Controlled dissolution from wax-coated aerosol particles in canine lungs

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Pillai, RaviRaj S., Donovan B. Yeates, Irving F. Miller, and Anthony J. Hickey. Controlled dissolution from wax-coated aerosol particles in canine lungs. J. Appl. Physiol. 84(2): 717–725, 1998.—Treatment of pulmonary and systemic diseases may be improved and toxicity reduced by pulmonary deposition of drug-containing aerosols exhibiting delayed dissolution. Aqueous disodium fluorescein and pentamidine aerosols were dried, concentrated, and condensation coated with paraffin wax. The apparent mass median aerodynamic diameters of the coated fluorescein particles were 2–4.0 µm. Wax-to-fluorescein ratios were 0.38–1.05. The dissolution half times determined using a single-pass flow system were 1.5 min for uncoated fluorescein and 0.8 min for uncoated pentamidine. These increased over threefold when the aerosols were coated with paraffin wax to maxima of 5.3 and 2.6 min, respectively. Wax-coated aerosols generated from fluorescein mixed with 99mTc-labeled iron oxide colloid delivered to the canine lungs demonstrated a 3.4-fold increase in the absorption half time of disodium fluorescein compared with uncoated fluorescein (11.2 vs. 38.4 min). The absence of changes in pulmonary function on inhalation of these wax-coated aerosols, together with a high drug load and delayed release, establishes a foundation for future therapeutic applications.

Paraffin wax; particle dissolution; aerosol concentrator; pentamidine; disodium fluorescein

Many drugs degrade or are metabolized after oral or intravenous administration. Systemic delivery to achieve therapeutic doses at the site of action may result in toxicity or side effects elsewhere. Also, many drugs are pharmacologically inactivated when administered by the conventional oral and parenteral routes. The lungs, with their large surface area, proximity to the vascular blood supply, and low local enzymatic activity in comparison to some other organs or tissues (3, 8, 12), present an ideal site for delivery of a wide variety of drugs with local and systemic activity. The advantages of delivering therapeutic agents as inhalation aerosols to the lungs are ease of administration, effectiveness in small doses for local therapeutic effect, frequently rapid response (e.g., bronchodilators), and absence of first-pass gastrointestinal or hepatic metabolism (12). The efficacy of inhaled agents in the respiratory tract depends on their site of deposition and physicochemical characteristics, which dictate their rate of dissolution, absorption, metabolism, and elimination (5, 17). The potential rapid absorption of the agent from the lungs could lead to transient peaks in drug action, resulting in adverse side effects. In addition, a therapeutic drug level may not be maintained for an extended time period. Therefore, it is desirable for toxicological and therapeutic reasons to formulate controlled-release aerosol systems capable of reducing high transient peak levels of drugs while extending their residence time in the lungs.

Formulation approaches to controlled drug delivery to the lung include coprecipitation, polymeric matrices, liposome encapsulation, crystal habit modification, and hydrophobic coating (12, 13). The major limitations of many of these techniques have been the small drug load that can be incorporated into the system to achieve controlled-release characteristics and the stability of the formulation after aerosolization. We have shown that coating hydrophilic aerosol particles with a hydrophobic material such as lauric acid by an evaporation-condensation technique yielded a product with a drug load >40% of the total mass (22). The rate of drug dissolution decreased with increasing mass of coating material associated with the particles (22). However, administration of these coated particles to the canine lungs caused irritation of the lungs in a dose-dependent manner. We wished to demonstrate predictable controlled release of a drug surrogate, disodium fluorescein, as well as pentamidine using a biologically inert coating.

Paraffin waxes are hydrophobic materials that have been used extensively in the formulation of oral controlled-release tablets. Dissolution studies performed on wax-coated tablets for oral administration showed a reduction in the rate of drug release (26). We present the release characteristics of disodium fluorescein and pentamidine from aerosol particles of these agents that have been wax coated from a paraffin wax vapor. In vitro and in vivo techniques are utilized and compared.

Materials

Disodium fluorescein (Fisher Scientific, Itasca, IL) is an amorphous hygroscopic orange-red powder, freely soluble in water, with yellowish-red color and intense yellowish-green.
fluorescence perceptible to a concentration of 0.002 parts/million (ppm). The concentration of fluorescein in solution was assayed spectrophotometrically at a wavelength of 489 nm (15).

