controlled release; Biodegradable polymers; Lung; Inhalation

1. Introduction

We are currently living through a revolution in biotechnology that is producing an abundance of potent new protein, peptide, and DNA-based drugs. A significant challenge facing scientists and engineers is the development of new delivery methods that maximize the therapeutic effect and convenience of administration of these new drugs [1].

Man has inhaled drugs for medicinal, recreational, and other purposes for centuries. Today's smokers, drug abusers, and asthmatics know that inhaled drugs act quickly, minimize the dose required, and are non-invasive. In fact, inhalation of aerosolized drugs has become a well-established means of treating localized

disease states within the lung, including the millions of people in the US that use fast-acting inhaled β_2 -agonists as treatment for unexpected asthma attacks [2]. It has recently been demonstrated that the lung may be an ideal site for the non-invasive delivery of therapeutic molecules, including peptides and proteins, to the systemic circulation as well [3–12]. Insulin, calcitonin, interferons, parathyroid hormone, and leuprolide are examples of proteins in clinical studies for systemic action following inhalation [10,13–16]. The lung is an attractive route for drug delivery owing to its enormous surface area for absorption (~ 100 – 140 m²) [17], highly permeable epithelium compared with the gastrointestinal tract [18–20], and favorable environment for protein drugs compared to the low pH and high protease levels associated with oral delivery. In addition, pulmonary drug delivery avoids first pass hepatic metabolism and is generally more acceptable to patients than an injection.

*Corresponding author. Tel.: +1-410-516-3484; fax: +1-410-516-5510.

E-mail address: hanes@jhu.edu (J. Hanes).

Although promising, delivery of therapeutics to the lungs faces several anatomical and physiological challenges [7]. To deposit in the lungs, drugs must traverse a complex lung structure that is heterogeneous in geometry and environment from patient to patient. Once deposited, natural clearance methods, including the “mucociliary escalator”, work to expel particles from the upper airways [7], while alveolar macrophages rapidly (often within minutes) engulf particles between 1 and 5 μm that reach the deep lungs [7]. Additional drug loss can occur in the inhaler device due to inefficient aerosolization, or in the mouth, throat, and upper airways due to sub-optimal aerosol characteristics or improper coordination of aerosol activation and breathing [21]. Consequently, aerosol design is vital to maximize delivery efficiency and eliminate irreproducibility that can limit the practicality of new pulmonary therapies.

Pulmonary drug delivery methods have traditionally focused on one of two strategies: (i) drug suspension/dissolution in liquid aerosol drops and (ii) mixtures of dry drug particulates with dry carrier particles typically composed of sugars. These methods, capable of delivering medicine quickly to the bloodstream or local tissue, have been studied for treatments ranging from asthma and pain relief [23] to influenza [24]. Although effective as immediate relief therapies, an inability to achieve sustained drug delivery with traditional methods has limited the scope of inhaled medicines [22,25].

The use of controlled release polymeric systems is an approach that holds promise for improving the duration and effectiveness of inhaled drugs, for both local and systemic action [21]. Micrometer- and nanometer-sized polymeric systems have been used to deliver precise amounts of drugs, including proteins and genes, over prolonged times to local tissues or the systemic circulation following injection [1]. Initial studies with polymeric aerosol systems showed that properly engineered, large porous particles (LPP) were also capable of delivering bioactive insulin to the blood of rats and control glucose levels for 96 h [26]. The previous longest sustained delivery of insulin to the blood via the lungs was only 6 h, using liposomes that were intratracheally instilled into rat lungs [27]. Since then, only limited examples of polymeric aerosol systems have been reported. For example, respirable poly(lactic-co-glycolic) acid (PLGA) microspheres containing rifampicin for the treatment of tuberculosis have been studied in a guinea pig model [28,29]. Cationic polymers, such as polyethyleneimine (PEI) and poly-L-lysine (PLL), complexed with DNA have also been tested in the airways as a method to achieve transient gene expression [30–32]. Although promising, transient gene expression would also require frequent administration to maintain a therapeutic effect [33,34]. Properly designed new poly-

meric aerosols, with the ability to target various regions of the lung, should prove beneficial for prolonged non-invasive treatment of both lung disorders, such as asthma or cystic fibrosis, and diseases requiring drug delivery to the systemic circulation.

Most previous studies of polymeric pulmonary drug delivery have utilized PLGA since it is readily available and has a long history of safety in humans. However, PLGA has many limitations as a carrier for drugs in the lungs. First, small PLGA microspheres degrade over the period of weeks to months, but typically deliver drugs for a shorter period of time [26,38]. Such a pattern would lead to an unwanted build-up of polymer in the lungs upon repeat administration. Second, bulk degradation of PLGA microspheres creates an acidic core, which can damage pH sensitive drugs such as peptides and proteins [39]. Surface eroding polymers, such as polyanhydrides, lessen the effect of acidic build-up by increased diffusion rates of soluble fragments away from the particle [40]. Third, PLGA microspheres have hydrophobic surfaces, which result in sub-optimal particle flight into the deep lung (due to particle agglomeration by van der Waals forces) [41]. Additionally, hydrophobic surfaces lead to rapid opsonization (protein adsorption), resulting in a rapid clearance by alveolar phagocytic cells [42].

