but different amounts of the polymer. The polymer mixture used in the test was a 1:1 mixture of the low-viscosity grade polymer and the medium-viscosity grade polymer. As expected, the drug was released from tablets more slowly with an increase in polymer contents; therefore, the release rate of the drug can be modified by changing the polymer contents in the tablets.

Salivary Levels Following Oral Administration—In Fig. 4, the average salivary levels of theophylline following oral administration of two kinds of sustained-release tablets were compared with those of fast-dissolving powders. After the administration of the tablets, salivary levels were lower at earlier hours but higher afterward compared with salivary levels following administration of powders. The lowest salivary levels observed after administration of the tablet prepared from the medium-viscosity grade polymer reflect a slow release rate, as shown in Fig. 2, demonstrating that different drug level profiles in body fluids are obtainable by modifying the release patterns of drug. Although it is shown that the rate of bioavailability is decreased with a decrease in release rate *in vitro*, the effect of release rate on the extent of bioavailability has to be examined by extending sampling periods to 36–48 hr.

The present study demonstrated that the release of theophylline from compressed tablets prepared from hydroxypropylcellulose can be modified by changing viscosity grades of the polymer, mixing ratios of two polymers with different viscosity grades, and changing polymer contents in the tablets. Sustained-release *in vitro* is reflected in drug level curves after oral administration of sustained-release tablets.

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ACKNOWLEDGMENTS

The authors are grateful to Nihon Soda Co., for generous gifts of hydroxypropylcellulose samples.

Characterization of Spray Patterns of Inhalation Aerosols Using Thin-Layer Chromatography

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Received November 27, 1981 from the *Institute of Pharmaceutical Sciences, Syntex Research, Palo Alto, CA 94304.* Accepted for publication May 5, 1982.

Abstract □ The spray pattern of an inhalation aerosol was characterized using photography and by observing the impaction pattern on a TLC plate. The aerosol plume was conical in shape, and its cross section increased with increasing distance from the actuator. Three puffs of the aerosol, at a distance of 3 cm between actuator and the TLC plate, produced a spot that had approximately the same diameter as the cross section of the aerosol plume at that distance from the actuator. The TLC technique with these parameters was selected to develop an assay characterizing the spray pattern of an inhalation aerosol because of its specificity, simplicity, and speed.

Keyphrases □ Aerosols, inhalation—spray pattern characterizations using photography and TLC □ TLC—characterization of spray patterns for inhalation aerosols □ Spray patterns—of inhalation aerosols, characterized by TLC and photography

Pressurized inhalation aerosols are generally used for drug administration into the lower respiratory tract. Only a minor part of the dose administered reaches the lung directly (1–3). Recently, using an *in vivo* radioactive technique, it was estimated directly that an average 8.8% of the administered dose was deposited in the lungs with 80% deposited in the mouth (4). The remainder of the drug (9.8%) was either exhaled or deposited in the aerosol actuator. Various test methods for the control of aerosol products have been developed (5–7). These include tests for net contents, medication delivered per dose, particle size distribution, valve delivery, vapor pressure, leakage rate, moisture contents, and spray pattern.

One of the important objectives in developing an aerosol product is to obtain the spray pattern best suited for the intended application. Various factors can affect the spray pattern. These factors are the design of the valve and the actuator, the pressure in the container, and its content composition (8, 9). The spray pattern is affected by the size and shape of the actuator orifice as well as by the valve (10). Therefore, characterization of spray patterns is important for evaluating the valve and actuator performances. In addition to its pattern, other tests generally used to characterize the spray are particle size distribution of the drug substance delivered and spray angle (11).

Several methods have been devised to record and compare the spray pattern of aerosol products. One method is based on the impingement of the spray on a piece of paper, glass, silica gel, or paper that has been treated with a dye–talc mixture (12, 13). Photographic (14) and laser holographic[1] methods also have been used. Miszuk *et al.* (15) recently described a technique that utilizes two orthogonal video images. These methods are best suited for solution aerosols in which the active ingredient is dissolved

[1] Laser Photographic Laboratories, Arlington Heights, Ill.

