Development of an Aerosol Dosage Form Containing Insulin

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Abstract □ An aerosol dosage form containing insulin was developed by suspending insulin zinc crystals in fluorocarbon propellant and using oleyl alcohol to improve the insulin suspension and to prevent valve clogging. A metered valve was used to control the amount of insulin delivered per actuation. The dose dispensed, sedimentation rate, and particle-size distribution of the aerosol formulation were evaluated. The potency of the insulin delivered from the aerosol containers stored at various temperatures and time intervals was determined using a radioimmunoassay. Based upon preliminary stability data, which were treated kinetically, it was noted that insulin aerosols stored in a refrigerator at 7° would have a predicted shelflife of approximately 19 years, whereas samples stored at 25 and 37° would have shelflives of 11 and 2 months, respectively. Long-term stability studies are indicated to establish this conclusion. On the basis of this study, it was concluded that an insulin-containing aerosol dosage form can be formulated and that a suitable dose of insulin can be dispensed using commercially available metered dose valves. The actual dose of insulin dispensed would have to be related to the degree of absorption of insulin one would normally expect when insulin is administered by the inhalation route.

Keyphrases □ Dosage forms—aerosol, insulin zinc suspension in fluorocarbon propellant, evaluation of dose dispensed, sedimentation rate, particle-size distribution, and insulin potency □ Aerosols—insulin zinc suspension in fluorocarbon propellant, evaluation of dose dispensed, sedimentation rate, particle-size distribution, and insulin potency □ Insulin zinc—aerosol dosage form, evaluation of dose dispensed, sedimentation rate, particle-size distribution, and insulin potency

Insulin, the drug of choice for diabetes mellitus, has been administered parenterally since its discovery. Clinical evidence has indicated that insulin can be delivered by inhalation, bringing about a reduction in the blood sugar level. The use of a liquefied gas as a vehicle to deliver insulin in the proper particle size and accurate dosage would make inhalation therapy simple and convenient compared to the use of atomizers or nebulizers. Since an aerosol dosage form is a self-contained complete unit, it can be used by the diabetic patient with little difficulty and should be readily acceptable for insulin administration.

The mechanism and molecular basis of action and clinical aspects of insulin were discussed previously (1). Since proteolytic enzymes inactivate the insulin molecule (2), insulin is completely ineffective when taken orally; subcutaneous injection has been the preferred route of administration. The effectiveness of insulin administered by inhalation was reported; a midget inhaler with an adapter with a narrow nozzle smaller than the opening of the patient's nostril was used (3).

In an animal study, insulin was administered in the form of fine spray (by nebulizer) to normal and hyperglycemic dogs (4), and the aerosol-administered insulin was almost as effective as a subcutaneous injection. Clinical studies were reported on the aerosol administration of insulin to human volunteers using a nebulizer[1] (5). The results indicated a hypoglycemic response in nondiabetic patients while the patients with diabetes mellitus showed an increase in plasma immunoreactive insulin and a decrease in plasma glucose concentration. It was suggested that insulin was absorbed through the respiratory mucous membrane and produced the hypoglycemic activity. Low absorption efficiency of insulin was found by the inhalation route, and approximately 10% of the aerosolized insulin was recovered based on comparisons of plasma immunoradioinsulin (6).

Methods for the *in vivo* detection of insulin have been described in the USP and by various workers (7–9). Several chromatographic methods also were reported (10–16).

The immunochemical assay (17) provided for the first time a procedure that could be used for commercial insulin. Baum *et al.* (18) modified the immunoassay method of Grodsky and Forsham (17) and Probst *et al.* (19) and reported that the method was accurate, fast, inexpensive, and specific for natural insulin.

The effect of temperature on the stability of commercial insulin preparations was studied using both a radioimmunoassay and the USP bioassay; the potency, as determined by the radioimmunoassay, compared favorably with data obtained by the USP rabbit assay (20).

The development of a suitable formulation system for an aerosol dosage form containing insulin and the physicochemical evaluation of the dosage form were the major concerns of this study. The physical evaluation of the suggested formulations was undertaken to determine the suitability of the formulation for clinical studies. Sedimentation, dose delivery, and particle-size distribution were deemed to be important aspects. In addition, the stability of the preparation under various temperature conditions was determined using a radioimmunoassay method.

EXPERIMENTAL

Insulin zinc crystals[2] USP and amorphous insulin powder were used. Each aerosol insulin preparation was formulated by adding 0.3 g (1.5%) of the appropriate insulin to a 30-ml plastic-coated glass aerosol container. A nonmetered aerosol valve (used as a closure) was crimped onto the bottle, and 19.7 g of dichlorodifluoromethane–dichlorotetrafluoroethane (50:50)[3] was added through the valve by the pressure fill method. Each aerosol was gently shaken and stored in a refrigerator at 7° for 24 hr.

