Development and Evaluation of a Dexamethasone Timed-Release Aerosol Formulation

W. FEINSTEIN and J. J. SCIARRA ˣ

Abstract □ Dexamethasone microcapsules were prepared and *in vitro* parameters were determined by sieve analysis which yielded materials retained on Nos. 80-, 100-, 200-, and 270-mesh sieves. Release rate and direct total determinations indicated that some measure of timed release appeared in all four sieve samples. The pH–release rate profiles of the microcapsules indicated that dissolution was pH independent. Colloidal silica and isopropyl myristate formulations provided the best suspending characteristics of all aerosol formulations evaluated. Delivery rate, pressure, and evacuation testing demonstrated the usability and functionality of the aerosol. An evaluation was made on the effect on urinary excretion levels of 17-hydroxycorticosteroid when a physical admixture of the drug and timed-release microcapsules were sprayed on rabbits. The significantly higher 17-hydroxycorticosteroid levels obtained in urine of animals treated with the admixture after 1 day may have been attributed to both an immediate suppression of the endogenous steroid and the "spilling over" of excess dexamethasone. A significant difference also was found at the 90% confidence level among the 24-, 48-, and 72-hr 17-hydroxycorticosteroid levels in the admixture and the microcapsules, which substantiated the delayed effect of the latter.

Keyphrases □ Dexamethasone—development and evaluation of timed-release aerosol formulation, preparation of microcapsules □ Aerosols—development and evaluation of dexamethasone timed-release formulation □ Timed-release formulations—dexamethasone aerosol, development and evaluation □ Topical preparations—dexamethasone timed-release aerosol formulation, development and evaluation

Sciarra and Gidwani (1) discussed the kinetics that influence the rate of release of substances from various polymeric films and found that several polymer films inhibited the release of the drug and may be suitable for producing a sustained-action aerosol spray. The film-forming agents, ethylcellulose and polyamide, are compatible with aerosol propellants and are suitable for spraying (2). The kinetics of drug release were studied from polymeric films formulated as an aerosol dressing (3).

The methods for delaying the activity of medicaments to produce orally and parenterally administered timed-release pharmaceuticals have been reviewed (4). The preparation of nylon microcapsules and their application to pharmaceutical dosage forms were reported (5), as well as a listing of the possible methods of microencapsulation (6). An *in vitro* test method for the characterization of oral timed-release materials appears in the National Formulary (7). The influence of particle size in an aerosol dosage form and the various factors influencing the particle size of a substance dispensed from an aerosol were reported (8).

Scoggins (9), using fluocinolone acetonide, reported depression of urinary steroid excretion caused by suppression of endogenous adrenocorticosteroid production, indicating absorption of the drug. The decrease in concentration of 17-hydroxycorticosteroids in the urine was suggested to reflect a partial shutdown of adrenal gland activity because of the absorbed steroid (10).

This study concerns the microencapsulation of dexamethasone by the phase separation method of Rowe (11). Various polymers were investigated to determine the most suitable one for coating the active drug. Since the particle size of the microencapsulated material is critical when used in an aerosol formulation, attention was given to methods for the separation of the microencapsulated substance into various particle-size ranges. The suspension, resuspension, and prevention of agglomeration and caking of the particles were considered using several dispersing agents. Finally, the 17-hydroxycorticosteroid urine level was determined in rabbits both before and after application of the spray to determine the extent of the release of dexamethasone from the film.

Dexamethasone was chosen for this study because it is a commonly used medicinal in topical products and can be easily detected. It has been shown that topical applications of steroids, when absorbed, bring about various detectable *in vivo* responses (12, 13).

EXPERIMENTAL

Preparation of Microcapsules—Methylcellulose, ethylcellulose, polyvinyl acetate, and polyvinyl alcohol were used. One major problem that occurred during the preparation of the microcapsules involved the separation of the mass into discrete particles. This problem was overcome by using ethylcellulose as the polymeric material, varying the order of mixing, and controlling the rate of addition of the solvents.

Fifty grams of ethylcellulose NF was added to 450 g of toluene by sprinkling it into the vortex of the toluene, using a laboratory mixer[1] at a speed of approximately 100 rpm. The solution was placed into a sealed container and allowed to stand overnight. This procedure produced a clear solution. Approximately 70–90 ml of petroleum ether[2] was added dropwise to the ethylcellulose solution contained in a 1000-ml beaker, using a 100-ml pipet, until a persistent turbidity appeared. Five grams of dexamethasone[3] was added and mixed well. The addition of the remainder of the petroleum ether was alternated between the addition of talc USP and ether in approximately three equal applications (a total of 400 ml of petroleum ether and 110 g of talc was used).

The coated particles were filtered in several small portions and washed several times with petroleum ether. The particles were separated and rack dried at room temperature. Microcrystals produced by this method were calculated to contain between 3.0 and 4.0% dexamethasone. Actually, 137 g of solids containing approximately 3.7% dexamethasone was obtained by this method. Figure 1 illustrates the type of particle obtained.

