(13) A. R. Midgley, Jr., R. W. Rebar, and G. D. Niswender, *Acta Endocrinol. Suppl.*, **142**, 247 (1969).
(14) F. C. Greenwood and W. M. Hunter, *Biochem. J.*, **89**, 114 (1963).
(15) G. D. Niswender and A. R. Midgley, in "Immunologic Methods in Steroid Determination," F. G. Peron and B. V. Caldwell, Eds., Plenum, New York, N.Y., 1970, pp. 149–173.
(16) D. Rosi, H. C. Neumann, R. G. Christiansen, H. P. Schane, and G. O. Potts, *J. Med. Chem.*, **20**, 349 (1977).
(17) G. E. Abraham, *J. Clin. Endocrinol. Metab.*, **29**, 866 (1969).
(18) G. E. Abraham, *Acta Endocrinol. Suppl.*, **183**, 1 (1974).
(19) G. W. Snedecor, *Biometrics*, **8**, 85 (1952).

ACKNOWLEDGMENTS

The authors thank M. Graham, S. Thorpe, and C. Barnick for technical assistance. They are also grateful to R. G. Christiansen and his associates for providing samples of related steroids.

Simulated Respiratory System for *In Vitro* Evaluation of Two Inhalation Delivery Systems Using Selected Steroids

JOHN J. SCIARRA ˣ and ANTHONY CUTIE

Received November 29, 1976, from the *Arnold and Marie Schwartz College of Pharmacy and Health Sciences of Long Island University, Brooklyn, NY 11201.* Accepted for publication February 7, 1978.

Abstract □ A simulated respiratory system was developed for the *in vitro* evaluation of two differently designed oral inhalation delivery systems. The deposition properties of a newly designed delivery system used for triamcinolone acetonide were compared to the more conventional, commercially available adapter utilized for an aerosol containing beclomethasone dipropionate. The simulated respiratory system was constructed so that the delivered dose of active ingredient could be classified into two fractions: the fraction that would be deposited in the oral cavity and throat and the fraction that would reach the desired site of activity in the respiratory tract. Based on this method, the newly designed system delivered more than 95% of the labeled dose to the desired site. The beclomethasone dipropionate aerosol system, which was observed to discharge the active ingredient with a greater intensity, delivered approximately 40% of the labeled dose. The particle-size distribution of the dose dispensed from the newly designed delivery system attached to the triamcinolone acetonide aerosol was determined using an impactor technique. No effort was made to correlate these results with an *in vivo* response.

Keyphrases □ Respiratory system, simulated—developed for *in vitro* evaluation of inhalation delivery systems □ Delivery systems, inhalation—evaluated *in vitro* using simulated respiratory system □ Inhalation delivery systems—evaluated *in vitro* using simulated respiratory system

Oral aerosol products used by inhalation are intended to deliver the active ingredient in the desired particle-size range to the proper portion of the respiratory system. In many instances, however, a fairly high percentage of formulation is retained in the oral adapter, mouth, and back of the throat instead of obtaining the desired deep penetration into the bronchi. This problem was discussed by Hayton (1). Since this loss is fairly consistent, many commonly used aerosol products contain an increased amount of active ingredient in the formulation so that the proper dose is delivered to the desired site of activity.

Various methods are useful for determining the dose of an aerosol delivered through an oral adapter in the desired portions of the lungs. Karig *et al.* (2) developed a compartmentalized lung chamber that could simulate different sized lung bronchi. They established an *in vitro* method for evaluating the penetration of solid particles in the lungs using several different aerosol generators. An air flow rate, based on a vacuum of 30.4 cm Hg, could be used to evaluate several different types of aerosols. The chamber size was based on literature values for the size of the human respiratory tract. Both solution- and suspension-type aerosols were studied, and it was concluded that it was possible to compare solution aerosols to suspension aerosols and to determine differences in their deposition.

The purpose of this study was to develop a suitable method for evaluating various oral adapters. In particular, the method should be capable of classifying the dispensed dose into a fraction that would be trapped in the oral cavity (mouth) and throat and a fraction that would reach the lungs. An artificial or simulated respiratory system was designed and constructed. A newly developed, different type of oral adapter, which may be capable of delivering a greater percentage of the active ingredient to the desired site, was attached to an aerosol product containing triamcinolone acetonide as the model drug and evaluated using the simulated respiratory system. For comparison, an aerosol product using a conventional oral adapter was also evaluated using the simulated respiratory system.