Pentamidine isethionate (Fujisawa, Melrose Park, IL) is a hygroscopic crystalline powder, soluble in water. It is an appropriate candidate for formulation as a controlled-release product because of its high toxicity. The amount of pentamidine in solution was assayed fluorometrically at an excitation wavelength of 290 nm and emission wavelength of 350 nm.

Paraffin wax (Fisher Scientific), a mixture of solid hydrocarbons with a melting point of 55°C, was used as a coating material. The high vapor pressure and inert nature of the long-chain hydrocarbons are desirable characteristics for condensation coating applications.

Methods

The apparatus used for condensing paraffin wax on the surface of hydrophilic aerosol particles consisted of three units: an aerosol generation-and-drying unit, a concentration unit, and an evaporation-condensation coating unit, the latter being a modified Sinclair-LaMer condensation generator. The design features of this apparatus are described elsewhere (22). Briefly, aerosol droplets were generated at a rate of 1 ml/min from 0.50 wt% disodium fluorescein or 0.50 wt% pentamidine solutions using a jet nebulizer (model CSL, Turbotak). The device used a pressurized liquid feed from a syringe pump (model 600-900 VDCM, Harvard Apparatus, Dover, MA) in conjunction with a compressed air supply from an air tank at a flow rate of 120 l/min. The droplets were dried in transit to a concentrator by a sheaf of warm dry dilution air. The aerosol was dried as it passed through a large-volume cylindrical drum (30 cm diameter, 110 cm long) placed vertically between the Turbotak jet nebulizer and the virtual impactor. The dilution airflow rate was 100–200 l/min. The dried aerosol particles were concentrated using a seven-orifice virtual impactor (21, 29) by extracting 80–90% of the impinged aerosol particles. The solution was filtered using a 0.2-µm-pore-size glass fiber filter (type A/E, Gelman Sciences, Ann Arbor, MI). Each disk, as well as the filter, was removed from the impactor, placed in a petri dish, and washed with 20 ml of phosphate buffer (pH 7.4) for disodium fluorescein or tris(hydroxymethyl)aminomethane phosphate buffer (pH 7.4) for pentamidine. The aerosol filter was subsequently washed with 20 ml of phosphate buffer, and the solution was heated in a water bath at a temperature close to the melting point of paraffin wax. This allowed complete dissolution of disodium fluorescein from the particles. The solution was filtered, and aliquots of the solution were assayed spectrophotometrically to estimate the concentration of disodium fluorescein. Calibration data showing the linear relationship between ultraviolet absorbance and concentration were obtained before the study began.

The size distributions of uncoated and paraffin wax-coated disodium fluorescein particles were determined using an inertial impactor (Anderson Non-Viable 1 ACFM Ambient Sampler, Graseby-Anderson, Smyrna, GA), consisting of eight impaction stages and a preseparator, operated at a flow rate of 28.3 l/min. Aerosols were collected at each of the eight impaction stages on stainless steel disks and on a 0.2-µm-pore-size glass fiber filter (type A/E, Gelman Sciences, Ann Arbor, MI). Each disk, as well as the filter, was removed from the impactor, placed in petri dishes, and washed with 20 ml of phosphate buffer (pH 7.4) for disodium fluorescein or tris(hydroxymethyl)aminomethane phosphate buffer (pH 7.4) for pentamidine. Each petri dish was shaken mildly at intervals for periods up to 2 h to dissolve disodium fluorescein or pentamidine from the deposited particles. The solution was filtered using a 0.2-µm filter (Millipore) to remove paraffin wax from the sampling solution. The cumulative mass fraction of disodium fluorescein or pentamidine on each stage was plotted against the effective cutoff diameter for that stage on logarithmic probability paper, and a log-normal distribution was calculated for the data by the method of least squares. The mass median diameter was taken as a point on the regression line that equally divided the mass. The geometric standard deviation (GSD) was defined as the ratio of the particle size below which 84.16%, by mass, of the distribution occurs to the mass...
median size. The ratio of dye/drug to paraffin wax may not be constant within the particle population. Thus the terms “apparent mass median aerodynamic diameter” (MMAD) and “apparent GSD” (GSD) were adopted to define the size and distribution of paraffin wax-coated disodium fluorescein and pentamidine particles.