Each insulin preparation was evaluated for redispersibility. Although both the insulin zinc crystals and the amorphous insulin

[1] Devilbiss Co., Toledo, Ohio.
[2] Beef insulin, Eli Lilly and Co., Indianapolis, Ind.
[3] Propellant 12/114 (50:50).

Table I—Evaluation of Selected Propellant Systems

Ingredient	Formula, % (w/w)									
	A	B	C	D	E	F	G	H	I	J
Insulin zinc crystals	2.18	2.18	2.18	2.18	2.18	2.18	2.18	2.18	2.18	2.18
Oleyl alcohol	6.54	6.54	6.54	6.54	6.54	6.54	6.54	6.54	6.54	6.54
Dichlorodifluoromethane—dichlorotetrafluoroethane (15:85)	91.28	—	—	—	—	—	—	—	—	—
Dichlorodifluoromethane—dichlorotetrafluoroethane (40:60)	—	91.28	—	—	—	—	—	—	—	—
Dichlorodifluoromethane—dichlorotetrafluoroethane (50:50)	—	—	91.28	—	—	—	—	—	—	—
Dichlorodifluoromethane—dichlorotetrafluoroethane (70:30)	—	—	—	91.28	—	—	—	—	—	—
Dichlorodifluoromethane	—	—	—	—	91.28	—	—	—	—	—
Difluoroethane	—	—	—	—	—	91.28	—	—	—	—
Monochlorodifluoroethane	—	—	—	—	—	—	91.28	—	—	—
Dichlorodifluoromethane—difluoroethane (90:10)	—	—	—	—	—	—	—	91.28	—	—
Dichlorodifluoromethane—monochlorodifluoroethane (80:20)	—	—	—	—	—	—	—	—	91.28	—
Dichlorodifluoromethane—monochlorodifluoroethane (90:10)	—	—	—	—	—	—	—	—	—	91.28
Suspension time, sec[a]	35	45	50	60	75	ND[b]	25	ND[b]	65	85

[a] Time required before first sign of settling noted. [b] ND = not dispersable.

could be easily redispersed, the insulin zinc crystals were selected for further study because this form exhibited greater redispersibility than the amorphous insulin.

To improve the suspendability of the insulin formulation, oleyl alcohol was added as a dispersing agent. Oleyl alcohol was also necessary as a lubricant for the metered valve to prevent valve clogging and to obtain uniform metering of the insulin dose. Insulin zinc (0.65 g) was accurately weighed (the amount was based on the assumption that 40 USP units of insulin would be dispensed when 70 mg of formulation was delivered through the aerosol valve) and transferred to a plastic-coated aerosol bottle. Oleyl alcohol, at levels of 5, 6.5, and 20%, was then added.

A 50-mg pressure fillable valve was crimped onto the bottle, and the propellant was added by the pressure method. The aerosols were gently shaken and stored in a refrigerator at 7° for 24 hr. The formulations were evaluated as to redispersibility immediately and at 1-, 2-, and 4-week intervals. Based upon these observations, 6.5% of oleyl alcohol was found to be suitable since the redispersion time (time necessary for the supernatant liquid to reach a value of 50%) increased from an initial value of 2.5 min to 12 min after 4 weeks.

Based upon these results, 2.18% of insulin zinc crystals and 6.5% of oleyl alcohol were used to determine the best propellant combination and concentration. Additional aerosols were prepared as previously indicated, except that varying propellant systems were used (Table I). Each aerosol was evaluated on the basis of the time required for settling to occur (Table I).

Evaluation of Formulation—On the basis of the results shown in Table I, the following formulation was selected for further study: insulin zinc crystals[4], 2.18% (w/w); oleyl alcohol[5], 6.54% (w/w); and dichlorodifluoromethane–monochlorodifluoroethane (90:10)[6], 91.28% (w/w).

Twenty grams of this formulation was placed into a 30-ml plastic-coated glass aerosol container along with the insulin zinc previously dispersed in the oleyl alcohol. The containers were purged of air by adding a few drops of propellant[3] to each container. A metered valve[7] (without a dip tube), which was previously determined to deliver approximately 70 mg of formulation or 40 units of insulin per actuation, was crimped onto each container.

Forty-eight containers were prepared, divided into three groups, and stored upright at 7, 25, and 37°. The dosage dispensed, sedimentation rate, and particle-size distribution of each sample was determined.