Two areas of focus in our laboratory include: (i) the synthesis of novel biomaterials as potential drug carriers and (ii) the design and optimization of polymeric materials into aerosol particles with desired physical properties for efficient and sustained drug delivery in the lungs. In this paper, we first describe the synthesis of a new class of polymers for pulmonary delivery, the polyether-anhydrides. The polymers are composed of the monomers sebacic acid (SA) and polyethylene glycol (PEG). Sebacic acid is FDA-approved for treatment in human brain tumors while PEG is approved for numerous medical applications. The safety of SA and PEG demonstrated in other tissues should improve their chances for approval in pulmonary applications. Polyether-anhydrides have significantly shorter degradation times, ranging from hours to many days depending on composition, and thus may be more appropriate for pulmonary delivery than existing polymers, such as PLGA. PEG was incorporated into the polymer backbone to reduce the interparticle adhesion forces and decrease the density of polymer aerosols, as well as to render the particles less susceptible to phagocytosis [43]. We then describe the production and optimization of polymer microparticulate aerosols, with a focus on unusually large, low-density polymeric systems. Such systems can be designed to target specific regions of the lung, and therefore allow controlled drug delivery to lung, or to the systemic circulation via the lung [26,35].

2. Materials and methods

2.1. Materials

All chemicals were purchased from Sigma-Aldrich (St. Louis, MO) unless otherwise noted. Sebacic acid was recrystallized three times from ethanol. Acetic anhydride was purified by distillation. Toluene and chloroform (J.T. Baker, Phillipsburg, NJ) were refluxed over and distilled from calcium hydride. Poly(ethylene glycol) bis(carboxymethyl) ether ($M_n = 600$) was dried by lyophilization. Cadmium acetate, polyvinyl alcohol (88 mol% hydrolyzed, $M_w = 25$ kDa, Polysciences Inc., Warrington, PA), bovine serum albumin (BSA), pyridine, phosphatidylcholine, and other reagents were used as received without further purification.

2.2. The synthesis and characterization of poly(sebacic anhydride-co-peg)

2.2.1. Sebacic acid (SA) prepolymer

SA (10.0 g) was refluxed in 100 ml acetic anhydride under dry nitrogen for 15 min, cooled to room temperature, and dried using a rotary evaporator. The crude prepolymer was recrystallized from dry toluene, washed with 1:1 anhydrous ethyl ether:petroleum ether (Fisher, Fair Lawn, NJ), and dried by vacuum.

2.2.2. Poly(ethylene glycol) (PEG) prepolymer

Polyoxyethylene dicarboxylic acid (10.0 g) was refluxed in 200 ml acetic anhydride for 30 min under nitrogen and evaporated to dryness using a rotary evaporator. The residue was extracted with anhydrous ether and dried under vacuum.

2.2.3. Polymer synthesis

A family of ether-anhydride copolymers was synthesized by melt polycondensation of SA and PEG prepolymers under high vacuum, as previously described [36]. The polymers were precipitated from chloroform into petroleum ether and dried by vacuum. Molecular weight was monitored by gel permeation chromatography (GPC) (JASCO AS-1555, Tokyo, Japan) with three columns in series (Waters, Milford, MA; Styragel guard column, 4.6 mm I.D. \times 30 mm; HR 3 column, 4.6 mm I.D. \times 300 mm; HR 4 column, 4.6 mm I.D. \times 300 mm) and polystyrene as standards (Fluka, Milwaukee, WI). The chemical structure of poly(PEG:SA) is shown in Fig. 1. Structure was

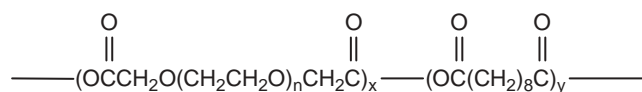


Fig. 1. Chemical structure of poly(PEG:SA).

confirmed by ^1H NMR recorded in CDCl_3 on a Varian UNITY-400 MHz spectrophotometer (Palo Alto, CA) and FT-IR with potassium bromide pellets on a Perkin-Elmer 1600 series spectrophotometer (Wellesley, MA).

2.3. Preparation of poly(sebacic anhydride-co-PEG) microparticles

Microparticles were prepared using a double-emulsion solvent-evaporation method [36]. The primary water-in-oil emulsion was created by probe sonication (Sonics and Materials Inc., Newtown, CT) of 100 μl aqueous solution (\pm bovine serum albumin) in 4 ml polymer solution in methylene chloride (\pm phosphatidylcholine). The primary emulsion was then poured into 100 ml of 1% (wt/vol) poly(vinyl alcohol) (PVA) solution and homogenized (Silverson Machines Inc., East Longmeadow, MA) at 6000 rpm for 1 min to form the double emulsion. Microspheres were stirred for 3 h to allow hardening, then collected by centrifugation, washed twice with deionized water, resuspended in 10 ml water, and freeze-dried.

2.4. Microparticle optimization via central composite design

The effects of five microparticle preparation parameters (homogenization speed of second emulsion, polymer concentration in methylene chloride (oil phase), PVA concentration in outer water phase, phosphatidylcholine concentration in oil phase, water/oil ratio in primary emulsion; see Table 1) on microparticle size, density and aerodynamic diameter were analyzed using a half-replicate central composite design (CCD) [37]. In a general CCD experiment, K input variables are assigned a center point value designated as 0, and a high and low value equidistant on either side of the center, (designated 1 and -1 , respectively) called the corner points. In this design, 2^K experiments were performed on all combinations of corner points. An additional 2^K experiments were performed on star points, which were a level $\pm 2^{k/4}$ for one variable and level 0 for all other variables. The remaining experiments were performed on center points, in which all variables are kept at level 0. Data was collected from each experiment and quadratic relationships were developed between all K input variables. Statistical significance ($p < 0.05$) was determined by analysis of variance (ANOVA) for each response variable (size, density, and aerodynamic diameter).