Disodium fluorescein solutions containing 99mTc-iron oxide colloids were sampled by inertial impaction to establish that the activity median diameter associated with the radioactive label was the same as the mass median aerodynamic diameter (MMAD) of the dye and that the ratio of the two substances on each of the eight stages of the impactor was the same.

The release characteristics of fluorescein or pentamidine from the uncoated and coated particles were assessed using a pressurized single-pass constant continuous flow-through system. An Amicon 52 cell (Millipore) fitted with a PM 30 Diaflow ultrafiltration membrane (Millipore) and connected via a concentration/dialysis selector (model CDS10, Millipore) to a pressurized reservoir (model RS 4, Millipore) served as the dissolution apparatus. The aerosol filter was placed over the ultrafiltration membrane, and a Parafilm cover with a 20-mm-diameter hole was centered over the top. The dissolution apparatus was designed to allow the dissolution medium (phosphate buffer or tris(hydroxymethyl)aminomethane phosphate buffer (pH 7.4)) to flow from the top, past the particles, and, after the aerosol filter and the ultrafiltration membrane consecutively. The flow of solution through the dissolution apparatus was adjusted by changing the applied pressure in the buffer reservoir. The eluate was collected at regular time intervals in a graduated cylinder applied pressure in the buffer reservoir. The eluate was analyzed using spectrophotometric and fluorometric techniques to estimate the concentration of disodium fluorescein and pentamidine, respectively. The half time ($t_{1/2}$) for dissolution was defined as the time for one-half of the total dye/drug load to be eluted in the aqueous medium. The data from three replicate experiments were combined to determine the $t_{1/2}$ values for dissolution.

Dog studies. The animal protocol and all the specific procedures in these experiments were approved by the Animal Care Committee of the Biological Resources Laboratory at the University of Illinois at Chicago, which is approved by the American Association for Accreditation of Laboratory Animal Care. Three conditioned male dogs, 3–5 yr old and weighing 9–12 kg, were used in each of three studies.

Each animal was fasted for 12 h before the experiment but was allowed free access to water. The dogs were sedated with 0.1 mg/kg of acepromazine maleate (Prom Ace, Aveco, Fort Dodge, IA) and anesthetized with an induction dose of 20 mg/kg of thiamyyl sodium (Surital, Parke Davis, Morris Plains, NJ) injected through an intravenous catheter. Lactated Ringer solution (100–400 ml; Travenol, Deerfield, IL) was provided through the intravenous line as the supporting fluid. The depth of anesthesia was maintained by intermittent thiamyyl sodium injections of 4–8 mg/kg. The dog was placed in the supine position with its upper jaw immobilized and intubated with a 9-mm-ID endotracheal tube with two sampling ports (Malinckrodt, Argyle, NY). The cuff of the endotracheal tube was inflated distal to the larynx. Eucapnic ventilation was confirmed by using an airway gas monitor (model 252, Datex, Helsinki, Finland), which indicated that end-expiratory CO2 was maintained between 4 and 5%. Rectal temperature was maintained at 37.5 ± 1.0°C with the help of heating pads (Casco Hospital Heating Pads, Valencia, CA).

Respiration rate, tidal volume, lung resistance, and dynamic lung compliance were monitored before and after inhalation of the test aerosols to ascertain any irritant response that may limit the usefulness of paraffin wax for therapeutic applications. To measure pulmonary function parameters, the end of the endotracheal tube was connected to a heated (37°C) pneumotach (model 3700, Hans Rudolph, Kansas City, MO). The pneumotach was coupled to a 2-cmH2O differential pressure transducer (model MP45-1-871, Validyne Engineering, Northridge, CA) for measurement of airflow. A catheterized esophageal balloon (1.5 × 10 cm) was inserted into the esophagus and connected to a 20-cm differential pressure transducer (model MP45-28-871, Validyne Engineering). The other side of the pressure transducer was connected to the largest side port of the endotracheal tube. The pressure difference between the two points was the transpulmonary pressure. Pressure and flow signals from the transducers were calibrated before the study. The pressure and flow signals from the pressure transducers were directed to a digital oscilloscope (model HP54501-A), which was interfaced to a 486-type microcomputer through an HPIB bus. The oscilloscope was programmed to sample 512 channels during each sequential 20-s sweep. The flow and pressure signals were processed in real time by using a customized iterative C program to derive tidal volume, lung resistance, and dynamic lung compliance. The dogs were exubated 90 min after exposure and maintained under sedation for up to 4 h for collection of blood and urine samples for evaluation of pharmacokinetics. A pattern of relatively slow (10–20 breaths/min) breathing was adopted to achieve peripheral lung deposition.