Dose Dispensed—Three aerosol units were tested initially and the tests were repeated for each storage condition after 24 hr and at 1-month intervals as follows. Each aerosol unit was fitted with an actuator, primed several times, and placed into a water bath at 21 ± 0.5° for at least 30 min. It was then dried, accurately weighed, actuated, and reweighed to determine the dose dispensed per actuation.

Repetitive doses were dispensed every 3 min. Ten readings for each unit were obtained; the average of 30 readings obtained at 7, 25, and 37° are shown in Fig. 1.

Sedimentation Rate—The sedimentation rate for the aerosol formulation was determined in the following manner. The outside of the plastic-coated aerosol bottles was calibrated with 10 equidistant lines. The containers were gently inverted five times to redisperse the solid. The time for the suspensoid to settle from the filling line by one, two, or more gradations was recorded, that is, the time for 10, 20%, *etc.*, of the supernatant liquid to appear.

The initial sedimentation rate was determined on the aerosol insulin samples stored at 7° for 24 hr. Monthly observations were made on the samples stored at 7, 25, and 37°. Samples stored at 7° were tested immediately after being taken from the refrigerator; samples stored at 25 and 37° were tested after being in a water bath at 21° for 30 min. The results shown in Table II represent the average of three determinations on each of three containers.

Particle-Size Analysis of Insulin Aerosols—The particle-size distribution of the aerosol insulin was determined by an impaction technique[8]. The sampling chamber (21, 22) was made from a 1000-ml round-bottom flask with an opening extending 45° to the body of the flask. An opening of approximately 7.6 cm was made in the bottom of the flask. The neck of the flask was about 4.4 cm in

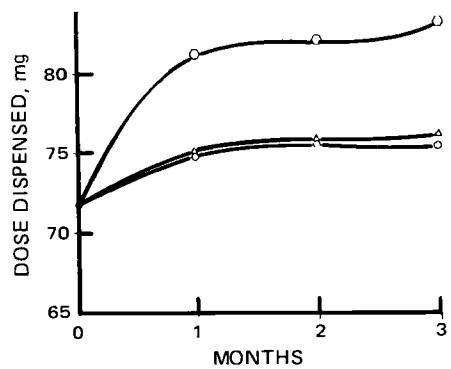

Figure 1—*Comparison of dose dispensed of insulin aerosols stored at different temperatures. Key:* ○, 7°; △, 25°; *and* ○, 37°.

[4] Lot 7AP06H, potency of 25.9 units/mg, Eli Lilly and Co.
[5] Adol 90, Lot 2237-M654-400, Ashland Chemical Co.
[6] Genetron 12/142b (90:10), Allied Chemical Co., Industrial Chemicals Division, New York, N.Y.
[7] B18F, 0.020 stainless steel stem, 50-mg housing, Neoprene gasket, VCA Inc., Bridgeport, Conn.
[8] Cascade impactor model DCI-6, Delron Research Products Co., Powell, Ohio.

Table II—Sedimentation of Aerosol Insulin Formulation at Varying Temperatures

Storage Time, Months	Supernatant Liquid, %	Time Required[a], min		
		7°	25°	37°
Initial	10	4.6	4.6	4.6
	20	5.7	5.7	5.7
	30	6.5	6.5	6.5
1	10	8.1	8.1	1.4
	20	13.6	2.3	1.7
	30	15.1	3.0	2.4
2	10	11.6	1.2	1.0
	20	15.3	1.5	1.5
	30	17.5	2.2	2.0
3	10	6.4	1.2	0.9
	20	9.5	1.6	1.3
	30	12.5	2.3	1.9

[a] Time required for 10%, 20%, *etc.*, of supernatant liquid to appear after gently shaking five times. Values are the averages of three determinations on each of three containers.

diameter, and its far end was slightly narrowed with an opening of 3.8 cm.

Samples were brought to ambient room temperature (23–25°). The impactor slides were accurately weighed and assembled in the impactor, and a vacuum of 44 cm Hg was applied. The weight of a sample was determined by weighing the aerosol container before and after removal of the sample.

Each container was fitted with an oral adapter, and the aerosol was actuated nine to 15 times into the sampling chamber. The time interval between each actuation was 20 sec so as to allow all of the sprayed sample to be drawn into the impactor. The vacuum was continued for 1 min after the last actuation and then slowly released. The glass slides were removed and accurately weighed to determine the difference in weight. This weight represented the amount of material deposited at each stage and is related to the particle size. The results shown in Table III represent the average of two determinations on each sample.