2.5. Characterization of polymeric microparticles

The mass-average size distribution of microparticles was determined using a Coulter Multisizer IIe (Beckman-Coulter Inc., Fullerton, CA). Approximately 2 ml

Table 1
Microparticle preparation variables and their levels in the central composite design

Levels in CCD	Homogenization speed (rpm)	Polymer conc. (mg/ml)	Water/oil phase ratio (% vol/vol)	PVA conc. (mg/ml)	Phosphatidyl-choline conc. (mg/ml)
-1.68	4000	25	1	1	0
-1	5000	56.25	7	13.25	2.5
0	6000	87.5	13	25.5	5
+1	7000	118.75	19	37.75	7.5
+1.68	8000	150	25	50	10

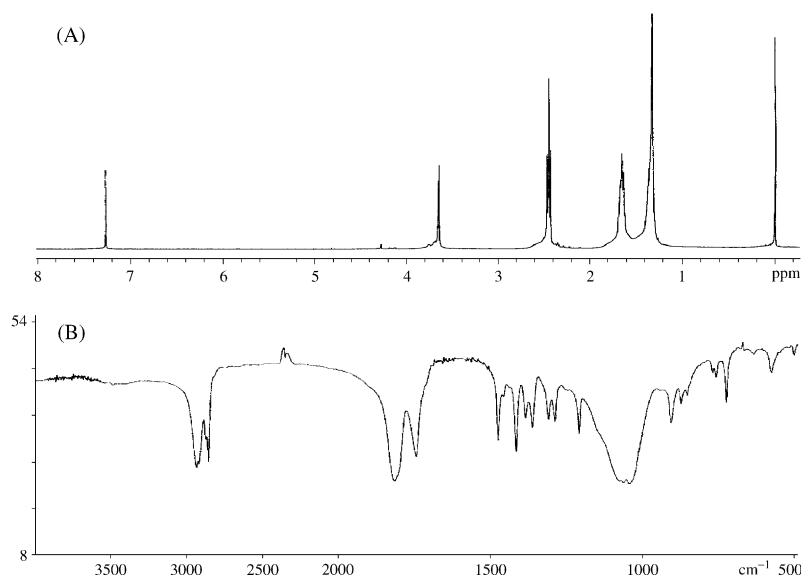


Fig. 2. (A) ¹H NMR spectra of poly(PEG:SA) 10:90 (10% PEG). (B) FT-IR spectra of poly(PEG:SA).

of isoton II solution was added to 5–10 mg microparticles. The solution was briefly vortexed to suspend the microparticles and then added dropwise to 100 ml isoton II solution until the coincidence of particles was between 8% and 10%. Greater than 100,000 particles were sized for each batch of microparticles to determine the mean particle size and size distribution. The bulk density of the particles was determined by tap density as described previously [26]. Degradation studies of poly(PEG:SA) were accomplished by placing a known amount (~10 mg) of microparticles in 1.0 ml phosphate buffered saline (0.1 M, pH 7.4), incubated at 37°C under rotary agitation. At predetermined time intervals, the molecular weight of the polymer within microparticles was monitored by GPC.

3. Results and discussion

3.1. Synthesis of polyether-anhydrides

Poly(ether-anhydrides) were synthesized by melt polycondensation of sebacic anhydride and poly(ethylene glycol) biscarboxylmethyl under high vacuum [36].

Copolymers of various compositions were characterized by ¹H NMR and FT-IR. The ¹H NMR (Fig. 2A) resonance line of the methylene protons of PEG appeared at 3.65 ppm, which indicated PEG was incorporated into the sample. The three peaks at 2.44, 1.65, and 1.32 ppm were attributed to the methylene protons of SA. Data from GPC (not shown) contained one peak corresponding to the molecular weight of the polymer, with no peak corresponding to the weight of free PEG. NMR studies combined with GPC data indicated that PEG chain was successfully copolymerized with SA. The actual weight percentage of PEG in the polymer was estimated by the area ratio of the PEG protons to methyl protons of SA. As shown in Table 2, the estimates were in good agreement with the feed ratio of PEG to SA added prior to polymerization. The typical anhydride IR double peaks appeared at ~1813 and ~1742 cm⁻¹ (Fig. 2B). The weight average molecular weight of poly(PEG:SA) ranged from >12 kDa with 50% PEG to nearly 20 kDa with 10% PEG in the feed. The present study showed that a polyanhydride molecular weight above ≈10 kDa is sufficient for efficient preparation of microparticles capable of controlled drug delivery.