EXPERIMENTAL

Design of Simulated Respiratory System—The simulated respiratory system (Fig. 1) was made of glass and was scaled to twice the relative dimensions of a normal respiratory system. It was designed to follow the normal structure of the respiratory system. The entrance to the simulated respiratory system opened into an area similar to the oral cavity. This area then opened into three separate chambers: the chamber above the opening represented the nasal passageway; another opening, below and to the left, represented the esophagus; and the third opening represented the entrance to the lungs. Removable ground-glass stoppers were fitted at the opening located at the base of the unit (esophagus) and to the simulated nasal passageway.

The simulated respiratory system was then fitted to a modified particle-size impactor[1] capable of classifying particles from 0.5 to 32 μm. However, the six stages of the impactor were removed, and only the last stage (0.5-μm size) was used. The portion of the simulated respiratory system representing the opening to the lungs was fitted to the opening of the impactor, and a vacuum was applied through this "lung" opening.

[1] Cascade impactor, model CI-S-6, Scientific Advances, Columbus, Ohio.

0022-3549/78/1000-1428$01.00/0

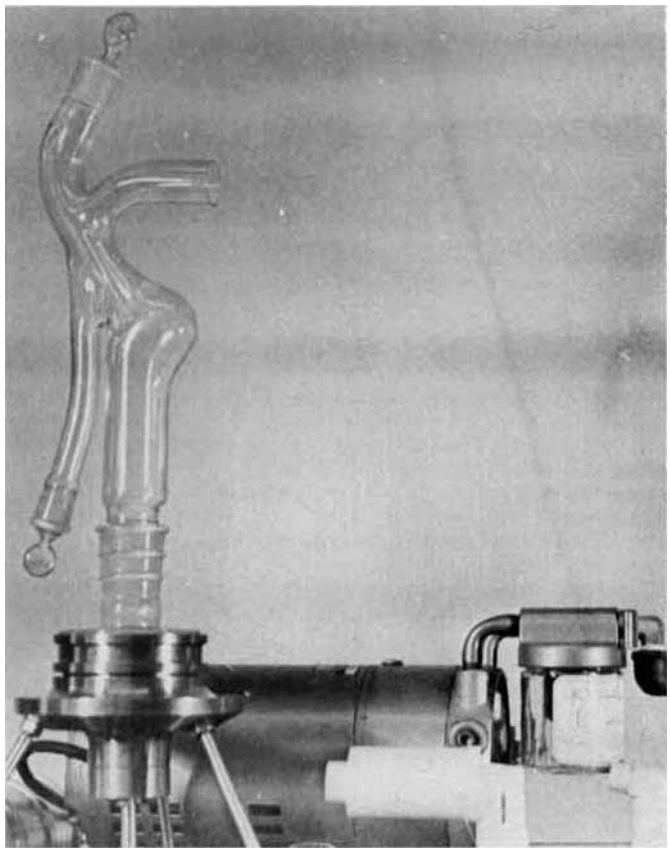

Figure 1—*Simulated respiratory system.*

A vacuum pump was connected to the impactor by a "T" valve (Fig. 1).

For this study, an air flow equivalent to a vacuum of about 75 mm Hg (19.8 liters/min) was utilized to simulate normal inspiration pressure (3, 4). Air flows equivalent to a vacuum of 50 mm Hg (16.9 liters/min) and 25 mm Hg (13.2 liters/min) also were utilized to determine the effect of air flow upon the dispensing characteristics of the product as it flowed through the oral adapter. A glass fiber filter was fitted onto the base of the simulated respiratory system so that all material reaching this point was collected. Spectrophotometric analysis of the material on this filter indicated the amount of active ingredient assumed to reach the desired site of activity.

Evaluation of Newly Designed Oral Adapter—An aerosol product containing triamcinolone acetonide[2] was fitted with an oral adapter (Fig. 2). This adapter consists of a mechanical breakup fitting through which the dose is dispensed into a relatively large chamber and allows for the expansion of the liquefied gas propellant and dispersion of the active ingredient. The product exits through a narrow opening intended to be placed into the patient's mouth. In this study, this opening was placed into the entrance of the simulated respiratory system.