Preparation of Reagents for Radioassay—The dose of insulin delivered upon dispensing of the aerosol formulation stored at different temperatures and at intervals of 1, 2, and 3 months was determined by a radioimmunoassay[9]. Each kit contained the following reagents in 10-ml vials.

Buffer Solution—The buffer was dissolved in 200 ml of redistilled water and provided a pH 7.4 solution.

Dextran Gel[10]–Anti-Insulin Complex—The content of the vial was quantitatively transferred to a beaker containing a magnetic stirring rod. The vial was rinsed with 100 ml of the buffer solution in several portions, and the rinsings were added to the beaker with gentle and continuous stirring to form a homogeneous suspension.

Insulin Standard Solution—A lyophilized insulin standard, having an activity of 320 μunits/ml, was reconstituted in 4.0 ml of redistilled water.

Labeled Insulin Reagent—The lyophilized ^{125}I-insulin was reconstituted by adding 10 ml of the buffer solution.

Preparation of Standard Solution—The insulin standard solution was diluted with buffer to obtain suitable concentrations of insulin within the range of 3–320 μunits/ml.

Radioimmunoassay—*Dilution of Insulin Aerosol Formulation*—Serial dilutions of the aerosol formulation were required so that the strength of each dose would be within the range for the radioimmunoassay. A transfer device consisting of 13-gauge stainless steel tubing, 8.3 cm in length and affixed to an actuator, was used. A diluting solution of 0.005 *N* HCl at pH 2.5 and 0.1% bovine albumin was prepared.

The aerosol insulin preparations stored at 7, 25, and 37° were placed in a water bath at 21 ± 0.5° for 30 min. The samples were weighed and fitted with the transfer device. The aerosol was gently agitated and actuated once into approximately 200 ml of cold 0.0005 *N* HCl in a 300-ml beaker, which also contained a magnetic stirring rod. The actuator and transferring device were removed and rinsed with 0.0005 *N* HCl, and the rinsing was placed into the beaker.

[9] Phodebas insulin test kit, Pharmacia Laboratory Inc., Piscataway, N.J.
[10] Sephadex.

Table III—Particle-Size Distribution of Insulin Aerosol

Storage Time, Months	Particle Size, μ	Cumulative Weight Percent		
		7°	25°	37°
Initial	1.0	3.0	3.0	3.0
	2.0	14.7	14.7	14.7
	4.0	39.4	39.4	39.4
	8.0	74.3	74.3	74.3
	16.0	100	100	100
1	1.0	2.5	2.4	3.3
	2.0	10.0	12.1	15.7
	4.0	31.7	34.7	40.7
	8.0	66.2	66.9	72.9
	16.0	100	100	100
2	1.0	3.2	2.4	2.3
	2.0	13.5	12.1	13.2
	4.0	36.8	34.1	38.0
	8.0	69.5	68.6	72.1
	16.0	100	100	100
3	1.0	3.0	2.8	3.5
	2.0	13.1	13.3	15.8
	4.0	35.1	34.1	40.8
	8.0	67.6	68.4	73.9
	16.0	100	100	100

The aerosol container was reweighed so as to determine the weight of product expelled by one actuation. Then the solution was brought to room temprature and gently stirred for approximately 20 min using a magnetic stirrer. This procedure ensured the removal of any remaining propellant. The acid solution was transferred to a 500-ml volumetric flask. The beaker containing the solution was rinsed twice with about 100 ml of 0.005 *N* HCl, and the rinsings were added to the 500-ml volumetric flask and brought to volume with 0.005 *N* HCl.

A 0.5-ml aliquot of this solution was pipetted into a 25-ml volumetric flask and diluted to volume with 0.005 *N* HCl. This dilution then became the acid stock solution of insulin aerosol to be assayed. A 0.1-ml aliquot of the acid insulin stock solution was pipetted into 5 ml of the buffer contained in a test tube and gently stirred. This buffer dilution is referred to as the "unknown" in the radioimmunoassay. One aerosol unit from each of the three storage temperatures was treated in a similar manner. The procedure was then repeated on samples stored for 1, 2, and 3 months at these temperatures.

Dilution of Raw Insulin Zinc—About 1.2 mg of insulin zinc was accurately weighed and diluted in 0.005 *N* HCl. This diluted acid stock solution of the raw insulin zinc was also referred to as the "unknown" for further use in the radioimmunoassay. This solution was used as the standard when the potency of the insulin zinc used to prepare the aerosol formulation was determined.

Test Procedure for Radioimmunoassay—Sufficient plastic centrifuge tubes with round bottoms were labeled for duplicate assays of Standards A–H (Series 1) and the unknowns (Series 2). The radioimmunoassay procedure follows.