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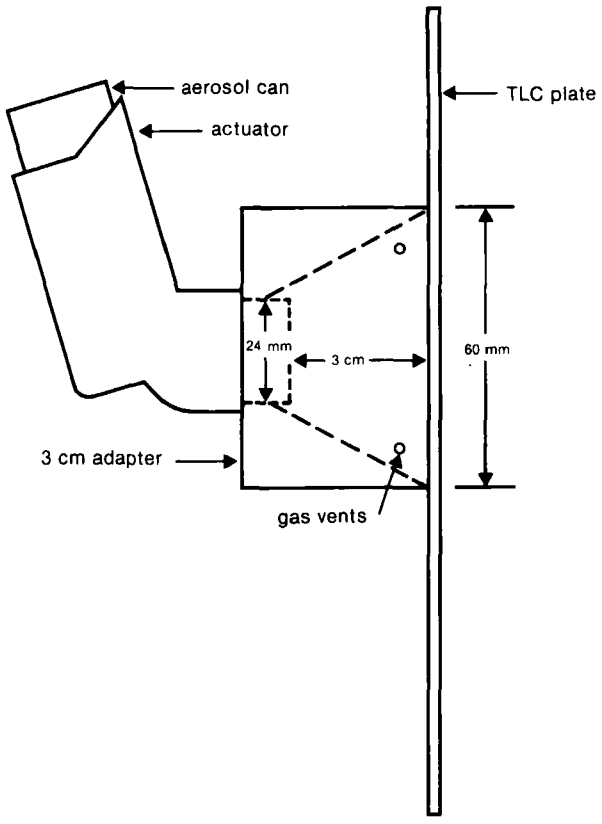

Figure 1—*Equipment and setup used to test the plume of corticosteroid aerosol by TLC method.*

in the solvent/propellant. In the case of heterogeneous aerosols (*e.g.*, suspensions), a major portion of the resulting spray consists only of the propellants. In such cases the aforementioned methods, being nonselective, do not provide sufficient information about distribution of the solid drug particles in various regions of the spray. The purpose of this study was to develop a specific method to characterize the spray pattern with respect to drug distribution in a plume pattern of an heterogeneous inhalation aerosol system.

EXPERIMENTAL

Materials—The aerosol formulations consisted of a suspension of micronized corticosteroid in a chlorofluorocarbon propellant system containing a surfactant. Dosage was controlled by a metered aerosol valve[2], with a 50-μl metering chamber and stainless steel stem. The valve gave a metered puff of formulation upon each actuation. The actuation of the valve was effected by a plastic actuator[2], also serving as a mouthpiece.

Four actuators were selected for this study. Three of them were modified as follows: the orifice diameter of actuator 2 was reduced to 0.483 mm; the orifice diameter of actuator 3 was enlarged to 0.864 mm; and the orifice exit angle with respect to the axis of the delivery tube for actuator 4 was changed and the orifice diameter was reduced to 0.406 mm. Actuator 1 was used as manufactured, with an orifice size of 0.533 mm.

Photography—Photographs of the spray were taken using a camera[3] with a 127-mm lens which was loaded with black and white film[4]. The aperture was set at f/16 and the shutter speed set at 0.5 sec. A flood light[5] was used to illuminate the aerosol plume from below. The actuator and shutter release were coordinated manually.

TLC Method—A plastic adapter was attached to the actuator. These were used to actuate an aerosol can to deliver a spray on a 20 × 20-cm silica gel TLC plate[6] from a fixed distance. The resulting spot was observed and outlined under UV light. Both the long and the short diameters of the spot were measured. The equipment and setup used is diagrammed in Fig. 1.

Corticosteroid Contents—In addition to measuring the diameters of the spots, corticosteroid contents in the spot were also determined. This was accomplished by scraping the silica gel corresponding to the spot from the plate and transferring it quantitatively into a test tube. This was followed by extraction of the corticosteroid with ethanol and quantitation by blue tetrazolium colorimetry (16).

Particle Size Distribution—The actuator was connected to the air sampler[7] by a stainless steel tubular throat. The nozzle of the aerosol can was inserted into the actuator, and a fixed number of puffs were sprayed into the eight-stage air sampler. The airflow through the air sampler was set at 28.5 liters/min. The acutator, throat, and the various stages were rinsed with ethanol. The corticoid deposited on the various stages was quantitated by reverse-phase high-performance liquid chromatography (HPLC) using an octadecylsilane column[8]. The mobile phase was water–acetonitrile (65:35) containing 1% acetic acid.

Table I—Cross Sections of Aerosol Plumes at Various Distances from the Actuator [a]

Distance from the Actuator, cm	Cross Section of Plume, mm							
	Actuator 1A			Actuator 1B			Mean	*SD*
1	15	14	14	15	14	17	14.8	1.2
2	18	18	16	17	15	18	17.0	1.3
3	22	20	18	20	19	20	19.8	1.3
4	25	23	23	24	22	25	23.7	1.2
5	30	27	27	28	25	28	27.5	1.6
6	34	31	30	33	30	33	31.8	1.7
7	38	35	34	37	35	37	36.0	1.5
15	75	80	75	80	82	80	78.7	2.9

[a] Measurements were taken from photographs.