Particle-Size Analysis—The particle-size distribution of the dried particles was determined[4] with Nos. 60-, 80-, 100-, 200-, and

[1] Lightnin.
[2] Boiling range of 30–60°, Specialty Chemicals Division, Phillips Petroleum Co., Bartlesville, Okla.
[3] Micromilled, S. B. Penick and Co., New York, N.Y.
[4] Pulverit Classification Equipment type RB3020, Geoscience Instruments Corp., Mount Vernon, N.Y.

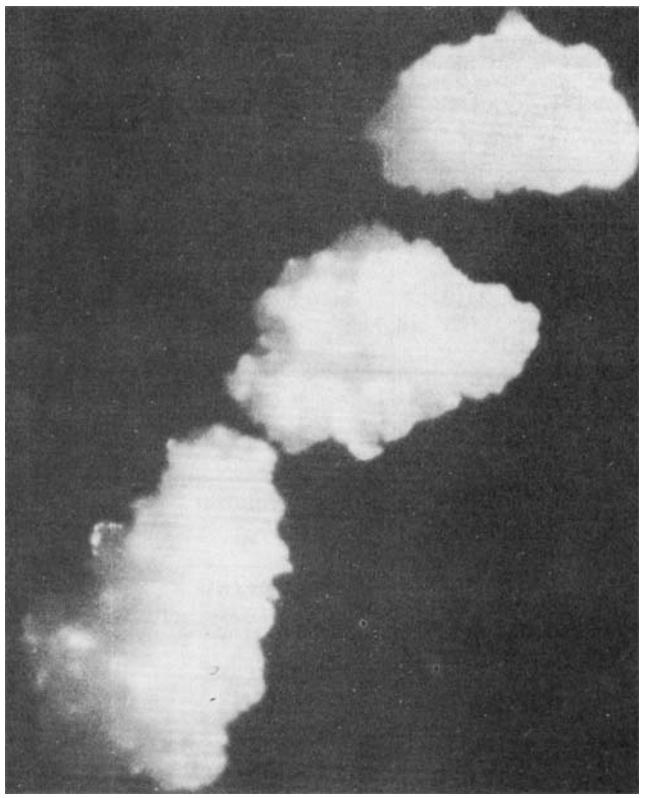

Figure 1—*Dexamethasone microcapsules (40×).*

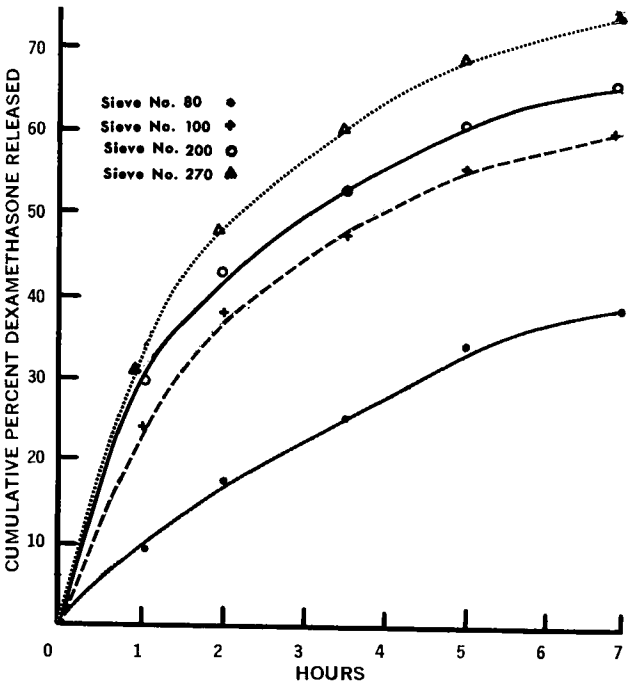

Figure 2—*Release rate from dexamethasone microcapsules of varying sieve sizes.*

270-mesh sieves. The portions collected on each screen were retained for further study. Additional microcapsules were prepared and the particles were classified as indicated so that a sufficient amount of material was available for the studies. Approximately 120 g of microcapsules was collected per batch and five batches were prepared.

Determination of Dexamethasone in Dexamethasone Microcapsules—The amount of dexamethasone in the microcapsules was determined by exposing the microcapsules to extracting fluids of varying pH. Simulated gastric fluid and intestinal fluid (7) were prepared without the inclusion of enzyme so as to obtain extracting fluids with pH values of 1.2, 2.5, 4.5, 7.0, and 7.5. Other fluids of varying pH could also have been used.

Two hundred and fifty milligrams of dexamethasone microcapsules was placed into a 200-ml bottle, and 150 ml of pH 1.2 fluid was added to the bottle, previously warmed to 37°. The bottle was tightly capped and rotated in a rotating-bottle apparatus at 28–30 rpm for 1 hr at 37 ± 1.0°. The pH 1.2 extracting fluid was decanted from the bottle through a 325-mesh stainless steel screen while the residue was retained in the bottle.

Residue collected on the screen was quantitatively returned to the original bottle with 150 ml of pH 2.5 extracting fluid, previously warmed to 37°. The bottle was tightly capped, returned to the apparatus, and rotated. This procedure was repeated at the end of 2 hr using pH 4.5 fluid, at the end of 3.5 hr using pH 7.0 fluid, and at the end of 5 hr using pH 7.5 fluid. After 7 hr of total rotation time, the pH 7.5 fluid was decanted from the bottle and the residue was retained for assay.