Blood and urine samples were collected and analyzed fluorometrically to estimate the systemic concentration of fluorescein. A two-way stopcock was inserted into the venous line to facilitate blood sampling. Urine samples were collected throughout the duration of study via a urethral catheter inserted into the bladder. Before and after inhalation of test aerosols, 0.5-ml blood samples were withdrawn at intervals using 1-ml heparinized syringes and emptied into heparinized collection tubes. Samples were centrifuged immediately, and the plasma was harvested. The amount of disodium fluorescein in the plasma (glycine buffered, pH 10.8) was subsequently quantified using a fluorometer (model MPF-2A, Hitachi). The $t_{1/2}$ values were calculated assuming a one-compartment model with first-order absorption and elimination process using nonlinear regression techniques (Scenitis, Micromath, Salt Lake City, UT). The areas under the plasma concentration-time curves (AUCs) reflect the dose of disodium fluorescein administered. The bioavailability of fluorescein was quantified by measuring the amount of fluorescein excreted in the urine. A small volume (50 ml) of urine was analyzed fluorometrically to estimate the concentration of disodium fluorescein.

In study 1, each of the three dogs underwent the following protocol on two occasions. Each dog was ventilated for 5 min with aerosol generated from a test solution containing 0.50% disodium fluorescein. Blood samples were taken at predetermined intervals, and urine samples were collected continuously for up to 3 h.

In study 2, each of the three dogs underwent the following protocol on two occasions, such that each animal was subjected to six experiments. The dogs were ventilated with disodium fluorescein aerosol particles condensation coated with paraffin wax at 100, 150, and 200°C. Aerosols were administered to the dogs in the supine position. Blood samples were taken at predetermined intervals, and urine samples were collected continuously for up to 5 h. Pulmonary function parameters were monitored throughout the duration of the study.
In study 3, each of the three dogs underwent the following protocol once. Aerosols were generated from a mixture containing 50 ml of 0.50 wt% disodium fluorescein and 0.5 ml of 2 wt% iron oxide colloid tagged with 99mTc (27). The concentration of iron oxide in the mixture was 0.01 wt%. The aerosols were dried and condensation coated with paraffin wax at 150°C. These aerosols were delivered to the animal lying supine above a gamma camera (model pho/Gamma, Searle Equipment, Des Plaines, IL). Deposition was monitored until a targeted value of 100 µCi was achieved in the lungs. Gamma emissions from deposited radiotagged particles in the lung were monitored sequentially for 1.5 h. The animal was allowed to recover from the anesthetic and returned to the animal housing facility. At 24 h after administration of the aerosol, the dog's thorax was viewed again using gamma-sctigraphy techniques, and the counts of radioactivity from the retained radiocolloid were measured.

Images on a visual display unit indicated the location of the deposited aerosols. Analysis of the location of radioactivity was performed using commercially available software routines (CMS ACE, Springfield, IL). A region of interest corresponding to the complete image of each dog's lungs was selected, and the total count in this region of interest at each time interval was corrected for background and radioisotope decay to analyze the clearance and retention data. The particle retention after 24 h was assumed to represent alveolar retention (1).

RESULTS

Physicochemical Characterization

The scanning electron photomicrographs of uncoated and paraffin wax-treated disodium fluorescein particles for comparison of their morphological characteristics are shown in Fig. 1. The shape of the particles in both cases appeared approximately spherical, with a majority of the particles having diameters <5 µm.

The signatory sodium peaks in the X-ray energy spectra obtained from an uncoated and a paraffin wax-coated particle are presented in Fig. 2, A and B, respectively. These peaks were indicative of the presence of sodium in disodium fluorescein. All the coated particles in the sample exhibited the signatory sodium peak. There was no detectable sodium peak in the X-ray energy spectrum obtained from a paraffin wax particle (Fig. 2 C).