RESULTS AND DISCUSSION

Photography—The photograph of an aerosol plume generated using actuator 1 (unaltered) shows that the plume is conical, symmetrical, and ~31–33 cm in length (Fig. 2). Photographs of six sprays using two actuators (1A and B) were taken. The widths of the aerosol plumes at various distances from the actuator were measured from these photographs. The data are given in Table I, and a plot of the plume width *versus* distance from actuator is shown in Fig. 3.

An ideal aerosol plume is considered to be a symmetrical cone of optimum apical angle to provide considerable clearance from the mouthpiece of the actuator (15). In addition, there should be only a few large particle dropouts from the spray cone (14). Actuator 1 yielded a plume consistent with the above criteria. However, the plumes produced by actuators 2, 3, and 4 (modified) had one or more deviations from such an ideal plume (Fig. 2). The actuator with the small orifice (actuator 2) yielded a misty and irregular-shaped spray with considerable amounts of streaks (dropouts). The apical angle appeared to be large and resulted in inadequate clearance from the actuator nozzle. The plume yielded by actuator 4 had about the same characteristics as 2. In addition, there was a displacement of the plume from the center and more dropouts. The effect of actuator 3 on the plume shape was not very obvious; however, the plume had a slightly larger apical angle and more dropouts than the regular actuator. This demonstrates that photography is capable of differentiating some defects in the actuators.

TLC Method—The spots effected by spraying the aerosol onto a TLC plate using the regular actuator (actuator 1) were circular in shape (Fig. 4). They consisted of an inner dark area surrounded by an outer diffused zone. The size of the spot varied with the number of puffs sprayed and the distance between the TLC plate and the actuator. The average diameter of the spot as a function of the number of puffs sprayed and the distance from the actuator is given in Table II, and graphical representations are shown in Fig. 3. For any fixed distance between the TLC plate and the actuator, the diameter of the spot increased with increasing number of puffs.

[2] Riker Laboratories, Northridge, Calif.
[3] Polaroid Land Camera CU-5, Cambridge, Mass.
[4] Landfilm type 107, ASA 3000, Polaroid Corp., Cambridge, Mass.
[5] Photoflood lamp G, G.E., Cleveland, Ohio.
[6] Analtech, Newark, Del.
[7] Andersen 2000 Inc., Atlanta, Ga.
[8] μBondapak, Waters Associates, Milford, Mass.