Release Rate Procedure—About 50 mg of dexamethasone standard was accurately weighed and quantitatively transferred to a 500-ml separator, and about 160 ml of water was added. The sample and the residue were transferred to individual 500-ml separators. These three samples were extracted with five 50-ml portions of chloroform. The chloroform layers were placed into 250-ml volumetric flasks by being passed dropwise through chloroform-wetted anhydrous sodium sulfate into a filtering funnel and were brought to volume with chloroform. An aliquot of each solution was pipetted into a 50-ml centrifuge tube and evaporated to dryness.

Assay of Samples—Twenty milliliters of ethanol was added to each tube, the solution was stirred for 5 min, and 2 ml each of blue tetrazolium solution (14) and tetramethylammonium hydroxide solution (15) was added to each tube. The tubes were then allowed to stand for 90 min. A blank was prepared in a similar manner. The absorbances of the sample and standard were read against the blank at 525 nm. The amount of dexamethasone was calculated as follows:

$$\frac{S}{Sa} \times \frac{C}{1000} \times \text{D.F.} = \text{mg dexamethasone released/g} \quad \text{(Eq. 1)}$$

where S = absorbance of sample at 525 nm, Sa = absorbance of standard at 525 nm, C = concentration of standard in micrograms per milliliter, and D.F. = dilution factors. Furthermore:

$$\frac{Bi}{\Sigma Bi} \times 100 = \text{\% dexamethasone released per time period} \quad \text{(Eq. 2)}$$

where Bi = mg dexamethasone/g released during the ith time period, and ΣBi = cumulative amount released in milligrams per gram. These results are shown in Table I and Fig. 2.

Direct Total Determination—Approximately 1 g of dexamethasone microcapsules was ground and finely powdered. An amount equivalent to 4 mg of dexamethasone was accurately weighed and transferred to a 100-ml volumetric flask, and 70 ml of ethanol was added. The solution was shaken for 30 min and brought to volume with ethanol. A 15-ml aliquot from each flask was transferred to a 100-ml volumetric flask and brought to volume with ethanol. The sample containing the No. 270 microcapsules was not diluted.

The standard was prepared by accurately weighing 50 mg of dexamethasone standard and dissolving it in enough ethanol to yield a total volume of 250 ml. A 3-ml aliquot was placed into a 100-ml volumetric flask and brought to volume with ethanol. Five-milliliter aliquots of each sample and 20 ml of the standard solution were pipetted into 50-ml centrifuge tubes. Each sample was then treated with the same reagents as previously indicated and the absorbance was determined as described under *Assay of Samples*. The direct total of dexamethasone was determined as follows:

$$\frac{S}{Sa} \times \frac{C}{1000} \times \frac{100}{W} \times \text{D.F.} = \text{mg dexamethasone/g} \quad \text{(Eq. 3)}$$

where S = absorbance of sample at 525 nm, Sa = absorbance of standard at 525 nm, C = concentration of standard in micro-

Table I—*In Vitro* Release Rate[a] and Direct Total Determinations of Dexamethasone Microcapsules

Hours	Sieve No. 80		Sieve No. 100		Sieve No. 200		Sieve No. 270	
	Determination Number		Determination Number		Determination Number		Determination Number	
	1	2	1	2	1	2	1	2
1	8.0	10.0	24.6	25.0	30.8	29.9	25.4	34.5
2	16.4	18.3	36.0	39.8	40.5	45.5	44.2	50.9
3.5	24.4	25.6	46.2	49.1	51.7	53.9	59.1	61.2
5	32.6	34.8	55.1	56.6	60.5	61.6	70.1	68.4
7	37.0	39.2	59.0	61.3	65.2	66.0	74.9	73.7
Residue	63.1	60.8	40.9	38.7	34.9	34.0	25.0	26.2
Direct total[b], mg/g	45.3		45.4		46.7		41.3	

[a] Cumulative weight percent dexamethasone released. [b] Average of two determinations.

Table II—Formulations Prepared

Ingredients	Formula Number					
	1	2	3	4	5	6
	Percent (w/w)					
Dexamethasone microcapsules	12.5	12.5	12.5	12.5	12.5	12.5
Fumed silica[a]	—	0.5	0.5	0.5	0.5	0.5
Oleyl alcohol	—	—	5.0	—	—	—
Isopropyl myristate	—	—	—	5.0	—	—
Talc USP	—	—	—	—	5.0	—
Mineral oil USP	—	—	—	—	—	5.0
Dichlorodifluoromethane NF–dichlorotetrafluoroethane NF (40:60)[b]	87.5	87.0	82.0	82.0	82.0	82.0

[a] Cab-O-Sil M-5, Cabot Corp., Boston, Mass. [b] Propellant 12/114.

grams per milliliter, W = sample weight in grams, and D.F. = dilution factors. These results are included in Table I.