A 0.1-ml aliquot of Standards A–H, each one in duplicate, was pipetted into the tubes in Series 1. A 0.1-ml aliquot of each unknown, each one in duplicate, was pipetted into tubes in Series 2. A 0.1-ml aliquot of the ^{125}I-insulin solution was pipetted into each of the tubes in Series 1 and 2. A 1.0-ml aliquot of dextran gel–anti-insulin complex suspension was pipetted into each tube in Series 1 and 2. The suspension was stirred slowly and continuously to avoid foaming.

The tubes in Series 1 and 2 were stoppered and incubated overnight at room temperature by placing the tubes horizontally in a shaker[11]. The tubes in Series 1 and 2 were centrifuged at 2000×*g* for 2 min to remove droplets from the stoppers, and the stoppers were then removed.

The centrifugation was repeated, and the supernatant liquid was cautiously aspirated to approximately 5 mm from the bottom of each tube in Series 1 and 2. For this purpose, a collared suction nozzle coupled to a water aspirator was used. The collar was adjusted vertically to the correct height to avoid removal of particles.

The washing procedure was performed by adding 2 ml of 0.9% saline to each tube, centrifuging the tubes at 2000×*g* for 2 min,

[11] Radioimmunoassay shaker, Labindustries, Inc., Berkeley, Calif.

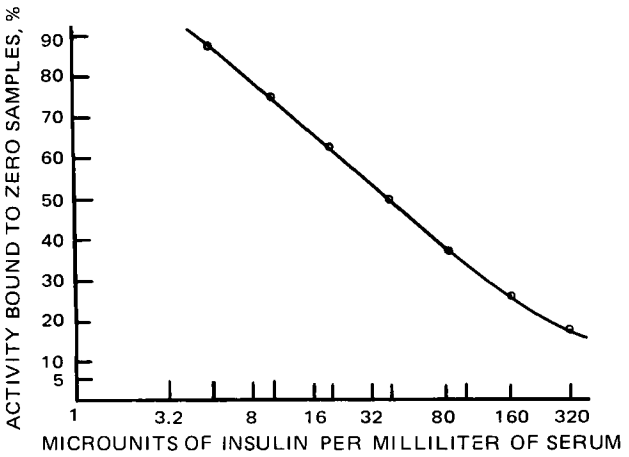

Figure 2—*Standard curve for radioimmunoassay of insulin aerosols: initial and 1-month samples.*

and withdrawing the supernatant liquid as just described. This washing procedure was performed three times, and the tubes were then stoppered.

The radioactivity in each tube in Series 1 and 2 was determined by using a gamma counter[12] set at a counting time of 4 min. The background radioactivity was checked by using an empty centrifuge tube.

Calculations—The mean count rate (*MCR*) for each standard was expressed as a percentage of the mean count rate of the "zeros." The background count was subtracted if significant. The percentage values of the mean count rate obtained for the insulin standards were plotted against the logarithm of insulin concentration on linear logarithmic paper, and a standard curve was constructed. The mean count rate for each "zero" and unknown was expressed in the same way as for the standards.

The concentration of insulin was read directly from the curve (microunits per milliliter) for each unknown sample. The value obtained was then multiplied by the dilution factor (1,250,000) to determine the amount of insulin delivered by one actuation of the aerosol. The results shown in Fig. 2 and Table IV represent the average of two determinations for each assay result.

RESULTS AND DISCUSSION

To improve the suspension of the insulin zinc crystals in the fluorocarbons, oleyl alcohol was added to the formulation. This dispersing agent served to wet the insulin zinc crystals, and its effectiveness was probably due to adsorption at the air–liquid and the solid–liquid interfaces, thereby reducing the contact angle and resulting in an improvement of wetting of the particles. Since each insulin particle was coated with a thin film of oleyl alcohol, which is miscible with fluorocarbons, this wetting process enhanced the dispersion of insulin zinc crystals in the fluorocarbons.

Furthermore, oleyl alcohol acts as a lubricant for the valve, thus preventing valve clogging. The quantity of oleyl alcohol used in this study was rather high. Lower percentages of the material resulted in a greater degree of caking of the insulin. By further reducing the particle size of the insulin, one possibly can reduce the amount of oleyl alcohol.

A preliminary study indicated that about 6.5% of oleyl alcohol would allow the insulin zinc to remain suspended for a long period when dichlorodifluoromethane was used as the propellant. However, further studies indicated that a blend of dichlorodifluoromethane–monochlorodifluoroethane (90:10) exhibited an even greater degree of suspension then dichlorodifluoromethane. This enhanced performance was attributed to the lower density of dichlorodifluoromethane–monochlorodifluoroethane than the density of dichlorodifluoromethane, so that the suspensoid was easier to suspend in the system and the duration of full suspension was longer. In addition, the combination of propellants is more polar than the pure propellant alone, thereby enhancing suspension. While other combinations of propellants could be used, this combination was best suited for the combination of insulin and oleyl alcohol.