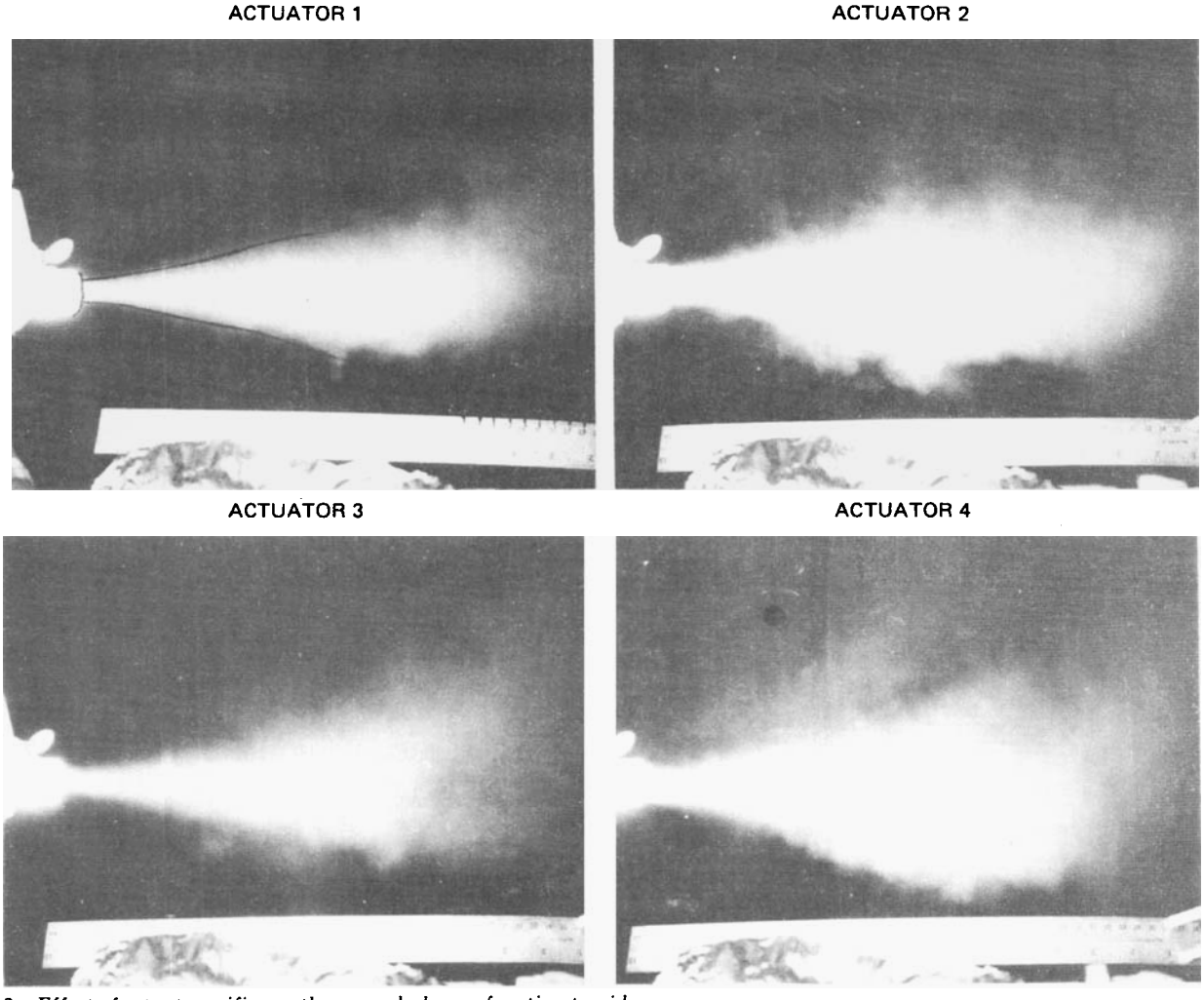

Figure 2—*Effect of actuator orifice on the aerosol plume of corticosteroid.*

Examination of the effect of varying this target distance, however, yielded some interesting facts. When three or fewer puffs were used, the diameter of the spot decreased with increasing distance from the actuator. On the other hand, the use of five or more puffs resulted in larger spot size with increase in distance from the actuator. Assuming that the aerosol plume has a conical shape (as shown by photography, Fig. 2), one would expect an increase in the size of the spot with increasing distance of the TLC plate from the actuator, irrespective of the number of puffs sprayed. This discrepancy could be due to the fact that photography does not distinguish between drug substance particles and propellant droplets in the plume. Thus, photography gives no indication of the drug substance density gradient in the aerosol plume (*i.e.*, between the center and outer part). On the other hand, the TLC method directly examines drug substance particles in the cross section of the plume, as the spot on the TLC plate (being the only UV absorbing species) is mainly produced by the drug. It is possible that the effective plume (containing the drug substance) is really a narrow jet burst rather than an expanding conical spray. Thus, the drug substance might be denser, to some extent, in the center of the plume cross section than at its outer zone. As more puffs are sprayed onto the TLC plate the concentration of the drug in the outer zone now increases and contributes to the observable diameter of the spot. This is consistent with the increase in the diameter of the TLC spot with increasing number of puffs sprayed.

The shapes of the spots (outlines) produced from the aerosol unit using four different experimental actuators are shown in Fig. 4. Using the regular actuator (actuator 1) a circular spot was produced. The modified actuators produced distinctly distorted spots. Actuator 3 with the largest orifice produced a slightly oval or kidney-shaped spot. Actuators 2 and 4 gave crescent-shaped spots indicating deflection of the spray from the actuator nozzle due to its displacement from the center. This information indicates that the TLC method is equal or better than photography in detecting defects in the size and shape of the actuator orifice. Moreover,

Table II—Diameters of the TLC Spots Produced by Spraying Various Puffs of the Inhalation Aerosol at Various Distances from the Actuator

Distance from the Actuator, cm	Mean Diameter[a] ± *SD*, mm				
	1 Puff	2 Puffs	3 Puffs	5 Puffs	10 Puffs
1.7	12.3 ± 0.37 (6)	14.3 ± 0.74 (6)	17.4 ± 0.64 (6)	20.4 ± 1.5 (10)	23.3 ± 1.85 (10)
2.8	11.9 ± 0.50 (6)	15.38 ± 1.13 (6)	17.57 ± 1.61 (6)	21.6 ± 1.8 (10)	
3.5	9.85 ± 0.22 (6)	13.8 ± 0.76 (6)	16.75 ± 0.69 (6)		
3.7	11.12 ± 0.88 (14)	15.0 ± 1.1 (12)	17.5 ± 1.25 (28)	24.3 ± 1.6 (10)	29.35 ± 2.17 (10)
5	7.6 ± 0.53 (6)	11.83 ± 0.75 (6)	16.36 ± 0.66 (6)	23.9 ± 1.3 (10)	30.7 ± 2.22 (10)
6	9.54 ± 0.48 (10)	12.6 ± 1.46 (12)	16.3 ± 1.1 (30)	24.8 ± 1.8 (10)	
7.5	5.92 ± 0.24 (6)	10.46 ± 0.32 (6)	15.2 ± 0.58 (6)	25.0 ± 1.7 (10)	33.27 ± 1.69 (10)

[a] The mean includes height and width for all the spots. The number of measurements is given in parentheses.