Table 1 summarizes the amount of paraffin wax associated with disodium fluorescein particles on a mass basis (g/g) and their MMADa at different paraffin wax bath temperatures. The ratio of paraffin wax to disodium fluorescein (by weight) increased from 0.38 to 1.05, with a corresponding increase in the MMADa from 2.8 to 4.0 µm as the paraffin wax bath temperature was elevated from 100 to 200°C. The increase in MMADa at elevated paraffin wax bath temperature was consistent with an increase in the mass of condensed paraffin wax at the surface of disodium fluorescein aerosol particles. The GSDa was 1.9–2.2 in all cases. These values appeared to decrease with increased coating thickness.

The MMAD of pentamidine aerosol particles from an initial nebulized 0.50 wt% solution generated at a rate of 1.0 ml/min was 2.5 µm, and the GSD was 2.1. The MMADa of pentamidine aerosol particles coated with paraffin wax at a bath temperature of 200°C was 4.1 µm, with a GSDa of 1.8.

The dissolution profiles for uncoated and paraffin wax-coated disodium fluorescein particles are shown in Fig. 3. A reduction in the rate of release of disodium fluorescein was observed as the paraffin wax bath temperatures increased from 100 to 200°C. The t for dissolution of uncoated disodium fluorescein particles was 1.5 min. The dissolution t values for disodium fluorescein particles coated at 100, 150, and 200°C with paraffin wax were 2.5, 3.7, and 5.3 min, respectively. The time required for the total dissolution of disodium fluorescein increased from 6 min for uncoated disodium fluorescein particles to 15 min for disodium fluorescein particles coated at 200°C with paraffin wax.

The release characteristics of coated disodium fluorescein particles (paraffin wax bath temperature 200°C) at three different dissolution medium temperatures are shown in Fig. 4. A reduction in the rate of release of disodium fluorescein from 30 to ~3.3%/min from the
coated particles was observed as the temperature of the dissolution medium was changed from 37 to 25°C. In addition, the maximum percentage of disodium fluorescein released decreased from 98 to 22% when dissolution studies were conducted for 12 min at these temperatures.

The release characteristics of uncoated and paraffin wax-coated pentamidine particles are shown in Fig. 5. The dissolution t<sub>diss</sub> increased from 0.8 min for uncoated pentamidine particles to 2.6 min for pentamidine particles coated at 200°C with paraffin wax. This represents a greater than threefold increase in the duration of total release for the coated particles.

**Dog Studies**

The average 24-h retention values of the paraffin wax-coated fluorescein-iron oxide 99mTc-tagged aerosol particles for two studies on the three dogs were 73, 70, and 68%, respectively. On the basis of the assumption that particles are cleared from the tracheobronchial airways by mucociliary clearance, the values are physiological indexes of peripheral deposition (1, 20). This is consistent with relatively uniform distribution of radiocolloid throughout the dogs’ lungs, as illustrated in previous scintigrams (21, 22).

The dose of disodium fluorescein deposited in the dog lung after inhalation of uncoated and paraffin wax-coated aerosol particles ranged from 300 to 500 µg, as estimated by fluorometric analysis of the urine samples. The plasma concentration of disodium fluorescein after absorption from a dog's lungs (Fig. 6) normalized to the peak plasma concentration demonstrated a rightward shift from 10 to 45 min and broadening of the peak plasma concentration-time profile.

The temporal changes in the plasma concentration of disodium fluorescein after absorption from uncoated and paraffin wax-coated particles deposited in the canine lungs were used to estimate the absorption and elimination t<sub>1/2</sub> values (24). Figure 6 shows the average

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**Table 1. Effect of increasing paraffin wax bath temperatures on characteristics of resultant disodium fluorescein aerosol particles**

<table>
<thead>
<tr>
<th>Coating Temp, °C</th>
<th>Wax/DF, g/g</th>
<th>MMAD, µm</th>
<th>GSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>0.38</td>
<td>2.8*</td>
<td>2.1†</td>
</tr>
<tr>
<td>150</td>
<td>0.65</td>
<td>3.2*</td>
<td>2.0†</td>
</tr>
<tr>
<td>200</td>
<td>1.05</td>
<td>4.0*</td>
<td>1.9†</td>
</tr>
</tbody>
</table>

Wax/DF, paraffin wax-disodium fluorescein ratio; MMAD, mean mass aerodynamic diameter; GSD, geometric standard deviation; NA, not applicable. *Apparent MMAD; †apparent GSD.
plasma concentration-time profiles for the uncoated disodium fluorescein after inhalation in three beagle dogs (n = 2). The pharmacokinetic parameters resulting from the analysis of plasma data are listed in Table 2. Peak plasma concentrations occurred within 16 min (T_{max}) of completion of inhalation with a maximum plasma concentration of 159 ± 9 (SD) ng/ml.