[12] Gammacord-Ames Co., Division of Miles Labs., Elkhart, Ind.

Table IV—Summary of Radioimmunoassay Results of Insulin Aerosol

Samples	Amount Sampled, mg	Theoretical Insulin, Units	Recovery		Stability, %
			Units	%	
Insulin zinc (standard)	1.79	46.36	39.88	86.02	92.54
Initial	64.30	36.31	33.75	92.95	100.00
7°, 1 month	75.10	42.40	36.25	85.50	91.98
2 months	76.60	43.32	38.75	89.45	96.23
3 months	76.10	42.97	39.50	91.92	98.89
25°, 1 month	76.10	43.02	38.25	88.91	95.65
2 months	76.90	43.42	38.13	87.82	94.48
3 months	78.80	44.49	38.75	87.10	93.70
37°, 1 month	83.10	46.92	41.00	87.38	94.00
2 months	84.60	47.76	37.63	78.79	84.76
3 months	83.30	47.03	36.25	77.08	82.93

As can be seen from Fig. 1, the weight of formulation dispensed per actuation of the metered valve increased after 1 month. The metering tank of the valves used in this study was made of plastic, and the slight increase in weight dispensed may have been due to the swelling effect of the formulation on the plastic housing. However, the effect was pronounced with samples stored at 37° for 1–3 months; a combination of a swelling effect and heat upon the plastic housing probably caused it to expand, resulting in a greater weight dispensed. This could be avoided through use of a metered valve with a stainless steel housing or metering tank.

Statistical treatment of the data shown in Fig. 1 indicated that uniform dose dispensing was obtained in the aerosol preparations stored at 7 and 25°. Mean values of the results obtained over 3 months were in the range of 71.9–76.5 mg/dose with a standard deviation of 0.73–2.50 mg/dose. Over this 3-month stability study, the insulin units delivered per actuation ranged from an initial 40.6 to 43.2 USP units/dose at the end of the 3rd month; the highest standard deviation was ±1.41 USP units.

This satisfactory result can be seen easily from a plot obtained when the mean of each value was plotted against time (Fig. 1). The plot for 25° almost overlapped the plot for 7°. The paired t-test for significant difference indicated that there was no significant difference at the 95% confidence level between the doses dispensed of aerosols stored at 7° and those stored at 25° over 3 months (at n = 2, t = 2.410, which is less than the distribution of t = 4.303 at the 0.05 probability level).

The samples stored at 37° had a wider variation of dose dispensed over 3 months. The dose ranged from 71.9 mg initially to 83.2 mg at the end of the 3rd month. The highest standard deviation was ±6.78 mg/dose. The dose ranged from an initial 40.6 to 46.9 USP insulin units delivered per dose at the end of the 3rd month; the standard deviation was ±3.83 USP units/dose. The paired t-test for significant difference indicated the t value of 17.759 at two degrees of freedom was far greater than the t value of 4.303 at the 0.05 probability level. This finding indicates that there was a significant difference between the dose dispensed from aerosols stored at 37° and that of those stored at 7° over the 3-month period.

An increase in the sedimentation rate (decrease in suspension time) was observed with the aerosols stored at higher temperatures (25 and 37°). A large difference in suspension time was noticed when these samples were compared with times of samples stored at 7° (Table II). The longer suspension time, which occurred in the 7° samples, probably was due to the gradual decrease in the density of the propellant blend. At first, in samples stored in a refrigerator, the density of the propellant blend was heavier than that of the suspensoid (insulin zinc dispersion in oleyl alcohol). The suspensoid was lighter and floated to the top.

When a sample was taken out of the refrigerator, the sample temperature rose gradually to ambient temperature, and the density of the propellant blend decreased with an increase in temperature. At one point, the density of the propellant blend became equal to that of the suspensoid, which would aid in helping the suspended solids. As the temperature approached room temperature, the density of the propellant became less than that of the sus-

pensoid, resulting in an increase in sedimentation rate. This phenomenon provided a longer suspension time for the insulin aerosol when the aerosol was used immediately after being removed from the refrigerator.