Table III—Effect of Distance Between the TLC Plate and Actuator on the Dimension and Drug Contents of the TLC Spot [a]

Distance from the Actuator, cm	Drug Recovered		Dimensions of Spot [b]	
	Mean, μg	Recovery, %[c]	Diameter, mm	Surface Area, mm²
1.7	671	89	17.43	238
3.7	633	85	17.50	240
5.0	542	72	16.36	210
6.0	538	72	16.30	209
7.5	446	60	15.20	181

[a] Spots produced by spraying three puffs from the aerosol unit onto the TLC plate. [b] Mean measurements of 3–15 spots. [c] Based on 250 μg/puff.

Table IV—Effect of the Number of Puffs Used on the Dimension and Drug Contents of the TLC Spots [a]

Number of Puffs	Drug Recovered		Dimensions of Spot [b]	
	Mean, μg	Recovery, %[c]	Diameter, mm	Surface Area, mm²
1	240	96	12	113
2	425	85	13.5	143
3	664	89	16.4	211
5	1207	97	20.2	322
10	2402	96	26	531

[a] Spots produced by spraying various puffs from the aerosol unit onto a TLC plate at a distance of 3 cm from the actuator. [b] Mean measurements of three spots. [c] Based on 250 μg/puff.

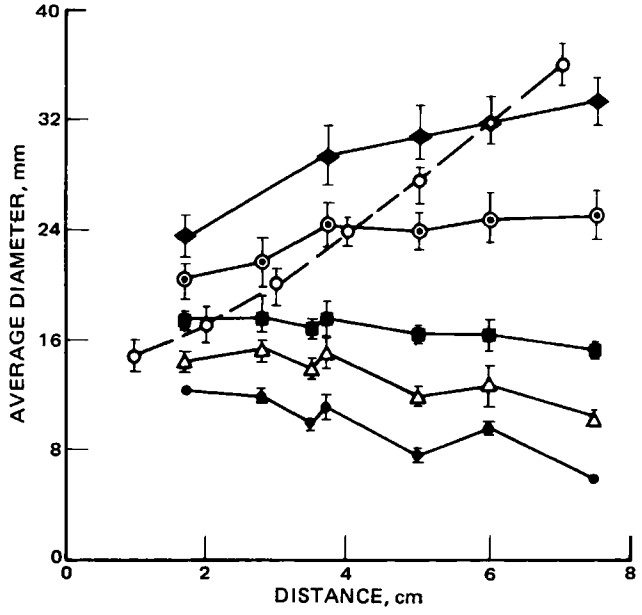

Figure 3—*TLC spot size and cross section of the aerosol plume as a function of distance from the actuator and number of puffs sprayed. Key: (○) cross section of plume by photography; diameter of the TLC spot using (●) one puff, (△) two puffs, (■) three puffs, (⊙) five puffs, and (◆) 10 puffs.*

the TLC method being specific for the drug substance itself provides direct information about the spray pattern of the latter rather than the propellant or solvent.

Correlation of Drug Contents and Dimensions of the TLC Spot—The contents of the drug substance in the TLC spot produced by spraying three puffs of the aerosol at various distances between the TLC plate and the actuator were quantified using blue tetrazolium colorimetry. The data are presented in Table III, and the correlation of the surface area of the spot with the drug recovered is shown in Fig. 5. The data indicate that the surface area of the spot appears to be a good indicator of the drug content in various sections of the observed plume. Table IV shows the recoveries of drug substances from TLC spots that were produced by spraying various puffs of aerosol from a distance of 3 cm from the TLC plate. The recovery of the drug appears to have a reasonably linear correlation (Table V) with the number of puffs, as shown in Fig. 6. This indicates that the drug is quantitatively deposited in the observed spot on the TLC plate under the conditions of the plume geometry test.