Table 2
Characterization of poly(sebacic anhydride-co-PEG) of different feed composition

PEG feed (wt%)	10	30	50
PEG (wt%) Calculated from ^1H NMR	8.5	28.8	48.8
M_w (Da)	20,294	19,495	12,504
M_n (Da)	8796	8504	6711

3.2. Poly(ether-anhydrides) as aerosol drug carriers

Microsphere size and density are crucial to dry powder aerosol design since it has been shown that particles deposit in the lungs based on their aerodynamic diameter. The aerodynamic diameter of a particle can be described as the in-flight diameter a spherical particle would possess assuming it had a density of 1 g/cm^3 . A quantitative relationship for the aerodynamic diameter (d_a) of a spherical particle derived from Stokes Law [45] is found to be

$$d_a = d\sqrt{\rho/\rho_a}, \quad (1)$$

where d = geometric diameter, ρ = particle mass density (g/cm^3), and ρ_a = water mass density (1 g/cm^3). Eq. (1) shows that a spherical particles' aerodynamic diameter relates its density and diameter into one parameter. Important early work by Landahl and coworkers showed that sedimentation and inertial impaction in the mouth, throat, and lungs uniquely depends on the aerodynamic diameter [46]. Sedimentation and inertial impaction are the two most important mechanisms of deposition in the lung of particles $> 1\text{ }\mu\text{m}$ in diameter.

Characterization of the polyether-anhydride microspheres revealed that their density and, therefore, aerodynamic diameter could be controlled by the amount of PEG incorporated into the polymer backbone. Density decreased from 0.344 to 0.077 g/cm^3 as the amount of PEG incorporated in the polymer increased from 0% to 30% PEG (Fig. 3A). The decrease in particle density may have resulted from the addition of the hydrophilic PEG, which encouraged water uptake and thus swelling of the microspheres. Swollen microspheres are frozen and lyophilized, leading to low-density structures [36]. An increase in PEG in the polymer backbone also decreased microsphere yield from 83% (0% PEG) to 40% (30% PEG). Lower yield may be due to an increase in the percentage of water-soluble polymer chains as the amount of PEG in the polymer backbone increases (Table 2). Shorter chains with a high percentage of hydrophilic PEG may dissolve and diffuse out of the microspheres during preparation.

The decrease in microsphere density with increasing PEG content resulted in a decrease in their average aerodynamic diameter (d_a) from 3.7 (0% PEG) to

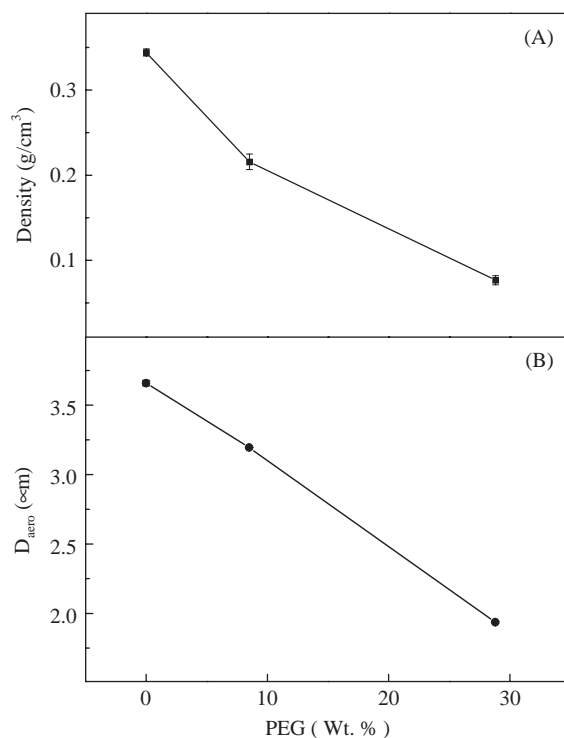


Fig. 3. Effect PEG content on PEG:SA microparticle (A) density and (B) aerodynamic diameter.

$1.9\text{ }\mu\text{m}$ (30% PEG) (Fig. 3B) while the average geometric diameters (not shown) were larger ($\sim 7\text{ }\mu\text{m}$).

Large, low-density particles, such as those easily produced with poly(ether-anhydrides), with aerodynamic diameters between 1 and $3\text{ }\mu\text{m}$ have shown considerable potential for alveolar deposition and systemic delivery [21]. Clearance rates of large particles by phagocytic cells are greatly reduced, allowing them to remain in the deep lungs and deliver drugs for extended periods of time. In addition, particle agglomeration due to van der Waals forces is greatly reduced with larger particles [38], resulting in an increase in aerosolization efficiency. Particles of aerodynamic diameter $> 5\text{ }\mu\text{m}$ deposit primarily in the upper airways or mouth and throat region, while a significant percentage of those $< 1\text{ }\mu\text{m}$ are exhaled [47]. Due to this region-specific deposition, poly(ether-anhydride) particles can be targeted to various areas in the lung by engineering particle aerodynamic diameter (also see Section 3.3). For example, particles with an aerodynamic diameter near $4\text{ }\mu\text{m}$ may be used as therapeutic carriers for bronchial delivery to treat lung disorders, such as asthma or cystic fibrosis.