Determination of pH Profile of Dissolution Rate of No. 100 Sieve Dexamethasone Microcapsules—The dissolution rate of the dexamethasone microcapsules, No. 100 sieve (149 μm), was determined as a function of pH. Two hundred and fifty milligrams of the dexamethasone microcapsules was exposed to pH 1.2, 2.5, 4.5, 7.0, and 7.5 fluids. The fluid and the method used were as previously described. Determinations were made at 1.0, 3.5, and 7.0 hr. The decanted media, as well as the residues, were treated, diluted, and assayed according to the procedures previously outlined. The results are reported as cumulative percent dexamethasone released at varying times and pH values (Fig. 3).

Formulation of Aerosol Containing Dexamethasone Microcapsules—Several formulations (Table II) were prepared in aerosol compatibility tubes calibrated in centimeters. The propellants were added by the pressure fill method, and the tube was inverted three times to disperse fully the suspended material. The dexamethasone microcapsules had an approximate concentration of 40 mg/g. The incorporation of 7.5 g/formulation was, therefore, equivalent to approximately 0.5% dexamethasone/tube.

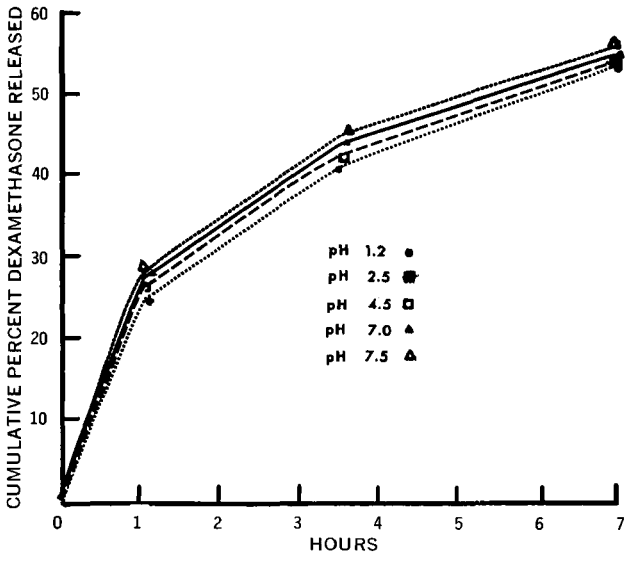

Figure 3—*Dissolution of dexamethasone microcapsules, sieve No. 100 material, at different pH values.*

The aerosols were prepared by mixing the dexamethasone microcapsules with the other solids present in the formulation. The dispersing agent was then blended with the solids and added to the compatibility tube, which was sealed with an aerosol valve[5]. Dichlorodifluoromethane–dichlorotetrafluoroethane (40:60) was added by the pressure process, and the tubes were allowed to stand 2–5 days and then evaluated. The sedimentation rate of each preparation was determined (Table III).

Evaluation of Aerosol Formulation—Based upon the results shown in Table III, Formulation 4 was selected for further study. Six aerosol containers were prepared as previously outlined, except that the samples were prepared in tin-plated aerosol cans. The valve previously described was also used for this portion of the study.

Approximately 60 g of product was placed into each container. The dip tubes were removed from the valves used on the samples for the assays to facilitate the removal of the propellant without discharging the microcapsules from the container. The samples were then evaluated for delivery rate, pressure, evacuation, and release of active ingredient.

Delivery Rate—The delivery rate was determined on two cans 5 days after filling. The aerosol product was sprayed, weighed, and equilibrated to 25 ± 1°. The can was set into position under the delivery apparatus (16). The actuator was set to deliver product for 10 sec, and then the can was cleaned and reweighed. Three determinations per container were made. The average of these readings produced a delivery rate of 1.75 g/sec.

Pressure Testing—The vapor pressure was determined on the two cans used for the delivery rate. The aerosol can was placed into a water bath at 21 ± 1°, and a gauge was prepressurized with

[5] Precision Valve Co., Yonkers, N.Y.; 5.08 × 0.05-cm (2 × 0.020-in.) orifice, radius core (nylon) stem, Buna N (regular durometer) gasket, type 302 stainless steel spring, 0.2-cm (0.08-in.) nylon body, flat dimpled tin plate, epon-coated mounting cup, standard dip tube, and 0.05-cm (0.02-in.) actuator.

Table III—Sedimentation Rate of Aerosol Formulations

Formula Number	Height of Supernate at $T_{30\ sec}$, cm	Height of Sediment at $T_{2\ hr}$, cm	Total Height at $T_{2\ hr}$, cm	$\frac{A}{C-B} \times 100$[a], %
1	1.4	1.6	3.1	93.3
2	0.5	2.0	3.1	45.5
4	0.45	2.0	3.5	30.0
5	0.5	2.0	2.9	55.6
6	1.1	2.0	3.5	73.3

[a] Ratio, in terms of percentage, of supernatant height at T = 30 sec to supernatant height at T = 2 hr. [b] Coagulation occurred with Formulation 3 and it was deleted from the study.