Usually a portion of the drug emitted from the valve deposits on the inside of the actuator and is unavailable to the patient. The medication delivered to the patient therefore, is, the amount of drug actually available at the mouthpiece of the actuator. This and other delivery characteristics of the regular (actuator 1) and the defective (actuators 2, 3, and 4) actuators were compared utilizing an air sampler. The air sampler consisted of seven stages. These stages can separate the aerosol particles into seven fractions ranging from an 11.0 to a 0.44-μm aerodynamic diameter. This sampling device can be used as an *in vitro* model for the respiratory tract. As such it simulates, to a reasonable extent, the distribution and retention of inhaled drug particles in the human lungs. The total recovery of the drug, medication delivered to the patient, and percent of drug deposited on the actuator, the tubular throat, and various stages of the air sampler are presented in Table VI. The total recovery of the drug substance was about the same for each actuator. The percent of drug substance deposited on the actuator was considerably higher using the defective actuators; consequently, the amount of medication delivered to the patient was markedly less for these defective actuators. These results are in excellent agreement with those obtained using the TLC method to define the spray pattern. Actuators 2, 3, and 4 produced distorted spots (Fig. 4), and therefore, it was expected that a greater portion of the drug emitted from the aerosol unit in these cases would be deposited on the actuator.

Another parameter of interest was the particle size distribution of the drug substance in the emitted aerosol. This is important since the indi-

Table V—Linear Correlation of Drug Recovered from the TLC Spot with the Surface Area of the TLC Spot and the Number of Puffs Sprayed

Parameters Correlated	Slope ± 90% Confidence Limits	Intercept ± 90% Confidence Limits	Standard Error of Regression	Correlation Coefficient
Drug contents and surface area of the spot [a]	0.270 ± 0.094	62.5 ± 54.0	5.2	0.98260
Drug content of the spot and number of puffs sprayed [b]	244 ± 0.0	−37.1 ± 0.0	82.4	0.99555

[a] From data in Table III and Fig. 5. [b] From data in Table IV and Fig. 6.

Table VI—Various Drug Delivery Parameters [a] of the Aerosol Unit Using Various Actuators

Actuator	Total Recovery, %[c]	Amount Delivered to Patient, %[d]	Drug Substance Recovery, %[b]			
			Actuator	Throat, Sampler Top, Stage 0 and Stage 1	Stages 2 and 3[e]	Stages 4–6[f]
1	109.0 ± 1.1	98.7 ± 2.0	23.0 ± 1.0	50.7 ± 0.6	21.0 ± 1.0	5.6 ± 0.6
2	105.6 ± 4.0	78.0 ± 4.5	37.6 ± 1.1	30.3 ± 2.1	25.0 ± 1.0	7.0 ± 0.0
3	110.3 ± 4.5	85.3 ± 4.5	34.3 ± 1.2	57.0 ± 2.6	6.3 ± 2.8	3.0 ± 0.0
4	110.3 ± 6.4	90.3 ± 6.6	30.6 ± 1.5	46.7 ± 2.3	16.6 ± 1.5	6.0 ± 0.0

[a] Average of three determinations. [b] Drug substance recovery on various stages of an air sampler. [c] Assuming 294 μg/puff. [d] Assuming 250 μg/puff. [e] Estimate to represent aerodynamic particle size in the range of 3.3–7.0 μm. [f] Estimate to represent aerodynamic particle size <3.3 μm.

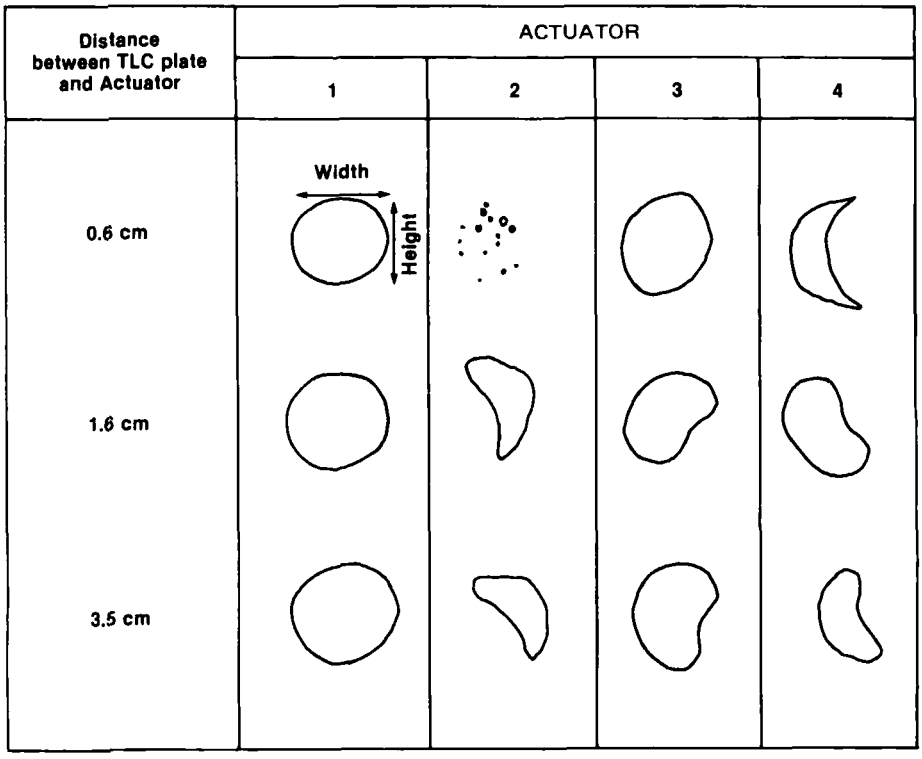

Figure 4—*Spot shapes produced by spraying from the aerosol unit on a TLC plate using the various actuators.*