The percent of PEG in the polymer backbone also had a significant effect on microsphere degradation times and model drug release kinetics. Degradation rates of polyanhydride or poly(ether-anhydride) microspheres (Fig. 4) in phosphate buffered saline (pH 7.4) were monitored by GPC. Poly(ether-anhydride) polymers of

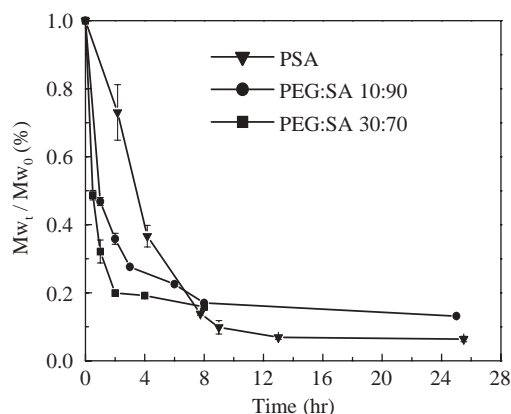


Fig. 4. Degradation profiles of PEG:SA microparticles in PBS (pH 7.4, 37°C).

various compositions degraded to within 20% of their original molecular weight in 8 h or less. Subsequently, slow dissolution and erosion of hydrophobic monomers controls the rate of drug release [44,48]. Degradation times that mirror drug release rates, as often achieved with hydrophobic polyanhydrides, would obviate the problem of polymer build-up in the lungs upon repeat administration. Polymers with a higher percentage of PEG showed increased degradation rates (Fig. 4). This could be explained by the increased hydrophilicity of the PEG-SA polymers, leading to more rapid water uptake. In a related study [44], we showed that the *in vitro* release of plasmid DNA could be controlled for up to 1 week by varying the amount of PEG in the polymer. It is interesting to note that DNA was released steadily for up to 6 days even though the polymer degraded within several hours. In a previous study with poly(anhydride-co-imide) microspheres, dissolution of degradation products (insoluble monomers) was shown to control the release of encapsulated macromolecules [48]. Therefore, as seen in our experiments, fast-degrading polymers with hydrophobic monomers allow sustained release of encapsulated drug, the rate of which can often be controlled by monomer dissolution rates.

3.3. Poly(ether-anhydrides) as injectable drug carriers

Although the focus of this paper is on pulmonary drug delivery, “stealth” liposomes with $\approx 5\%$ PEG by mass have been shown to provide significantly enhanced circulation times following intravenous injection [43]. Therefore, it is expected that PEG:SA polymers, containing as much as 50% PEG by mass, may also serve as materials for long-circulating “stealth” particles following intravenous injection [43]. In this case, such systems with enhanced degradation rates compared to PLGA may deliver a higher percentage of their therapeutic payload prior to removal by the reticuloendothelial system.

3.4. Systematic design of polymeric aerosols via central composite design

We used a two-level factorial design to investigate the effects of various particle preparation variables on important physical properties of our polymeric aerosol particles (microparticle size, density, and aerodynamic diameter). Useful in many fields, this methodology allows one to minimize the number of experiments performed and still obtain quantitative relationships between many design inputs. The relationships can then be used as predictive tools to obtain results that were not explicitly tested in the original design [49]. The encapsulation parameters optimized were: the polymer concentration in the organic phase, the concentration of phosphatidylcholine in the organic phase, the ratio of aqueous drug phase to polymer organic phase in the first emulsion, the speed of emulsification of the second emulsion, and the surfactant concentration in the final aqueous phase (see Fig. 5 and Table 1). Using this approach, we developed protocols by which to produce polymer microspheres with mass-average diameters ranging from 1 to 30 μm and aerodynamic diameters ranging from 1 to 9 μm .

Homogenization speed and polymer concentration had the largest effects on the microsphere size (Table 3).

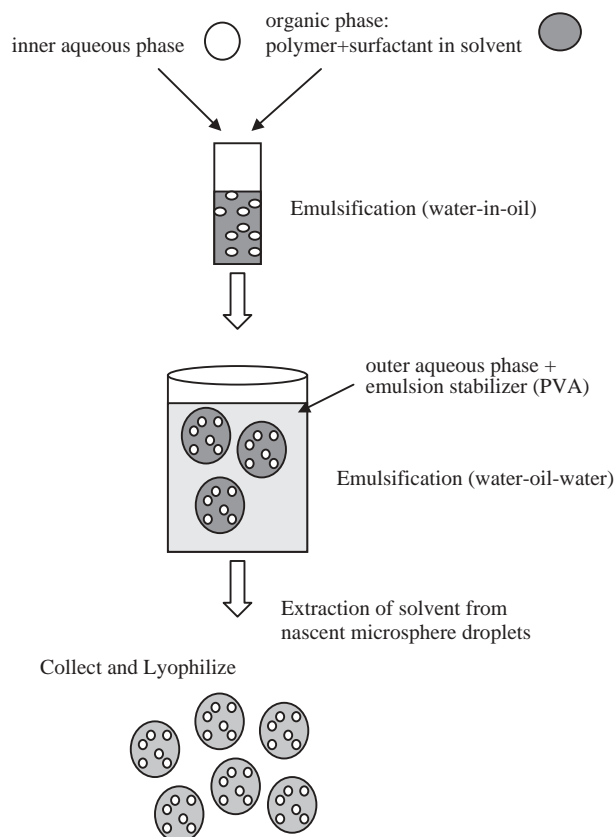


Fig. 5. Simplified schematic of the microsphere preparation process.