Table IV—Assay of Dexamethasone Microcapsules from an Aerosol

Hours	Cumulative Percent		
	Determination Number 1	2	Control
1	36.4	31.5	34.5
2	56.2	49.1	50.9
3.5	71.4	65.6	61.2
5	78.7	76.1	68.4
7	84.1	84.0	73.7
Residue	15.8	15.9	26.2
Direct total, mg/g	39.6[a]		41.3

[a] Average of two determinations.

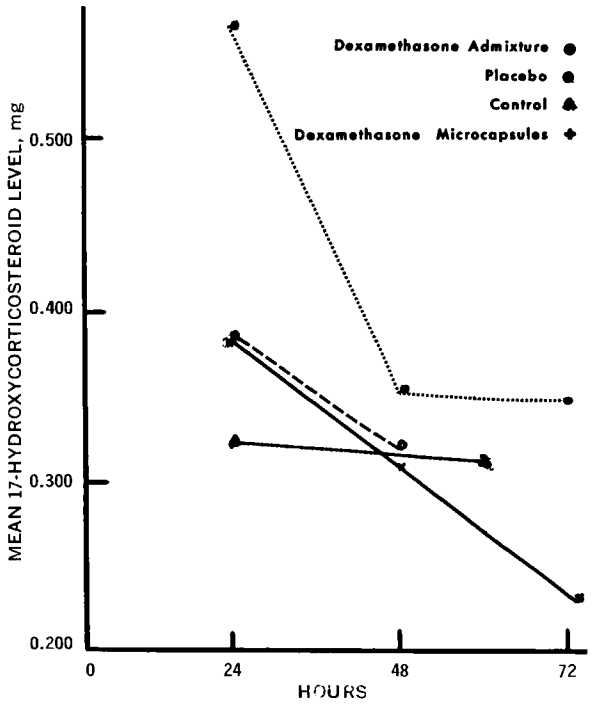

Figure 4—*Mean 17-hydroxycorticosteroid levels in rabbit urine.*

nitrogen. The pressure was then obtained with the use of the prepressurized gauge (17), and the average of two determinations on each can was 49 psig.

Evacuation Testing—Two containers, 5 days after preparation, were weighed and completely evacuated over a 24-hr period. Each can was reweighed, and the loss was determined by difference. This value was 57.7 g, representing the average for the two cans.

Analytical Testing—An assay was performed on two containers, 48 hr after preparation, and the result was compared to a control representing the analysis of the initial dexamethasone microcapsules, No. 270 sieve material, from which the aerosol products were originally prepared (Table IV).

***In Vivo* Evaluation of Aerosol**—To ascertain the release of the active ingredient from the encapsulated material sprayed from the aerosol container, five male New Zealand rabbits, approximately 4 months old, were fed rabbit pellets[6] and were allowed water *ad libitum*. They were placed in separate cages placed at an angle to allow the urine to flow into vessels suspended at the lower end of the cages. The urine passed through a fiberglass filter before flowing into the vessels.

The backs of the animals were shaved and the necks were collared[7]. The aerosol product was sprayed onto their backs at a distance of approximately 20.3 cm (8 in.), and the test areas were then occluded by wrapping[8]. The urine volume was recorded, and the samples were frozen for subsequent assay.

Three aerosol preparations were used: *(a)* a placebo container in which the inactive ingredients were mixed together and then pressurized, *(b)* a container in which the drug and the inactive ingredients were admixed, and *(c)* the delayed drug release microcapsules, No. 270 sieve, in an aerosol can. This phase of the study was carried out as follows:

1. A control urine sample was collected from each of the five rabbits after 24 and 48 hr. The placebo container was then sprayed for 2 sec. Urine samples were collected after 24 and 48 hr and assayed (Table V).

2. Two days after the last collection, the container with the nondelayed preparation was sprayed for 2 sec onto each of the five rabbits. Urine samples were collected after 24, 48, and 72 hr and assayed (Table VI).

3. Seven days after the last collection of the nondelayed preparation, urine samples were collected from each rabbit to determine if a return to the mean placebo value had occurred (Table VI).

4. After the urine collection in Step 3, the timed-release product was sprayed for 2 sec onto each of the five rabbits. Urine samples were collected after 24, 48, and 72 hr and assayed as previously described (Table VII). The data were then treated statistically (Table VIII).

DISCUSSION

A photograph, at 40× magnification, taken with reflected light (Fig. 1), identifies the microcapsules formed in this process as irregular-shaped particles containing a wall of polymer film. This shape is typical of microcapsules produced by this method. While it is desirable to have uniform particles, the results shown in Table I indicate the range of particles produced by this method. Usable microcapsules chosen for this study were those retained on Nos. 80-, 100-, 200-, and 270-mesh sieves, although the larger sized particles would tend to settle faster and may cause valve clogging. In most cases, particles 60 μm and less in diameter tend to present a minimum amount of valve clogging problems when formulated as an aerosol.

The selection of the most suitable method for the preparation of the dexamethasone microcapsules was performed experimentally. The major problem encountered was related to capsule wall agglomeration. Ethylcellulose, when wet, produced a sticky material which appeared to enhance agglomeration. Once the solvent evaporated from the agglomerated material, it was impossible to bring about separation. The use of a mineral silicate, talc (11), minimized but did not eliminate this problem. However, by repeated washings with petroleum ether, this problem was reduced to a minimum.