The average plasma concentrations plotted against time after inhalation of wax-coated disodium fluorescein at 100, 150, and 200°C are shown in Fig. 6. The peak plasma concentrations achieved by these samples were 147 ± 18 ng/ml (T_{max} = 22 min), 135 ± 16 ng/ml (T_{max} = 30 min), and 132 ± 9 ng/ml (T_{max} = 40 min) for disodium fluorescein coated at 100, 150, and 200°C, respectively.

The AUC to infinity (AUC_{0-\infty}) calculated using the trapezoidal rule increased with wax temperature (particle size) from 123 ng·h·ml^{-1} for the uncoated disodium fluorescein to 215 ng·h·ml^{-1} for the sample coated at 200°C (Table 2).

Curve fitting using least-squares nonlinear regression methods indicated that the fall in plasma concentrations after inhalation of the uncoated and wax-coated formulations was best described by a monoexponential decay with first-order absorption. The evaluation of the goodness of the fit was performed by comparing the respective model selection criteria (where values < 1 are unacceptable). Model selection criteria values were 2.21 ± 0.23, 2.91 ± 1.36, 2.22 ± 0.39, and 2.81 ± 0.95 (SD) for the uncoated disodium fluorescein and the disodium fluorescein coated at 100, 150, and 200°C, respectively. These values indicate a good fit to the data.

The log plasma concentration-time plots for the samples coated at 150 and 200°C showed a decline in plasma concentrations with a defined t_{1/2} longer (34 and 40 min) compared to the uncoated disodium fluorescein.

### Table 2. Pharmacokinetic parameters for disposition of uncoated and paraffin wax-coated disodium fluorescein after administration as an aerosol

<table>
<thead>
<tr>
<th>Pharmacokinetic Parameter</th>
<th>Uncoated DF</th>
<th>100°C</th>
<th>150°C</th>
<th>200°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>K_e, min^{-1}</td>
<td>0.042 ± 0.01</td>
<td>0.04 ± 0.01</td>
<td>0.05 ± 0.02</td>
<td>0.04 ± 0.02</td>
</tr>
<tr>
<td>t_{1/2}, min</td>
<td>17.4 ± 5.4</td>
<td>20.2 ± 2.5</td>
<td>16 ± 6.6</td>
<td>18.4 ± 7.2</td>
</tr>
<tr>
<td>K_a, min^{-1}</td>
<td>0.06 ± 0.02</td>
<td>0.04 ± 0.01</td>
<td>0.022 ± 0.01</td>
<td>0.02 ± 0.01</td>
</tr>
<tr>
<td>t_{1/2}, min</td>
<td>11.2 ± 2.9</td>
<td>15.5 ± 2.6</td>
<td>34 ± 12</td>
<td>38.4 ± 13</td>
</tr>
<tr>
<td>AUC, ng·h·ml^{-1}</td>
<td>123 ± 1</td>
<td>152 ± 6</td>
<td>192 ± 10</td>
<td>215 ± 44</td>
</tr>
<tr>
<td>C_{max}, ng/ml</td>
<td>159 ± 9</td>
<td>147 ± 18</td>
<td>135 ± 16</td>
<td>132 ± 9</td>
</tr>
<tr>
<td>T_{max}, min</td>
<td>16.3 ± 2.9</td>
<td>21.7 ± 5.8</td>
<td>30 ± 0.0</td>
<td>40 ± 0.0</td>
</tr>
</tbody>
</table>

Values are means ± SD for 2 experiments in 3 beagle dogs. K_e, elimination rate constant; t_{1/2}, half time of elimination; K_a, absorption rate constant; t_{1/2}, half time of absorption; AUC, area under plasma concentration-time curve; C_{max}, maximum plasma concentration; T_{max}, time at which peak plasma concentration occurs.
38 min, respectively, see Table 2) than that previously reported after intravenous administration of fluorescein ($t_{1/2} = 17.9$ min) (4). This observation suggests a “flip-flop” case, which occurs when absorption, rather than elimination, dictates the decline in plasma concentrations. The terminal $t_{1/2}$ values for the uncoated disodium fluorescein and the disodium fluorescein coated at 100°C were 17.4 and 20 min, respectively.