The particle-size distribution of the insulin aerosols was determined. A cutoff point was made at the 16-μm level, since particles with a diameter of less than 10 μm are more significant for inhalation therapy; this figure also represents the upper limit of this technique. As indicated by Porush *et al.* (23), the optimum particle diameter for penetration into the pulmonary system is between 0.5 and 7 μm. Therefore, further analysis of the data of particle-size distribution (Table III) indicated that approximately 65–75% of the total sprayed insulin particles would have a particle size below 8 μm.

The "percent deposited" represents the percent of the total sprayed insulin that deposited on the lower four slides of the impactor, which, in turn, corresponded to a particle size of 1.0–8.0 μm. Particles in this size range can penetrate deep into the lungs and produce a biological effect. Therefore, if a higher percentage deposited would be more desirable, the particle size of insulin zinc crystals should be reduced further.

Table IV indicates a recovery of insulin by the radioimmunoassay method of 77–93%. The percentage of recovery was based on the assay result and its theoretical value. The low recovery also occurred in the initial assay of insulin zinc crystals and was due to normal assay variation. The variation is, however, within the limits established by the USP for insulin with a 95% fiducial limit of 87–115%. This assay variation for a single assay in this study was found to be ±6.54% at the 95% confidence level.

The precision of the radioimmunoassay was influenced by two major sources: (*a*) the variation encountered in preparing the standard curve, which was largely dependent upon volumetric errors and incomplete washing; and (*b*) the variation that occurred due to the diluting procedure and that attributed to the huge dilution factor in the successive dilution of insulin aerosols. Incomplete washing would be experienced if the washing procedure was performed twice instead of three times or if the collared suction nozzle was not placed deep enough to remove the unbound portion. If the nozzle was placed too deep, the dextran gel particles would then be removed. A lower count rate would result.

Another variation experienced during the dilution process of the insulin aerosol was the adsorption of insulin onto the glass walls. Since all volumetric flasks and beakers were made of Pyrex glass, the chance for insulin adsorption was relatively high. Therefore, 0.1% of bovine albumin was added to the diluent. The bovine albumin served as protein to precoat the glass walls and thus minimize insulin adsorption.

Within the scope of this study and in recognition of assay variations, the radioimmunoassay data demonstrated that insulin aerosols stored at 7° remained stable over 3 months while insulin aerosols stored at 25° showed some drop in potency. Samples stored at 37° showed a decline in potency. Based on percent stability, declines in potency of 6.0, 15.2, and 17.2% were observed in the 1st, 2nd, and 3rd months of storage, respectively, at 37°. However, for the purpose of predicting shelflife, the results of the radioimmunoassay for the insulin aerosols were analyzed using chemical kinetics.

Since the decomposition reaction was noted to follow first-order kinetics, the chemical stability of the insulin aerosol could be determined using the following equation:

$$\log \frac{C_0}{C} = \frac{kt}{2.303} \qquad \text{(Eq. 1)}$$

where C_0 is the initial potency, C is the potency at time t, and k is the rate constant.

According to the least-squares method and linear regression analysis, a new set of values for C_0 and C (in terms of percent stability) were calculated (Table V). By using the new values for C_0 and C in the analysis, estimates for $k_{7°}$, $k_{25°}$, and $k_{37°}$ were calculated according to Eq. 1.

The $k_{7°}$ result had a negative value, indicating an increase in potency with time. Apparently, this increase was due to the variation within the assay. Therefore, a better estimation for $k_{7°}$ can be obtained by the Arrhenius equation:

$$\log \frac{k_2}{k_1} = \frac{E_a(T_2 - T_1)}{2.303R(T_2T_1)} \qquad \text{(Eq. 2)}$$

Table V—Regression Analysis of Radioimmunoassay Results[a]

Storage Conditions of Samples	Stability, %	
	Original Values	New Values
7°, 1 month	91.98	92.245
2 months	96.23	95.700
3 months	98.89	99.155
25°, 1 month	95.65	95.585
2 months	94.48	94.610
3 months	93.70	93.635
37°, 1 month	94.00	92.765
2 months	84.76	87.230
3 months	82.93	81.695

[a] Data derived from results reported in Table IV.

where R is the gas constant, T is the absolute temperature, E_a is the energy of activation, and k_1 and k_2 are specific rate constants at T_1 and T_2, respectively.

In this case, E_a should first be calculated by using the known values for $k_{37°}$ and $k_{25°}$. Then the value for $k_{7°}$ should be calculated by using the known values for $k_{25°}$ and E_a. The results are as follows: $k_{25°} = 9.9 \times 10^{-3}$ (month^{-1}), $k_{37°} = 6.3 \times 10^{-2}$ (month^{-1}), $E_a = 28.3$ kcal/mole, and $k_{7°} = 4.58 \times 10^{-4}$ (month^{-1}).