The methodology used in studying the two aerosol products was varied to some extent to compensate for differences in oral inhalation delivery systems and in the apparent solubility of the steroids in the methanol–water solutions.

Assay for Triamcinolone Acetonide—A standard UV spectral curve for triamcinolone acetonide was prepared by dissolving triamcinolone acetonide, 97.2% purity (used as reference standard), in a 6% methanol–water solution (spectroanalytical grade). Six dilutions of this solution were made to obtain varying concentrations of triamcinolone acetonide. The solutions were read[3] at 237 nm. A linear curve was obtained indicating compliance with the Beer–Lambert law.

Triamcinolone Acetonide Delivered through Valve Stem (No Oral Adapter)—The amount of triamcinolone acetonide delivered through the valve stem was determined by making an incision across the plastic valve stem (notch) and inverting the unit into 6 ml of methanol contained in a glass beaker. The aerosol unit was then actuated 3, 6, 9, 12, and 18 times (at least 1-min intervals between actuations) into the methanol, which was then diluted with distilled water to 100 ml. The propellant was allowed to escape from the solution, and each solution was read[3] at 237 nm.

Table I—Triamcinolone Acetonide Actuation Delivered through Simulated Respiratory System at Various Air Flows [a]

Number of Actuations	Amount Delivered to Simulated Respiratory System		Amount Retained in Simulated Oral Cavity		Amount Retained in Oral Inhalation Adapter	
	±2 μg	±4% [b]	±2 μg	±4% [b]	±2 μg	±4% [b]
			At 75 mm Hg			
6	105	52.5	—[c]	—[c]	98	49
12	100	50.0	—[c]	—[c]	—	—
18	100	50.0	—[c]	—[c]	—	—
			At 50 mm Hg			
9	96.6	48.3	3.4	1.7	100	50
			At 25 mm Hg			
9	94.3	47.2	5.7	2.8	100	50

[a] Average of three determinations. [b] Calculated on the basis of 200 μg/actuation delivered through valve. [c] Negligible amount, could not be determined.

Triamcinolone Acetonide Delivered through Oral Adapter—Before proceeding with this determination, a sample of triamcinolone aerosol was dispensed directly onto the glass fiber placed over the lowest stage of the impactor (0.5-μm stage). The aerosol product was fitted with a regular aerosol actuator, and the product was actuated 6, 12, and 18 times directly onto the glass fiber filter. A vacuum of 75 mm Hg was used to collect all active ingredients and to allow for complete vaporization of the propellant. The glass fiber filter was then placed into 6 ml of methanol and gently swirled to aid extraction of the active ingredient. The solution was diluted to 100 ml with distilled water and read at 237 nm. This procedure also was carried out in a vacuum of 400 mm Hg.

The amount of triamcinolone acetonide delivered through the newly designed oral adapter was determined by dispensing 6, 12, and 18 doses directly onto the lowest stage of the impactor fitted with a glass fiber filter paper, as previously described. A vacuum of 75 mm Hg was used. The filter paper was then removed and placed into 6 ml of methanol, and the mixture was swirled to aid extraction. The solution was diluted to 100 ml with distilled water and read at 237 nm. The impactor base was rinsed with 6 ml of methanol, diluted to 100 ml with distilled water, and read at 237 nm. The amount of steroid found on the base was added to the amount collected on the glass fiber.

The simulated respiratory system was then placed over the lower stage of the impactor, and the procedure was repeated by dispensing the triamcinolone aerosol through the simulated respiratory system. Six, 12, and 18 doses were dispensed into the simulated respiratory system at a vacuum of 75 mm Hg. The material collected in the glass fiber filter was determined using the spectrophotometric procedure. The oral adapter was rinsed with methanol, and the amount of active ingredient was determined spectrophotometrically. To determine any possible effect of

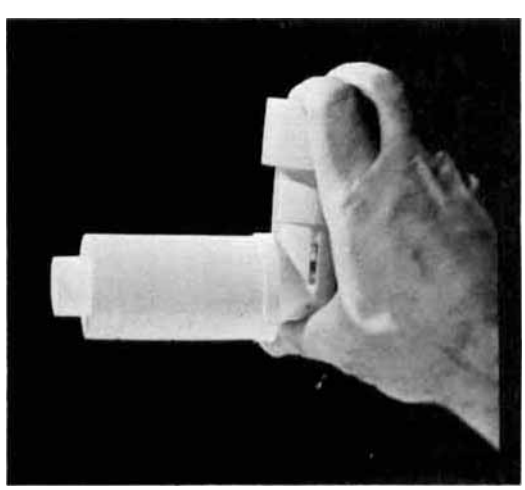

Figure 2—*Newly designed aerosol oral adapter.*

[2] Aristocort Aerosol, Lederle Laboratories, Pearl River, N.Y.
[3] Spectronic 200, Bausch & Lomb Optical Co., Rochester, N.Y.