The values for the *in vitro* release rate and direct total determinations are shown in Table I. The results indicate that some measure of timed release appeared in all four sieve samples tested according to the methods noted. As can be seen from Fig. 2, as the particle size of the microcapsule was decreased, the release rate increased; after 7 hr, approximately 74% of the dexamethasone contained in the material on the No. 270-mesh screen was released while only about 38% of dexamethasone was released from the No.

[6] Ralston Purina Co., St. Louis, Mo.
[7] Medicollar, EVSCO Pharmaceutical Corp., Oceanside, N.Y.
[8] Saran Wrap, Dow Chemical Co., Midland, Mich.

Table V—17-Hydroxycorticosteroid Levels in Rabbit Urine (Control and Placebo)

	Control						Placebo					
	24-hr Sample			48-hr Sample			24-hr Sample			48-hr Sample		
Rabbit Number	Urine Volume/ 24 hr, ml	Concentration/ ml, μg	Concentration/ 24 hr, mg	Urine Volume/ 24 hr, ml	Concentration/ ml, μg	Concentration/ 24 hr, mg	Urine Volume/ 24 hr, ml	Concentration/ ml, μg	Concentration/ 24 hr, mg	Urine Volume/ 24 hr, ml	Concentration/ ml, μg	Concentration/ 24 hr, mg
1	192	1.94	0.373	162	2.01	0.326	274	1.66	0.455	105	3.28	0.344
2	222	1.44	0.320	240	2.07	0.497	242	2.04	0.494	252	1.32	0.333
3	65	2.99	0.194	110	2.19	0.241	82	4.35	0.357	76	4.44	0.337
4	122	3.06	0.373	85	3.85	0.327	75	4.29	0.322	60	4.13	0.248
5	130	2.70	0.351	42	4.19	0.176	70	4.14	0.290	94	3.49	0.328

Table VI—17-Hydroxycorticosteroid Levels in Rabbit Urine after Treatment with Dexamethasone Aerosol Admixture

	24-hr Sample			48-hr Sample			72-hr Sample			24-hr Control (7 Days after 72-hr Sample)		
Rabbit Number	Urine Volume/ 24 hr, ml	Concentration/ ml, μg	Concentration/ 24 hr, mg	Urine Volume/ 24 hr, ml	Concentration/ ml, μg	Concentration/ 24 hr, mg	Urine Volume/ 24 hr, ml	Concentration/ ml, μg	Concentration/ 24 hr, mg	Urine Volume/ 24 hr, ml	Concentration/ ml, μg	Concentration/ 24 hr, mg
1	216	1.71	0.369	133	2.70	0.359	200	1.44	0.288	240	1.39	0.334
2	418	1.88	0.786	285	1.30	0.371	340	1.16	0.394	266	1.80	0.479
3	150	4.52	0.678	93	3.73	0.347	104	3.11	0.323	142	2.08	0.295
4	126	4.16	0.524	142	2.67	0.379	158	2.21	0.349	174	2.11	0.367
5	156	3.02	0.471	87	3.48	0.303	82	4.57	0.375	80	4.18	0.334

80-mesh sieve. The fact that this pattern of release of steroid was obtained is not surprising, since the release would be expected to be dependent upon particle size. It is also possible that some polymer wall material may have been ruptured during the separation process, resulting in a premature release.

Assays were performed on the four sieve sizes selected using the rotating-bottle apparatus and method desribed. There appears to be good reproducibility between the assays performed, although variations in the *in vitro* release rate between the different particle-size fractions were noted. The proximity of values in the direct total determinations for all four sieve sizes indicates a measure of uniformity in the different samples. Therefore, since the amount of drug content is approximately the same, the variation in release rate appears to be related to variations in the polymer wall deposition.

The data shown in Fig. 3 indicate that there is essentially no difference in the dissolution rate of dexamethasone microcapsules at various pH values. Dexamethasone dissolution from these microcapsules apparently is pH independent under the conditions studied. In light of the apparent pH independency of the microcapsules, there should be essentially no difficulty in using the NF (7) oral release rate method to characterize microcapsules for topical use from a pH point of view. Since the pH of skin is approximately 4–6 and the study was carried out in this and other pH ranges, these results should be applicable for a topical preparation.

Of the various dispersing agents studied, a combination of fumed silica and isopropyl myristate seemed to be the most useful (Table III). It was necessary to provide a formulation that would have good suspending characteristics to obtain a uniform delivery of the drug from the system. In addition, no evidence of valve clogging or leakage was noted.

To determine whether there would be any adverse effect on the *in vitro* delayed release of the drug from the microcapsules when packaged in the aerosol containers, a sample of the microcapsules from the aerosol was assayed. These results (Table IV), when compared against the initial results for the No. 270-mesh material, indicated that there was relatively little, if any, effect upon the delayed release of the drug. Evidently the coating produced by this technique is not destroyed or damaged in the presence of the propellant and the dispersing agents studied.