vidual drug particles are destined for delivery to the lung, and efficiency of the bronchial delivery is dependent on the effective aerodynamic particle size. It was suggested that the effective aerodynamic particle size of drugs for delivery into the lung is <5 μm (17). The percent of drug substance deposited on the various stages of air sampler is included in Table VI. The effective aerodynamic size of particles deposited on stages 2–6 of the air sampler is considered to be <7 μm[9]. Actuator 3 delivered substantially fewer particles <7-μm aerodynamic size because of higher depositions on the throat and actuator. When actuator 2 was used a crescent-shaped spot was observed on the TLC plate (Fig. 4), and the largest amount of the drug substance was deposited on the actuator while the least amount was deposited on the throat. However, this did not affect the amount of particles expected to reach the lungs. Actuator 4, which also yielded a crescent-shaped spot on the TLC plate, showed slightly lower recoveries of particles <7 μm due to larger deposit on the actuator (compared with actuator 1) as well as on the throat stage (compared with actuator 2).

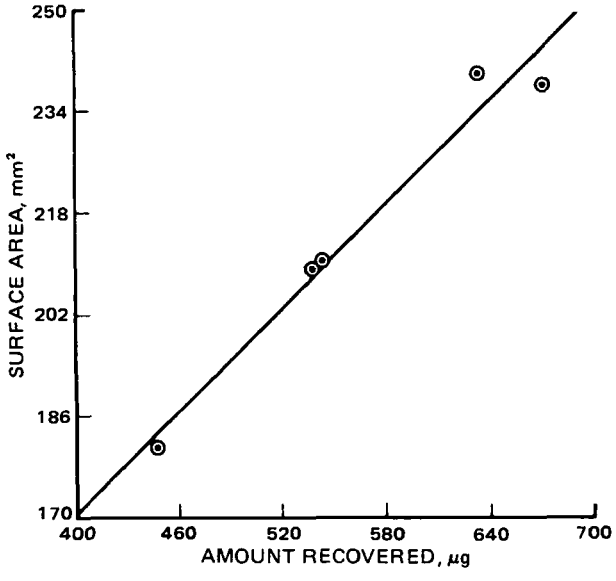

Figure 5—*Correlation of surface area and drug substance content recovered from TLC spots produced by spraying three puffs from the aerosol unit.*

[9] "Particle Fractionating Sampler Bulletin 176-3" Andersen 2000 Inc., Atlanta, Ga.

CONCLUSIONS

The TLC method for characterization of the spray pattern of inhalation aerosols is specific for the drug substance. Therefore, this method provides direct information about the distribution of the drug particles in the spray rather than the general pattern of the propellant or solvent in the spray. Such information is extremely useful in cases of heterogeneous aerosols. In addition, the TLC method is capable of detecting defects in the size and shape of the actuator orifice. Thus, the TLC technique, because of its specificity, simplicity, and speed, can be used as a good incoming quality control tool to evaluate actuators and valve performances.

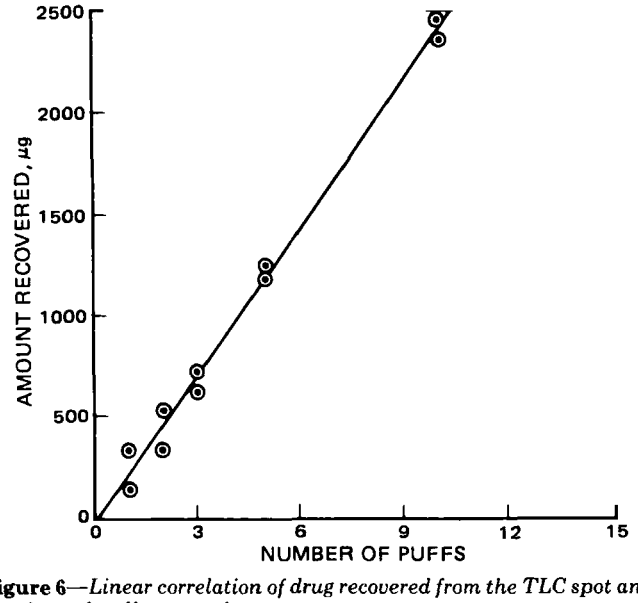

Figure 6—*Linear correlation of drug recovered from the TLC spot and numbers of puffs sprayed.*

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ACKNOWLEDGMENTS

The authors acknowledge Dr. R. Jones' and Mrs. G. Harringer's contributions in adapting the Andersen Air Sampler to determine particle size distribution of the corticosteroid in the inhaled aerosol.