Table II—Beclomethasone Dipropionate Actuation Delivered through Simulated Respiratory System at Various Air Flows [a]

Number of Actuations	Amount Delivered to Simulated Respiratory System		Amount Retained in Simulated Oral Cavity	
	±2 μg	±4% [b]	±2 μg	±4% [b]
	At 75 mm Hg			
15	20.90	41.8	29.10	58.2
20	20.32	40.6	29.68	59.4
30	18.75	37.5	31.25	62.5
	At 50 mm Hg			
15	22.0	44.0	28.0	56.0
20	21.5	43.0	28.5	57.0
30	20.6	41.2	29.4	58.8
	At 25 mm Hg			
15	16.7	33.4	33.3	66.6
20	18.8	37.6	31.2	62.4
30	20.6	41.2	29.4	56.8

[a] Average of three determinations. [b] Calculated on the basis of a labeled amount of 50 μg/actuation. From 2.9 to 3.5% of active ingredient remained trapped in the oral adapter.

air flow through the simulated respiratory system, the procedure was repeated at vacuums of 50 and 25 mm Hg.

Triamcinolone Acetonide Delivered through Conventional Actuator—The newly designed oral adapter was removed from the aerosol, and the valve stem was fitted with a conventional actuator. Nine doses of the aerosol were dispensed into the simulated respiratory system attached to the impactor, and the amount of triamcinolone acetonide delivered was determined spectrophotometrically as previously indicated.

Evaluation of Conventional Oral Adapter—A commercially available aerosol containing beclomethasone dipropionate fitted with a conventional oral adapter[4] was used for comparison with the newly designed oral adapter.

Assay for Beclomethasone Dipropionate—A standard UV spectral curve, using a reference sample of beclomethasone dipropionate (98.0%) in 50% methanol–water, was established at 239 nm. Beer–Lambert curves were prepared using 6 and 12% methanol–water solutions, but subsequent studies showed that these percentages of alcohol were inadequate to dissolve appreciable quantities of beclomethasone dipropionate.

Beclomethasone Dipropionate Dispensed through Valve Stem—The procedure described for triamcinolone acetonide was used for this determination. The delivery of the labeled amount (50 μg/actuation) was verified by making an incision across the plastic dip tube, inverting the aerosol, and actuating the metered valve 15, 20, 30, and 60 times into 50 ml of methanol, which was subsequently diluted to 100 ml with distilled water. The propellant was allowed to vaporize, and the solutions were read spectrophotometrically at 239 nm.

To verify that the propellant was not solubilized and contributing to the total absorbance of these solutions, the beclomethasone dipropionate aerosol was actuated for 15, 20, 30, and 60 times into a 1000-ml Florence flask that had its neck removed so atomization could be accomplished with minimum loss of active ingredient. The contents of the flask were

Table III—Dose of Triamcinolone Acetonide Dispensed through Conventional Actuator and Reaching Simulated Respiratory System [a]

Vacuum, mm Hg	Retained Dose in Simulated Respiratory System (Esophagus Portion), %	Dose Reaching Simulated Respiratory Portion, %	Average, %
75	47.5	52.5	—
75	51.6	48.4	—
75	40.1	59.9	53.6
50	46.6	53.4	—
50	49.9	50.1	—
50	48.3	51.7	51.7
25	44.9	55.1	—
25	46.6	53.4	—
25	48.3	51.7	53.4

[a] Average of two determinations.

[4] Becotide Aerosol, Allen and Hanburys, Ltd., London, England.

Table IV—Amount of Triamcinolone Acetonide Delivered through Valve Stem [a]

Number of Actuations	Absorbance at 237 nm	Triamcinolone Acetonide per Actuation, μg	Percent Recovered per Actuation [b]
3	0.21	210	105.0
6	0.41	205	102.5
9	0.59	197	98.5
12	0.78	195	97.5
18	1.20	200	100.0

[a] Average of two determinations. [b] Label indicated 200 μg of triamcinolone acetonide/actuation.

removed with 50 ml of methanol, diluted to 100 ml with distilled water, and read spectrophotometrically at 239 nm.