Table 3
Summary of effects of microparticle preparation parameters on important particle physical properties

Input variables (range)	Effect on diameter	Effect on density	Effect on aerodynamic diameter
Homogenization speed (5000–7000 rpm)	Significance* (diameter range) $p < 0.001$ (15.8–12.0 μm)	Significance* (density range) $p = 0.54$ (not sig.) (0.190–0.179 g/cm^3)	Significance* (aero. diameter range) $p < 0.001$ (6.95–9.03 μm)
Polymer concentration (56.25–118.75 mg/ml)	$p < 0.001$ (10.8–15.5 μm)	$p = 0.77$ (not sig.) (0.212–0.218 g/cm^3)	$p < 0.001$ (4.73–7.17 μm)
Ratio of water/oil phase (7–19 ml/ml)	$p < 0.001$ (13.0–14.0 μm)	$p = 0.79$ (not sig.) (0.186–0.191 g/cm^3)	$p < 0.02$ (5.57–6.13 μm)
PVA concentration (13.25–37.75 mg/ml)	$p < 0.001$ (14.8–12.4 μm)	$p = 0.15$ (not sig.) (0.173–0.201 g/cm^3)	$p < 0.007$ (6.19–5.51 μm)
PC concentration (2.5–7.5 mg/ml)	$p < 0.09$ (not sig.) (13.3–13.8 μm)	$p < 0.001$ (0.244–0.162 g/cm^3)	$p < 0.001$ (6.51–5.50 μm)

* p -values < 0.05 are statistically significant.

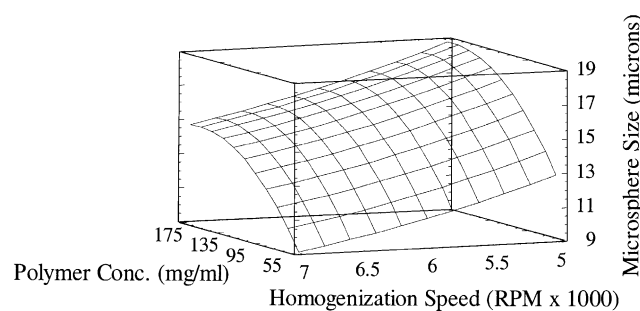


Fig. 6. Effect of polymer concentration and homogenization speed on microparticle size.

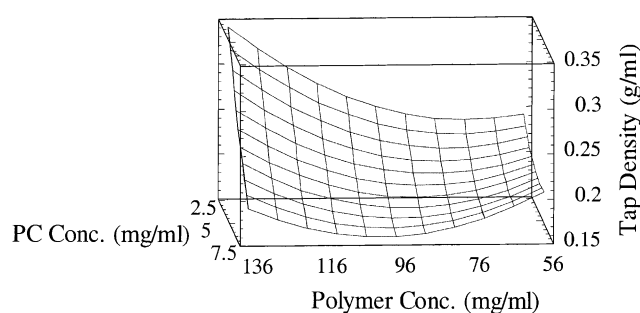


Fig. 7. Effect of polymer and PC concentrations on microparticle density.

A modest increase in homogenization speed from 5000 to 7000 rpm caused a decrease in the microsphere size from 15.8 to 12.0 μm , whereas a decrease in polymer concentration from 118.75 to 56.25 mg/ml caused a decrease in microsphere size from 15.5 to 10.8 μm (Table 3). The ratio of aqueous drug phase to organic phase had little effect on microsphere diameter, with the diameter increasing from 13.0 to 14.0 μm with a ratio increase from 7 to 19 ml/ml . The surfactant (PVA) concentration in the final aqueous phase had an inverse relationship with microparticle size (Table 3). The PC concentration did not have a significant effect on the microsphere size. An example response surface showing the effects of homogenization speed and polymer concentration on microparticle size (Fig. 6) shows that particles from 9 to 19 μm could be produced (using a set water/organic volume ratio of 0.13, PVA concentration of 2.5% (wt/vol) and PC concentration of 5 mg/ml). The response surface can then serve as a predictive template to control particle diameter, a critical parameter for targeting aerosols to different regions of the lung.

The homogenization speed, polymer concentration, ratio of aqueous drug phase to organic phase, and PVA concentration had no significant effect on microsphere density (Table 3). As the PC concentration increased, however, the microparticle density decreased as much as

three-fold (Fig. 7). The response surface showing the effects of polymer and PC concentration on microparticle density shows that densities of 0.15–0.35 g/ml can be achieved (with a set homogenization speed of 6000 rpm, water/organic ratio of 0.13 and polymer concentration of 87.5 mg/ml). Lower densities can be achieved upon simultaneous optimization of all of the encapsulation parameters.

Homogenization speed and polymer concentration also had the largest effect on the aerodynamic diameter of microspheres (Table 3). As the homogenization speed increased or the polymer concentration decreased, the aerodynamic diameter decreased. By increasing either the PC or PVA concentration, a significant ($p < 0.001$ for PC concentration, $p < 0.007$ for PVA concentration) decrease in aerodynamic diameter was achieved; whereas an increase in the ratio of water to organic phase increased the aerodynamic diameter. Similar studies, aided by CCD, on poly(ether-anhydride) microsphere formulations have been recently completed (J. Fiegel, J. Fu, J. Hanes, unpublished). We have also used CCD to optimize polymeric cationic particles for DNA delivery to the lungs (E. Krauland, J. Hanes, unpublished).

It should be noted that the ability to fully optimize new formulations for pulmonary drug delivery has been

limited by our inability to closely mimic the conditions particles encounter in various regions of the lung. Conventional “complete immersion” methods of particle characterization (particles submersed in buffer) may greatly overestimate the hydration, degradation, and drug release kinetics of microparticles that deposit on a thin fluid film on the lung surface. This is particularly important in alveoli where the fluid thickness bathing the epithelium is $\approx 0.07\ \mu\text{m}$ [7], or two-orders of magnitude smaller than our particles. In collaboration with Lehr and coworkers, we have begun utilizing air-interfaced lung epithelial cell monolayers that secrete mucus or surfactant on their apical surface in particle characterization studies to more closely mimic the thin fluid layer found in vivo [50].