Esterase-Like Activity of Human Serum Albumin II: Reaction with *N-trans*-Cinnamoylimidazoles

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Received March 1, 1982 from the *Faculty of Pharmaceutical Sciences, Nagoya City University, Tanabe-dori, Mizuho-ku, Nagoya, 467, Japan.* Accepted for publication September 17, 1982.

Abstract □ To elucidate the details of the esterase activity of human serum albumin, the reaction of *N-trans*-cinnamoylimidazoles with albumin was investigated kinetically at various pHs at 25°. The reaction consisted of the acylation of albumin (probably the tyrosine-411 residue) by the substrate and the deacylation of cinnamoyl-albumin. The acylation was ~10–100-fold faster than the spontaneous hydrolysis of the substrate over the pH range examined. The pH profile for the deacylation rate constant indicated the participation of a group having a pK_a of ~9.4. The deacylation was subjected to the effect of deuterium oxide. The electron-withdrawing substituent facilitated the deacylation; the Hammett ρ value was 1.63. These results suggest that the deacylation proceeded *via* general base catalysis by this group.

Keyphrases □ Albumin, human serum—esterase-like activity, acylation with *N-trans*-cinnamoylimidazoles, kinetics □ Cinnamoylimidazoles—acylation of albumin, kinetics □ Kinetics—acylation of albumin with *N-trans*-cinnamoylimidazoles at the tyrosine-411 residue binding site

Studies involving the binding of drugs to human serum albumin are pharmacologically and clinically important, since this binding influences the *in vivo* distribution, availability, and elimination of drugs (1). Localization of drug binding sites on the albumin molecule and the classification of drugs with respect to the binding sites can be used to predict the displacement of one drug by another when two or more drugs are administered concurrently (2). It was reported previously that albumin exhibits esterase activity toward phenyl esters (3, 4). The active site involved when the substrate is *p*-nitrophenyl acetate was found to be one of the most important drug binding sites on albumin, and was named the R site (5–7). The R site is located near the reactive tyrosine-411 residue (5, 8–12). Previous studies (5–7) examined the inhibition of the esterase activity by several drugs, and led to the classification and identification of the various binding sites involved.

The details of the esterase activity of albumin have not been described. The activity of albumin toward amide substrates as well as the mechanisim of the deacylation of the acyl-albumin are not known. Since many drugs possess the amide linkage, we have investigated the activity of

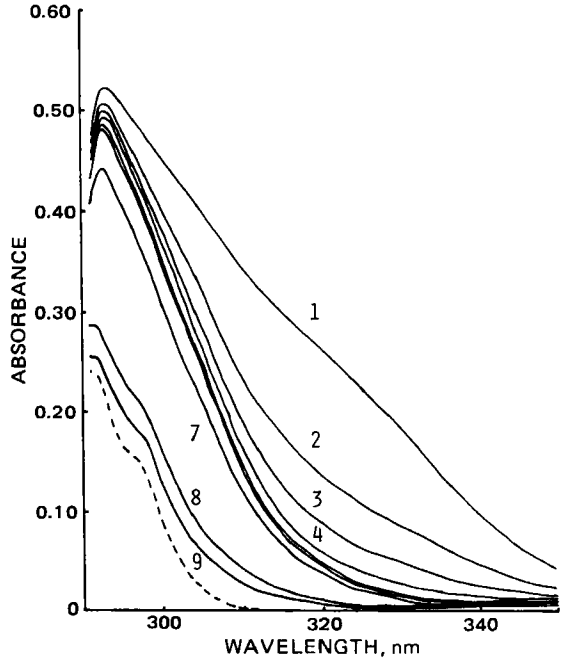

Figure 1—*Periodic difference spectrum of the reaction mixture (albumin + I)* versus *albumin at pH 8.41 and 25°. The dotted line is the spectrum of the mixture of* trans-*cinnamic acid and imidazole. Key: (1) 1.0, (2) 3.0, (3) 5.0, (4) 9.0, (5) 30, (6) 60, (7) 240, (8) 1440, and (9) 2580 min. The initial concentrations of I and albumin are 4.00 × 10^{-5} M and 2.00 × 10^{-4} M, respectively.*

0022-3549/83/0400-0385$01.00/0