Beclomethasome Dipropionate Delivered through Simulated Respiratory System—The beclomethasone dipropionate aerosol was then actuated 15, 20, and 30 times through the entire simulated respiratory apparatus at air flows equivalent to vacuums of 25, 50, and 75 mm Hg. The material collected in the simulated esophagus was removed by washing with 50 ml of methanol. The 50 ml of methanol containing the active ingredient was diluted to 100 ml with distilled water and read at 239 nm. The oral adapter was also assayed for active ingredient by rinsing with methanol as previously indicated.

RESULTS AND DISCUSSION

The results indicate the applicability of the simulated respiratory system in determining the efficiency of certain oral inhalation adapters. The simulated respiratory system constructed was scaled to twice the relative dimensions of a normal respiratory system to accommodate the oral adapter from the aerosol product as well as to allow for a greater atomization and dispersion of the sample. The system was designed to follow the normal structure of the respiratory system and, in particular, the upper respiratory tract (nasal passage), the back of the mouth, and the esophagus. In this way, the system was capable of separating the dispensed dose into two fractions: the fraction trapped in the simulated oral cavity and throat and the fraction reaching the simulated desired site.

Of particular interest is the fact that the particles emitted into the simulated respiratory system can be classified into two broad ranges. These ranges include those particles small enough to be carried by the airstream into the respiratory system and those particles that may be trapped in the back of the throat. The latter may be due to the particle size itself or to the velocity with which the spray is emitted through the oral inhalation adapter.

In this simulated respiratory system, the entrance into the respiratory system is separated from the opening to the esophagus. It is anticipated that material adhering to the back of the unit would collect in the simulated esophagus area rather than be transferred to the area having the direct opening to the lungs. By attaching the simulated respiratory system to a modified particle-size impactor, a uniform air flow would be obtained through the simulated respiratory system when a vacuum is applied to the outlet of the impactor. By adjusting the vacuum at several different levels, varying air flows through the simulated respiratory system could be achieved. This system also allowed for the determination of the effect of inhalation pressure on the amount of dispensed dose collected in each fraction since these determinations could be carried out at varying pressures.

The glass simulated respiratory system allowed for the classification of particles (Tables I and II). To evaluate this system further, two aerosol inhalation products were studied. The triamcinolone acetonide aerosol

Table V—Dose of Triamcinolone Acetonide per Actuation Delivered through Oral Inhalation Adapter [a]

Number of Actuations	Absorbance at 237 nm	Triamcinolone Acetonide Delivered per Actuation, μg	Percent Recovered per Actuation [b]
6	0.20	100.0	100.0
12	0.39	97.5	97.5
18	0.60	100.0	100.0

[a] Average of three determinations at vacuum of 75 mm Hg. [b] Label indicated that 100 μg/actuation would be delivered through the oral inhalation adapter.

Table VI—Amount of Beclomethasone Dipropionate Delivered through Valve Stem [a]

Number of Actuations	Absorbance at 239 nm	Beclomethasone Dipropionate per Actuation, μg	Percent Recovered per Actuation [b]
15	0.250	52.1	104.2
20	0.535	52.5	104.6
30	0.490	51.0	102.0
60	0.975	49.8	99.8

[a] Average of two determinations. [b] Label indicated 50 μg of beclomethasone dipropionate/actuation.

was selected on the basis of the newly designed oral inhalation adapter used with the product. This adapter decreased spray velocity as it was emitted from the valve stem. Beclomethasone dipropionate aerosol was fitted with a conventional oral inhalation adapter and was studied to determine the usefulness of the simulated respiratory system in evaluating the dispensing characteristics of the two adapters.

The spray emitted from the newly designed oral adapter was of decreased velocity since very little, if any, of the droplets were trapped in the simulated esophagus portion of the simulated respiratory system (Table I). Approximately 100% of the delivered dose reached the simulated respiratory system. When the newly designed oral adapter fitted to the triamcinolone aerosol was replaced with a conventional actuator, only about 50% of the delivered dose reached the simulated respiratory system (Table III). These results clearly indicate that the newly designed adapter reduces the velocity of the emitted dose and allows the dose to be carried in the airstream to the simulated respiratory system. These values can be compared to approximately 58–62% of the delivered dose of beclomethasone dipropionate trapped in the simulated esophagus portion of the simulated respiratory system (Table II). With both products, the amount trapped in the simulated esophagus and oral cavity of the simulated respiratory system increased as the vacuum (air flow) was decreased from 75 to 25 mm Hg (Tables I and II).