The absorption $t_{1/2}$ after the inhalation of uncoated dye, calculated from the estimate of the absorption rate constant ($K_a$) after curve fitting, was 11.2 min and is in agreement with previously reported values of 12.2 min (3) for a polydisperse disodium fluorescein aerosol (MMAD = 3.5 µm).

Multiple comparisons of absorption $t_{1/2}$ among treatments by using Tukey’s test revealed significant differences (P < 0.05) between uncoated disodium fluorescein and disodium fluorescein coated at 150 or 200°C, whereas no significant difference was found between uncoated samples and samples coated at 100°C or between samples coated at 150 and 200°C (Table 2). These results suggest the capacity of wax coating at 150 and 200°C to produce extended pulmonary residence times for which less frequent inhalation would be required to maintain therapeutic concentrations of drug in the lungs.

The ratios of $t_{1/2}$ coated to $t_{1/2}$ uncoated for in vitro dissolution and in vivo absorption are itemized in Table 3. The average respiration rate, tidal volume, lung resistance, and dynamic lung compliance for each of the three dogs for the 5 min before and 15 min after inhalation of the test aerosol particles are tabulated in Table 4. There were no appreciable changes in the pulmonary function parameters before and after inhalation of the paraffin wax-coated disodium fluorescein particles.

**DISCUSSION**

A technique was developed to condense hydrophobic material onto the surface of hydrophilic aerosol particles. The evidence of coating was obtained from scanning electron microscopy and energy-dispersive X-ray microanalysis. The coated particles were <5 µm in diameter with a drug load >40% of the total mass. The rates of drug/dye release from the coated particles were inversely related to the mass of condensed paraffin wax. Administration of paraffin wax-coated particles to the canine lungs showed an increase in the absorption $t_{1/2}$ as the paraffin wax bath temperature increased from 100 to 200°C. Thus the efficacy of coating to reduce the rate of drug release was demonstrated.

The scanning electron micrographs of the wax-coated disodium fluorescein particles appeared to show undulations on the surface. This characteristic may be attributed to condensed paraffin wax. The sodium peak in the energy spectra obtained from all the coated particles examined indicated detectable amounts of disodium fluorescein. The sodium peak was absent in the spectra from aerosol particles consisting of paraffin wax alone. If it is assumed that the samples are representative of the aerosols, these observations indicate that condensed paraffin wax particles, if present, represent only a small fraction of the particle population.

Adjusting the concentration and flow rate of the test solution to be aerosolized (21) and adjusting the paraffin wax bath temperature resulted in production of coated aerosol particles in the respirable size range of 2.7–4.1 µm, and drug load >40% of the total mass. These delivery systems may be useful in practical inhalation therapy, which requires delivery of small bolus doses of drug to the lung. Although such particles have a high deposition efficiency in the human lung periphery (5), in the diseased lung, smaller particle sizes and slow inhalation rates will likely optimize alveolar deposition. However, it must be remembered that the mass delivered increases as the cube of the particle radius. On this basis, it can be predicted that waxes or other coating materials with much greater resistance to dissolution would be required if much smaller aerodynamic diameters were desired.

The characteristics of the dissolution system used in these studies allowed most of the surface area of the individual particles to be exposed to the dissolution medium. The in vitro dissolution characteristics of the coated particles obtained using the pressurized single-pass flow-through system were consistent with the experimentally determined in vivo absorption patterns in the lower respiratory tract.

The release kinetics of disodium fluorescein from particles coated with paraffin wax at high (>150°C)
and low (100°C) bath temperatures may be explained as follows. When the coating thickness is small, the surface layer is eroded rapidly, resulting in dissolution of the remaining disodium fluorescein particles at a rate comparable to that of uncoated particles. At higher coating thickness the wax layer is eroded slowly, resulting in prolonged disodium fluorescein release. The release approximates first-order kinetics in both cases.

If similar doses of disodium fluorescein were administered in each of the experiments at increasing temperatures, no differences in AUC_o-∞ should have been observed. The observed increases in AUC_o-∞ with increasing wax bath temperatures (Table 2) are likely due to the increases in fractional aerosol deposition with increasing particle size (Table 1), as can be predicted (5).