Seven days was considered an adequate interval to allow for full excretion of the drug from the time the admixture and microcapsule preparations were applied. As can be seen from Tables V, VI, and VIII, the concentrations of 17-hydroxycorticosteroid in 24 hr for the control and the control taken 7 days after the 72-hr admixture was applied were not significantly different ($p > 0.3$).

Tables VI and VII provide the values for the urine volume, 17-hydroxycorticosteroid concentration per milliliter of urine, and total 17-hydroxycorticosteroid levels found in the 24-hr periods when spraying immediate release and timed-release material. Figure 4 shows the difference obtained when plotting mean value *versus* time. The high 17-hydroxycorticosteroid level in animals treated with the admixture may be attributed to both an immediate suppression of the endogenous steroid and the "spilling over" of

Table VII—17-Hydroxycorticosteroid Levels in Rabbit Urine after Treatment with Dexamethasone Aerosol Microcapsules

	24-hr Sample			48-hr Sample			72-hr Sample		
Rabbit Number	Urine Volume/ 24 hr, ml	Concentration/ml, μg	Concentration/24 hr, ml	Urine Volume/ 24 hr, ml	Concentration/ ml, μg	Concentration/24 hr, mg	Urine Volume/ 24 hr, ml	Concentration/ml, μg	Concentration/24 hr, mg
1	222	1.48	0.329	182	1.98	0.360	202	0.92	0.186
2	370	1.69	0.625	300	1.03	0.309	312	0.51	0.159
3	158	2.06	0.326	160	2.05	0.328	120	3.04	0.365
4	159	1.85	0.294	126	2.81	0.354	103	1.37	0.141
5	60	4.98	0.299	56	3.39	0.190	90	3.42	0.308

Table VIII—Levels of Significance of 17-Hydroxycorticosteroid in Rabbit Urine Samples

Samples Compared (Paired Data)	Mean ± Standard Error of Mean	17-Hydroxycorticosteroid Levels	
		t	p
24-hr Control and 24-hr Placebo	0.322 ± 0.035 0.384 ± 0.039	1.212	>0.25
48-hr Control and 48-hr Placebo	0.313 ± 0.054 0.318 ± 0.018	0.081	>0.9
24-hr Placebo and 24-hr Admixture	0.384 ± 0.039 0.566 ± 0.074	2.536	>0.05
48-hr Placebo and 48-hr Admixture	0.318 ± 0.018 0.352 ± 0.013	1.280	>0.25
24-hr Placebo and 24-hr Dexamethasone microcapsules	0.384 ± 0.039 0.375 ± 0.063	0.213	>0.8
48-hr Placebo and 48-hr Dexamethasone microcapsules	0.318 ± 0.018 0.308 ± 0.031	0.248	>0.8
24-hr Dexamethasone admixture and 24-hr Dexamethasone microcapsules	0.566 ± 0.074 0.375 ± 0.063	3.772	>0.02
48-hr Dexamethasone admixture and 48-hr Dexamethasone microcapsules	0.352 ± 0.013 0.308 ± 0.031	2.171	>0.1
72-hr Dexamethasone admixture and 72-hr Dexamethasone microcapsules	0.346 ± 0.019 0.232 ± 0.044	2.284	>0.1
24-hr Control and 24-hr Control (7 days after 72-hr admixture collection)	0.322 ± 0.035 0.362 ± 0.031	1.035	>0.3

excess dexamethasone, with a subsequent rapid increase in the 17-hydroxycorticosteroid content in the urine, hence the significant increase from the placebo as noted in Fig. 4.

However, a difference is seen in release and absorption between the dexamethasone admixture and microcapsules (Fig. 4). The values for the microcapsules for Days 1 and 2 are approximately equal to the control and placebo values, indicating that there is a delay in the release of dexamethasone. Not until Day 3 is a difference noted as indicated by the suppression of the 17-hydroxycorticosteroid content.

To treat the results statistically, the results were evaluated as paired data (Table VIII). While the general level of biological significance is 0.05 (95% confidence level) or less, it is believed that, with the number of experiments conducted in this evaluaton, any value less than 0.1 (90% confidence level) could be of possible significance.

From Table VIII the p values for the 24-hr control and placebo are not significantly different, indicating that the handling and treatment of the animals with the placebo had no effect upon the 17-hydroxycorticosteroid content of the urine.

The p values for the 24-hr placebo and dexamethasone admixture show significant variation, attributed to the effect of the immediate release material. The values for the 48-hr placebo and admixture are not significant, indicating the return to the norm. The p values for the 24-hr placebo and dexamethasone microcapsules show no significant variation which can be attributed to the prevention of the absorption of the dexamethasone from the microcapsules. The p values for the 24-, 48-, and 72-hr dexamethasone admixture and microcapsules show significant differences at the 90% confidence levels.

CONCLUSIONS

On the basis of the obtained results, the following may be concluded:

1. An aerosol preparation containing a microencapsulated drug can be prepared. Dexamethasone microcapsules, prepared by the phase separation technique using an ethylcellulose film, exhibit timed release in a pH range of 1.2–7.5 and the release appears to be pH independent.

2. The phase separation technique produces microcapsules with a suitable size distribution for dispensing as an aerosol dosage form. Those particles in the range of about 50 μm in diameter are most desirable for this application.