Another important aspect in determining the effectiveness of the particles being delivered through an aerosol system is a determination of the amount of active ingredient remaining behind in the oral inhalation adapter. As can be seen from Table I, approximately 50% of the dose delivered through the valve stem (Table IV) actually was emitted through the newly designed oral inhalation adapter. This value of 50% retention in the adapter was consistent based on the analysis of at least 10 units. Table IV indicates the amount of triamcinolone dispensed through the valve stem (~200 μg), and Table V shows that approximately 100 μg of active ingredient actually was delivered through the oral inhalation adapter to the entrance to the simulated respiratory system. Approximately 50 μg of beclomethasone dipropionate was delivered through the valve stem when actuated directly into methanol (Table VI), and approximately the same amount was delivered through the commercially available adapter when actuated onto the Florence flask (Table VII).

Table VII—Recovery of Beclomethasone Dipropionate Delivered through Commercially Available Adapter Using Florence Flask as Collection Chamber [a]

Number of Actuations	Absorbance at 239 nm	Beclomethasone Dipropionate per Actuation, μg	Percent Recovered per Actuation [b]
15	0.225	46.9	97.8
20	0.325	50.8	101.6
30	0.490	51.0	102.0

[a] Average of three determinations. [b] Label indicated 50 μg of beclomethasone dipropionate/actuation.

Table VIII—Amount of Triamcinolone Acetonide Recovered from Glass Fiber Filter Paper [a]

Number of Actuations	Absorbance at 237 nm	Triamcinolone Acetonide per Actuation, μg	Percent Recovered per Actuation [b]
3	0.20	200	100.0
6	0.38	190	95.0
9	0.59	197	98.5
12	0.76	190	95.0
18	1.20	200	100.0

[a] Average of two determinations. Label indicated 200 μg of triamcinolone acetonide/actuation. [b] Similar results were obtained at vacuums of 75 and 400 mm Hg.

The reliability of the analytical procedures was determined in several ways (Tables VI and VIII). The values for the triamcinolone acetonide and beclomethasone dipropionate corresponded to the labeled quantities of active ingredient indicated for each product.

No essential change in the quantity of steroid extracted from the glass fiber filter was noted as the vacuum was varied (Table VIII). Therefore, it was assumed that the steroid did not escape through the collection filter at high pressures and that the steroid was not misdirected from the filter at lower pressures. A vacuum of 75 mm Hg was selected since it simulated the normal lung inspiration capacity. These results also indicated that there was no interference in the spectrophotometric reading by either the propellant or any methanol-soluble extractive from the filter paper.

CONCLUSIONS

On the basis of the results, this simulated respiratory system can be used to evaluate the deposition properties of the dose delivered through the oral inhalation adapter. The newly designed adapter delivered almost 100% of the dose dispensed from the adapter while the commercially available adapter delivered about 38–42% of the spray through the simulated respiratory system. This newly designed oral inhalation adapter can be useful in delivering a greater portion of the emitted dose to the desired area of the respiratory system.

REFERENCES

(1) W. L. Hayton, *J. Am. Pharm. Assoc.*, **NS 16,** 201 (1976).

(2) A. W. Karig, G. E. Peck, and G. J. Sperandio, *J. Pharm. Sci.*, **62,** 811 (1973).

(3) J. S. G. Cox, J. E. Beach, A. M. J. N. Blair, A. J. Clarke, J. King, T. B. Lee, D. E. E. Loveday, G. F. Moss, T. S. C. Orr, J. T. Ritchie, and P. Sheard, *Adv. Drug Res.*, **5,** 115 (1970).

(4) A. C. Guyton, "Textbook of Medical Physiology," 2nd ed., Saunders, Philadelphia, Pa., 1961, p. 517.

ACKNOWLEDGMENTS

Presented at the Industrial Pharmaceutical Technology Section, APhA Academy of Pharmaceutical Sciences, Orlando meeting, November 1976.