Pharmaceutical products are required to have extended storage and shelf-life. The dissolution rates and apparent retention of dye at lower dissolution medium temperatures indicate a formulation advantage of this method of treatment of hydrophilic aerosol particles. Long-term storage at ambient or reduced temperatures appears possible for these coated particles.

The coated test aerosol particles were delivered to the canine lung as a surrogate for the human lung (6). The peripheral deposition of the uncoated and coated particles indicates that mucociliary transport would have little effect on the bioavailability of the drug. Retention studies of radiotagged iron oxide particles in canine lungs for a more proximal deposition pattern (24-h retention of 56% compared with 70% in these experiments) showed that only 14% cleared from the lungs in 2 h (20). Thus mucociliary clearance is unlikely to account for a substantial portion of the clearance of disodium fluorescein in these studies. Disodium fluorescein is not metabolized in the lung (3); therefore, its bioavailability is equal to the mass deposited in the lung. The dose of fluorescein delivered as uncoated and paraffin wax-coated particles was estimated from a urinary excretion method to be 300–500 µg. This dose is within the therapeutic range for drugs commonly administered by inhalation.

Wax coating retarded the dissolution of pentamidine. Slow release of this drug in the air spaces could reduce its toxicity while providing continuous exposure to Pneumocystis carinii, thereby potentially enhancing its therapeutic effect (11). Dog studies were not performed with pentamidine, as the drug is sequestered by lung tissue (8, 18) and is cleared slowly (8, 10), rendering pharmacokinetic analysis meaningless with respect to therapeutic activity. Consequently, a pharmacokinetic assessment such as that described for disodium fluorescein was not feasible. A pharmacodynamic model is required to evaluate the effectiveness of pentamidine. In this respect, an animal model of P. carinii pneumonia could be employed to assess pharmacological efficacy of wax-coated pentamidine.

In previous studies, lauric acid was selected as a coating material (22). Inhalation studies performed on beagle dogs showed that, with lauric acid, coated disodium fluorescein particles gave rise to transient irritation in the dog’s lungs, as indicated by changes in the pulmonary function parameters. A moderate increase in lung resistance and a decrease in lung compliance occurred after inhalation. Inhalation of paraffin wax-coated disodium fluorescein particles caused no deviations in the pulmonary function parameters from baseline values, even at high paraffin wax bath temperatures (>200°C), indicative of the inert characteristics of the coating material. These experiments were designed only to evaluate any short-term irritant effect of the wax or any potential degradation products. The clearance mechanisms and any long-term hazards associated with paraffin wax in alveolar regions of lung were not investigated and are still unknown. Waxes have been used to control the release of drugs in microsphere products (16, 25). In addition, waxes have been used to monitor lung deposition and clearance mechanisms. Among these, studies using carnauba wax are most notable (2). Oral (19, 23) and inhalation (14, 28) studies of toxicity have failed to demonstrate toxicity of waxes. Carnauba wax is a naturally occurring product derived from the leaves of Copernicia cerifera. Despite its use as a pharmaceutical excipient, this wax was not selected for these studies because of its complex composition and narrow specifications. Paraffin wax was selected, as it can be obtained in a number of forms (although only one was used in these studies) with various melting points (vapor pressures) and well-defined compositions.

The duration of drug release and its residence time in the lungs are related to its intrinsic dissolution and disposition properties, which may be modified by hydrophobic coating, as indicated in these studies. The similarity of the in vitro and the in vivo ratios indicates that, although the absolute in vitro rates are faster, the relative in vitro dissolution ratios are reflective of the in vivo processes. The importance of these observations is in the demonstration of the relative increase in the dissolution time rather than the absolute time delay. In preliminary experiments, the dissolution of disodium
fluoroscein alone and from particles coated with carnauba wax at 200 and 300°C was compared (Fig. 7). The sample coated at the low temperature had a 2.7-μm median diameter and exhibited a dissolution rate equivalent to a similarly treated paraffin wax-coated aerosol. The sample coated at a higher temperature had a 4.0-μm median diameter and exhibited a dissolution rate, of ~1 h, with complete dissolution occurring at 200 min.

These data indicate the potential for waxes to be incorporated into a drug-containing respirable aerosol with controlled release characteristics when deposited within the lungs. Such particles are without any identifiable toxicity. By the judicious selection of wax matrices and/or coating temperatures, wax coatings can be designed that retard the candidate drug dissolution rate from minutes to hours and attain the desired pharmacological/toxicological response profiles.

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