4. Conclusion

The use of polymeric systems to achieve controlled drug delivery in the lung is still in the early stages of development. New polymers specifically designed for pulmonary delivery are needed to overcome the limitations of currently available off-the-shelf polymers such as PLGA. We have reported the synthesis of a family of PEG-based poly(ether-anhydrides) that are aimed to correct several problems inherent with PLGA. Further, we approached particle preparation systematically to engineer particle physical properties suitable for delivery to the various regions of the lung. Continued advances in material synthesis, particle engineering, particle characterization techniques and mathematical modeling, should improve the likelihood of the future development of suitable polymeric carriers for controlled drug delivery in the lungs. Such systems will likely find applications in both local therapies (lung as target) and systemic therapies (delivery to the blood).

Acknowledgements

The authors are thankful to the Whitaker Foundation (grant RG-99-0046). Partial support for Jennifer Fiegel came from a National Science Foundation Graduate Fellowship (grant DGE-9616062).

References

- [1] Langer R. Drug delivery and targeting. *Nature* 1998;392(6679): 5–10.
- [2] www.lungusa.org/asthma/cited_12/01.
- [3] Wall DA. Pulmonary absorption of peptides and proteins. *Drug Del* 1995;2:1–20.
- [4] Niven RW, Lott FD, Cribbs JM. Pulmonary delivery of powder and solutions containing granulocyte colony-stimulating factor (rhg-CSF) after intratracheal instillation to the hamster. *Pharm Res* 1993;10:1060–604.
- [5] Patton J, Platz R. Pulmonary delivery of peptides and proteins for systemic action. *Adv Drug Del Rev* 1992;8:179–96.
- [6] Laube B, Benedict G, Dobs A. The lung as an alternative route of delivery for insulin in controlling postprandial glucose levels in patients with diabetes. *Chest* 1998;114:1734–9.
- [7] Patton JS. Mechanisms of macromolecule absorption by the lungs. *Adv Drug Del Rev* 1996;19:3–36.
- [8] Wang J, Ben-Jebria A, Edwards DA. Inhalation of estradiol for sustained systemic delivery. *J Aerosol Med* 1999;12:27–36.
- [9] Choi WS, Murthy GGK, Edwards DA, Langer R, Klibanov AM. Inhalation delivery of proteins from ethanol suspensions. *Proc Natl Acad Sci USA* 2001;98:11103–7.
- [10] Agu RU, Ugwoke MI, Armand M, Kinget R, Verbeke N. The lung as a route for systemic delivery of therapeutic proteins and peptides. *Respir Res* 2001;2:198–209.
- [11] Pettis RJ, Hall I, Costa D, Hickey AJ. Aerosol delivery of muramyl dipeptide to rodent lungs. *AAPS Pharmsci* 2000;2:U50–9.
- [12] Patton JS. Deep-lung delivery of therapeutic proteins. *Chemtech* 1997;27:34–8.
- [13] Damms B, Baines W. The cost of delivering drugs without needles. *Bio-Technology* 1995;13:1438–40.
- [14] Selam JL. Inhaled insulin: clinical results in type 2 diabetic patients. *Diabetes Metab* 2001;27:S28–SA32.
- [15] Patton JS, Bukar J, Nagarajan S. Inhaled insulin. *Adv Drug Del Rev* 1999;35:235–47.
- [16] Patton JS. Pulmonary delivery of drugs for bone disorders. *Adv Drug Del Rev* 2000;42:239–48.
- [17] Altieri RJ, Thompson DC. Physiology and pharmacology of the airways. In: Hickey AJ, editor. *Inhalation aerosols*. New York: Marcel Dekker, 1996. p. 233–72.
- [18] Folkesson HG, Westrom BR, Karlsson BW. Permeability of the respiratory tract to different-sized macromolecules after intratracheal instillation in young and adult rats. *Acta Physiol Scand* 1990;139:347–54.
- [19] Patton JS, Trincherro P, Platz R. Bioavailability of pulmonary delivered peptides and proteins: α -interferon, calcitonins and parathyroid hormones. *J Controlled Release* 1994;28:79–85.
- [20] Patton JS. Inhalation: the other “oral” route for delivery of molecules with low gastrointestinal bioavailability. *Abstr Pap Am Chem Soc* 2000;219:175.
- [21] Edwards DA, Abdelaziz B-J, Langer R. Recent advances in pulmonary drug delivery using large, porous inhaled particles. *J Appl Phys* 1998;85:379–85.
- [22] Zeng X, Martin G, Marriott C. The controlled delivery of drugs to the lung. *Int J Pharm* 1995;124:149–64.
- [23] Dershwitz M, Walsh JL, Morishige RJ, Connors PM, Rubsam RM, Shafer SL, Rosow CE. Pharmacokinetics and pharmacodynamics of inhaled versus intravenous morphine in healthy volunteers. *Anesthesiology* 2000;93:619–28.
- [24] Ehlers M, Silagy C, Fleming D, Freeman D. New approaches for managing influenza in primary care. *Clin Drug Invest* 2001;21:443–52.
- [25] Sanjar S, Matthews J. Treating systemic diseases via the lung. *J Aerosol Med* 2001;14:S51–8.
- [26] Edwards DA, Hanes J, Caponetti G, Hrkach J, Ben-Jebria A, Eskew ML, Mintzes J, Deaver D, Lotan N, Langer R. Large porous particles for pulmonary drug delivery. *Science* 1997;276:1868–71.
- [27] Liu F, Shao Z, Kildsig D, Mitra A. Pulmonary delivery of free and liposomal insulin. *Pharm Res* 1993;10:228–32.
- [28] Suarez S, O’Hara P, Kazantseva M, Newcomer CE, Hopfer R, McMurray DN, Hickey AJ. Respirable PLGA microspheres containing rifampicin for the treatment of tuberculosis: screening in an infectious disease model. *Pharm Res* 2001;18:1315–9.