Once the rate constant (k) is obtained, it can be used to estimate the time required for 10% of the insulin aerosol to degrade. This $t_{10\%}$ value is regarded as the shelflife of the product and can be calculated from:

$$t_{10\%} = \frac{0.104}{k} \qquad \text{(Eq. 3)}$$

Therefore, by using Eq. 3, the predicted shelflife of the insulin aerosol would be 19 years at 7°, 11 months at 25°, and only 2 months at 37°. The long predicted shelflife at 7° indicates that the insulin aerosol should be stored in a refrigerator. However, these predictions are based upon preliminary results obtained by assaying a limited number of samples. Additional samples should be studied under accelerated conditions and long-term storage.

SUMMARY AND CONCLUSIONS

Based upon the results obtained in this study, it was concluded that a stable aerosol dosage form containing insulin could be developed by suspending insulin zinc crystals in a propellant system with the aid of a dispersant and that this aerosol should be stored in a refrigerator. The dose dispensed per actuation was in the range of 40–43 USP insulin units for the samples stored at 7 and 25° while a higher dose was dispensed from the samples stored at 37°.

The particle-size distribution measured by the impactor was reliable and reproducible as long as the operating conditions were standardized. Approximately 65–75% deposition was obtained with the insulin aerosol stored at 7 and 25°. This percent deposited was defined as the percentage of total sprayed drug with particle diameters of 1–8 μm and able to penetrate deep into the pulmonary cavity to produce a biological effect.

As indicated by the radioimmunoassay, insulin zinc crystals can be formulated into an aerosol system and remain stable. The results indicated that the insulin potency of samples at 7 and 25° remained unchanged over 3 months. However, a decline in potency was noticed with the samples stored at 37° for 3 months. This decline would be simply due to the effect of heat on the insulin. No chemical incompatibilities between insulin zinc and oleyl alcohol or the propellant system were deduced from the results of the chemical stability study.

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Effect of Thermal Gelation on Dissolution from Coated Tablets

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Abstract □ Tablets with a methylcellulose coating were found to exhibit lower dissolution profiles than those coated with a hydroxypropyl methylcellulose coating at 37°, and the cause was investigated. The differences are attributed to thermal gelation of the methylcellulose at temperatures near 37°, which creates a barrier to the dissolution process and essentially changes the dissolution mechanism. This mechanism is substantiated by the fact that at temperatures below the gel point and at increased agitation, the effect disappears. The retarded dissolution effect is not peculiar to the drug involved.

Keyphrases □ Thermal gelation—effect on dissolution of methylcellulose-coated tablets □ Dissolution—methylcellulose-coated tablets, effect of thermal gelation □ Dosage forms—tablets, methylcellulose coated, effect of thermal gelation on dissolution □ Methylcellulose—tablet coating, effect of thermal gelation on dissolution

Polymers, particularly the cellulose polymers, are used in pharmacy as film formers for tablet coatings and as binding agents in a granulation step.

During the development of a coated tablet, several different polymers may be evaluated for various properties and their effects on the dosage form. Ideally, unless applied for a specific purpose, the coating used for a drug delivery system should not affect the efficiency with which the drug is delivered to the target site (1).

During a coating investigation, it was observed that identical core tablets coated with two different polymers exhibited widely different dissolution profiles. Differences in the polymers, methylcellulose[1] and hydroxypropyl methylcellulose[2], were investigated in an attempt to rationalize the observed dissolution differences.

EXPERIMENTAL

Core tablets containing the following components were prepared by standard direct compression techniques: dibasic calcium phosphate dihydrate, lactose USP, starch USP, purified wood cellulose[3], colloidal silicon dioxide[4], stearic acid, and magnesium stearate. Active ingredients included either aspirin or amitriptyline hydrochloride.

The coating consisting of only the polymer, and a yellow lake was applied by normal film-coating techniques. Approximately 2.9 mg of polymer was deposited per tablet. Dissolution measurements were carried out in the USP apparatus at 150 rpm, unless noted otherwise, in 0.1 *N* HCl. Solubility was measured according to the USP procedure.

RESULTS AND DISCUSSION

The dissolution profiles (Fig. 1) for the aspirin core tablet and the two corresponding coated tablets at 37° illustrate the difference in the methylcellulose coating. Aside from the lag time for the tablet coated with the hydroxypropyl polymer, the profile parallels that of the core tablet. The methylcellulose-coated tablet exhibits a slower release profile.

[1] Methocel MC 25 cps.
[2] Methocel 60 HG.
[3] Solka-Floc, B.W. 2030.
[4] Cab-O-Sil.