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Suspending Agent Effects on Steroid Suspension Dissolution Profiles

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Abstract □ Dissolution profiles and particle-size analyses were determined for two lots of prednisolone acetate. The effects of common suspending agents on dissolution and particle-size distributions of these suspensions also were investigated. Lot-to-lot variation in the prednisolone acetate dissolution rate was observed and was apparently related to the percentage of fine particles within the distribution. Carboxymethylcellulose sodium inhibition of prednisolone acetate dissolution occurred with only one lot of raw material and seemed to be related to aggregation of the fine particles. Hydroxypropyl methylcellulose inhibited both prednisolone acetate lots and was observed with or without small particle aggregation. The dissolution variations observed have important implications in suspension formulation.

Keyphrases □ Dissolution—suspending agent effects on steroid suspensions □ Steroids—prednisolone acetate suspensions, effects of suspending agents on dissolution □ Suspensions—steroids, effects of suspending agents on dissolution

Suspensions, capsules, and tablets share dissolution as a rate-limiting step for absorption and bioavailability. *In vitro* suspension dissolution has been related to the absorption rate (1, 2). Several investigators showed that the relationship between the dissolution rate and the formulation is most important (3–9). In particular, suspending agents are important in suspension dissolution (6–9).

The dissolution of handmade and commercial prednisolone acetate suspensions has been investigated (6). Hydroxypropyl methylcellulose inhibits prednisolone acetate dissolution, and this inhibition might be reflected in dissolution differences among commercial suspensions. Similar inhibition of sulfa drug dissolution was found with increasing carboxymethylcellulose sodium concentrations (7).

The purpose of the present study was to investigate systematically the dissolution-retarding effects of common agents on steroid suspensions (*e.g.*, prednisolone acetate). Information regarding the retardation mechanism as well as concentration dependence and viscosity effects was desired. By testing a number of agents, compounds with minimal retarding effects could be recognized. Such information should help to improve steroid suspension formulation.

EXPERIMENTAL

Dissolution data were obtained using a device reported by Shah *et al.* (10) with the sample basket removed. This apparatus featured a large-volume fluid container, a rotating filter assembly, and an external variable-speed magnetic stirrer. The rotating filter assembly provided variable mild laminar liquid agitation, and it also functioned as an *in situ* nonclogging filter to permit efficient intermittent or continuous sample filtration during dissolution. A 0.5-μm stainless steel filter was used.

One liter of distilled water was the dissolution medium. The temperature was maintained at 37°, and the filter assembly stirring speed was 300 rpm. A strobe lamp was employed to standardize the stirring speed.

Filtered fluid samples were withdrawn continuously at a 100-ml/min rate and were circulated through a spectrophotometer[1] for assay and then back into the dissolution flask. Air bubbles were released periodically with an air trap. The system was allowed to equilibrate at least 15 min before the sample was added.

Dissolution experiments were initiated by injecting 0.5 ml of a 0.25% prednisolone acetate suspension at a fixed position in the beaker with a syringe fitted with an extra-long needle. This concentration is below 10% saturation for prednisolone acetate. The chart paper was marked at the exact time the suspension was introduced. The absorbance of dissolved prednisolone acetate was recorded from the digital display of the spectrophotometer at 5-sec intervals for 1500 sec and then periodically for ~2 hr. Usually, a final 24-hr reading was taken.

[1] Cary model 118, Varian Instrument Division, Palo Alto, Calif.

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Table I—Composition of Lot A Suspensions [a]

Suspension	Active Ingredient, %	Suspending Agent, %
Prednisolone acetate	0.25	—
Prednisolone acetate containing carboxymethylcellulose sodium	0.25	0.5
Prednisolone acetate containing hydroxypropyl methylcellulose 4000 cps	0.25	0.5
Prednisolone acetate containing hydroxypropyl methylcellulose 4000 cps	0.25	0.1
Prednisolone acetate containing hydroxypropyl methylcellulose 50 cps	0.25	0.5

[a] The amount used of each suspension in the dissolution tests was 0.5 mg/liter.

The prednisolone acetate was assayed at 246 nm. The absorbance of the suspending agents was accounted for by assaying similar dilutions of suspending agents as found in the dissolution flask but without prednisolone acetate.

The described suspensions were prepared with a glass mortar and pestle. This method provided reproducible results and was easier to use than any other small-scale suspension methods attempted. The composition of the suspensions is listed in Table I.

The particle-size distribution data were obtained using an electronic flow counter[2] with a population accessory. A convenient aspect of this apparatus is that the volume distribution is obtained automatically with no calculation by the operator. The population accessory also allows for a direct determination of a numbers distribution. A meter is used to maintain sufficient dilution to prevent multiple counts.

A commercially available balanced electrolyte[3] was saturated with prednisolone acetate. Particulate contamination of the electrolyte was avoided by filtering the saturated solutions twice through a 0.22-μm filter.

RESULTS AND DISCUSSION

Dissolution profiles and particle-size analyses were determined for two lots of prednisolone acetate (Lots A and B[4]). Lot A was studied initially.