- [29] O'Hara P, Hickey AJ. Respirable PLGA microspheres containing rifampicin for the treatment of tuberculosis: manufacture and characterization. *Pharm Res* 2000;17:955–61.
- [30] Fajac I, Allo JC, Souil E, Merten M, Pichon C, Figarella C, Monsigny M, Briand P, Midoux P. Histidylated polylysine as a synthetic vector for gene transfer into immortalized cystic fibrosis airway surface and airway gland serous cells. *J Gene Med* 2000;2:268–378.
- [31] Bragonzi A, Dina G, Villa A, Calori G, Biffi A, Bordignon C, Assael BM, Conese M. Biodistribution and transgene expression with nonviral cationic vector/DNA complexes in the lungs. *Gene Ther* 2000;7:1753–60.
- [32] Gautam A, Densmore CL, Golunski E, Xu B, Waldrep JC. Transgene expression in mouse airway epithelium by aerosol gene therapy with PEI-DNA complexes. *Mol Ther* 2001;3:551–6.
- [33] Ferrari S, Pettenazzo A, Garbati N, Zacchello F, Behr JP, Scarpa M. Polyethylenimine shows properties of interest for cystic fibrosis gene therapy. *BBA-Gene Struct Express* 1999;1447:219–25.
- [34] Matthews CB, Jenkins G, Hilfinger JM, Davidson BL. Poly-L-lysine improves gene transfer with adenovirus formulated in PLGA microspheres. *Gene Ther* 1999;6:1558–64.
- [35] Hanes J, Evora C, Edwards DA, Langer R. Particles incorporating surfactants for pulmonary drug delivery. US Patent No. 5855913, 1999.
- [36] Fu J, Fiegel J, Hanes J. Synthesis and characterization of PEG-based ether-anhydride terpolymers: Novel polymers for pulmonary drug delivery, in preparation.
- [37] Peng PC. The design and analysis of scientific experiments. Reading, MA: Addison-Wesley, 1967. p. 163–71.
- [38] Batycky RP, Hanes J, Langer R, Edwards DA. A theoretical model of erosion and macromolecular drug release from biodegrading microspheres. *J Pharm Sci* 1997;86:1464–77.
- [39] Mader K, Gallez B, Liu KJ, Swartz HM. Non-invasive in vivo characterization of release processes in biodegradable polymers by low-frequency electron paramagnetic resonance spectroscopy. *Biomaterials* 1996;17:457–61.
- [40] Shieh L, Tamada J, Chen I, Pang J, Domb A, Langer R. Erosion of a new family of biodegradable Poly(anhydrides). *J Biomed Mater Res* 1994;28:1465–75.
- [41] Visser J. Vanderwaals and other cohesive forces affecting powder fluidization. *Powder Technol* 1989;58:1–10.
- [42] Tabata Y, Ikada Y. Effect of the size and surface-charge of polymer microspheres on their phagocytosis by macrophage. *Biomaterials* 1988;9:356–62.
- [43] Gref R, Minamitake Y, Peracchia MT, Trubetskoy V, Torchilin V, Langer R. Biodegradable long-circulating polymeric nanospheres. *Science* 1994;263:1600–3.
- [44] Fu J, Fiegel J, Hanes J. Large, light polyether-anhydride microspheres: a new carrier for pulmonary drug delivery. *Proc Int Symp Controlled Rel Bioact Mater* 2001;28:393–4.
- [45] Bird RB, Stewart WE, Lightfoot EN. Transport phenomena. New York: Wiley, 1960, p. 59.
- [46] Landahl H. On the removal of air-borne droplets by the human respiratory tract. I. *Lung Bull Math Biophys* 1950;12:43–56.
- [47] Darquenne C, Brand P, Heyder J, Paiva M. Aerosol dispersion in human lung: comparison between numerical simulations and experiments for bolus tests. *J Appl Physiol* 1997;83:966–74.
- [48] Hanes J, Chiba M, Langer R. Degradation of porous poly(anhydride-co-imide) microspheres and implications for controlled macromolecule delivery. *Biomaterials* 1998;19:163–72.
- [49] Zeng XM, Martin GP, Marriott C. Tetrandrine delivery to the lung: the optimization of albumin microsphere preparation by central composite design. *Int J Pharm* 1994;109:135–45.
- [50] Fiegel J, Ehrhardt C, Lehr C-M, Hanes J. Air-interfaced lung epithelial cell monolayers for characterization of large light polymer aerosols. *Ann Biomed Eng* 2001;29:S141.