3. A formulation consisting of dexamethasone microcapsules (53 μm in diameter) together with suitable dispersing agents and propellants can be dispensed without rapid sedimentation and valve clogging.

4. A statistically significant reduction in the urinary level of 17-hydroxycorticosteroids in rabbits can be obtained from a timed-release dexamethasone aerosol when sprayed topically and compared to a dexamethasone preparation in which the ingredients are simply admixed.

5. No release of dexamethasone from the microcapsules was obtained over 24–48 hr, but reduction in the 17-hydroxycorticosteroid level (indicating absorption of dexamethasone) was noted over 72 hr. The use of higher concentrations of dexamethasone initially in the microcapsules could possibly have shown a more significant effect.

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[x] To whom inquiries should be directed. Present address: Brooklyn College of Pharmacy, Long Island University, Brooklyn, NY 11216

Effect of Flavoring Oils on Preservative Concentrations in Oral Liquid Dosage Forms

PRAMOD B. CHEMBURKAR [x] and ROBERT S. JOSLIN *

Abstract □ The partitioning of methyl-, ethyl-, propyl-, and butylparabens into flavoring oils from aqueous systems was studied. The partitioning is dependent on the concentration of the flavoring oil, the pH of the aqueous medium, and the nature and concentration of additives to the aqueous medium.

Keyphrases □ Flavoring oils—effect on preservative concentrations in oral liquid dosage forms, partitioning of parabens □ Preservatives—effect of flavoring oils on concentrations in oral liquid dosage forms, partitioning of parabens □ Parabens—partitioning from aqueous systems, effect of flavoring oils on concentrations in oral liquid dosage forms □ Interactions—parabens–flavoring agents in aqueous medium, effect on preservative concentrations, oral liquid dosage forms

Although the search for effective preservatives continues unabated, esters of *p*-hydroxybenzoic acid are still the most frequently used preservatives in oral pharmaceutical preparations. Several recent articles discussed the conditions in which various preservatives are effective and various conditions or agents that reduced or destroyed their effectiveness (1–3).

The effect of partitioning on the antimicrobial activity of preservatives was discussed (4). In a mixed system containing an oil phase, emulsified or as a separate layer, the concentration of antimicrobial agent required was higher than that in a completely aqueous system (5). Higher concentrations of preservatives are required to compensate for the quantities made unavailable for antimicrobial action due to adsorption, binding, or solubilization by nonionic surfactants or partitioning into a nonaqueous phase (6–11).

The theory behind partitioning of a preservative in an oil–water system was discussed (12). Several reports (7, 8, 10, 13) also described the partitioning of a preservative between two phases and the equilibria involved in partitioning and binding of a preservative to surfactants. Patel and Romanowski (12) further showed microbiologically the partitioning and binding effect of the oils and the surfactants on the preservatives. It has been noted that creams and emulsions are more difficult to preserve than aqueous solutions (14–18). The significant part played by partitioning of the preservatives between aqueous and nonaqueous phases, allowing availability of only a portion of the total quantity of the preservatives in the aqueous phase where contaminants normally multiply, has been stressed (19–22).

Flavoring agents are used in most oral liquid pharmaceutical preparations. These flavoring agents are composed of aromatic oils, natural or synthetic, and other ingredients to make them compatible with aqueous systems.

Preliminary observations in this laboratory showed that the solubility of parabens in these flavoring agents was extremely high. Since parabens are used in low concentrations, their high solubility in flavoring agents may cause depletion of the preservatives from the aqueous phase, with a consequent reduction in overall preservative activity. The purpose of this paper is to report the interaction between parabens and flavoring agents in an aqueous medium.

EXPERIMENTAL

General Partitioning Study—A 0.15% solution of methylparaben was prepared in a buffer solution of specified pH. Fifty-milliliter aliquots of the solution were transferred to 125-ml erlenmeyer flasks, and 0.5 ml of flavoring oil (1% of the total volume) was added to each flask. Respective blanks were also prepared containing flavoring oil but no methylparaben in the buffer solutions. The flasks were mounted on a wrist shaker[1] and shaken for 12 hr. The insoluble oily component in the mixture was then separated by a combination of centrifugation and column filtration technique.

The mixture was centrifuged at 10,000 rpm, corresponding to approximately 12,000 rcf. in a high-speed analytical centrifuge[2] for about 30 min. After centrifugation, the aqueous layer was separated from the floating oily layer using a Pasteur pipet. Aqueous layers from different tubes corresponding to one sample were pooled. This aqueous portion was then passed through an acid-washed kieselguhr column. The columns were prepared as follows.

A 10-ml disposable plastic syringe was plugged with a wad of glass wool of about 10-mm constant thickness. Two grams of acid-washed dry kieselguhr was weighed into the syringe. The column was lightly tapped down to a 35-mm column height, corresponding to the 6-ml mark on the plastic syringe, and the mixture was passed through the column. The first 5 ml of the effluent was re-

[1] Burrell.

[2] I.E.C. international centrifuge model H-T; rcf. = reciprocal centrifugal force.