The dissolution profiles for suspensions of Lot A prednisolone acetate and Lot A prednisolone acetate containing 0.5% carboxymethylcellulose sodium are shown in Fig. 1. Carboxymethylcellulose sodium retarded

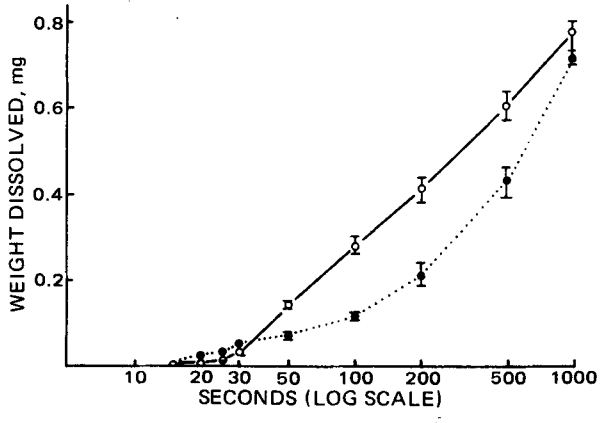

Figure 1—*Dissolution profiles for suspensions of Lot A prednisolone acetate (○) and Lot A prednisolone acetate containing 0.5% carboxymethylcellulose sodium (●) (n = 3; bars show extremes).*

[2] Coulter counter model TA II with a 200-μm aperture tube, Coulter Electronics, Hialeah, Fla.

[3] Isoton, Coulter Diagnostics, Miami Springs, Fla.

[4] Lot A was code 66010 MI 19636 and Lot B was code 66010 MI 70425, Schering Corp., Kenilworth, N.J.

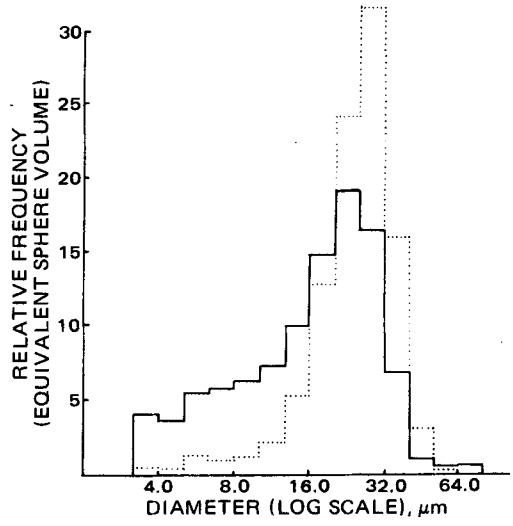

Figure 2—*Particle-size distributions of suspensions of Lot A prednisolone acetate (—) and Lot A prednisolone acetate containing 0.5% carboxymethylcellulose sodium (·····) (n = 3).*

prednisolone acetate dissolution. Dissolution of 0.4 mg required 500 sec when carboxymethylcellulose sodium was present, whereas an equal amount with no suspending agent dissolved in 200 sec.

Since dissolution characteristics are highly dependent on particle size, the particle-size distributions for the two suspensions were compared (Fig. 2). The distribution with the plain prednisolone acetate suspension showed an approximate mean diameter of 23 μm, and the suspensions containing 0.5% carboxymethylcellulose sodium had slightly larger particles with an approximate mean diameter of 29 μm. The major difference between the two distributions was the volume of fine particles found in each sample. The prednisolone acetate suspension without carboxymethylcellulose sodium had a considerably higher volume percent of fine particles when compared with the steroid suspension containing the suspending agent.

Dissolution experiments were run using 0.5 and 0.1% hydroxypropyl methylcellulose 4000 cps and 0.5% hydroxypropyl methylcellulose 50 cps as suspending agents (Fig. 3). The dissolution curves show that each of these suspending agents inhibited prednisolone acetate dissolution. The particle-size distributions were essentially identical to those in Fig. 2, where the suspensions containing suspending agents had somewhat larger mean particle diameters than the suspension without a suspending agent. The suspensions containing a suspending agent again contained fewer fine particles than the suspensions without one.

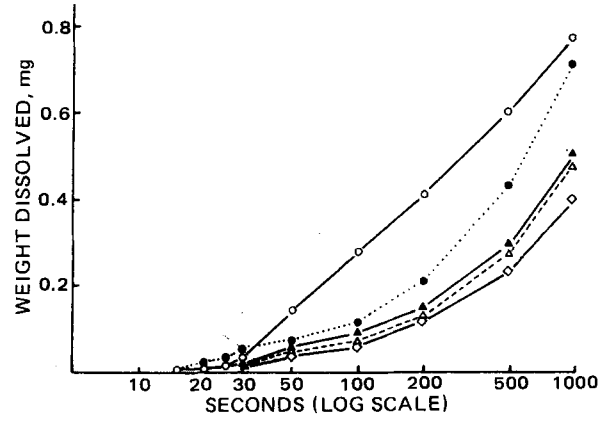

Figure 3—*Suspension dissolution profiles. Key: ○, Lot A prednisolone acetate; ●, Lot A prednisolone acetate containing 0.5% carboxymethylcellulose sodium; ▲, Lot A prednisolone acetate containing 0.5% hydroxypropyl methylcellulose 4000 cps; △, Lot A prednisolone acetate containing 0.1% hydroxypropyl methylcellulose 4000 cps; and ◇, Lot A prednisolone acetate containing 0.5% hydroxypropyl methylcellulose 50 cps.*

Table II—Particle-Size Analyses of Hydroxypropyl Methylcellulose Solutions and Lot A Prednisolone Acetate Suspensions (n = 3)

Hydroxypropyl Methylcellulose, 0.5%		Plain Prednisolone Acetate Suspension, 0.25%		Suspension of 0.25% Prednisolone Acetate Containing 0.5% Hydroxypropyl Methylcellulose		Particle-Size Diameter, μm
Percent Total Volume	Counts/ Channel	Percent Total Volume	Counts/ Channel	Percent Total Volume	Counts/ Channel	
Too few	1474	1.8	17,219	0.4	9,825	3.17
counts to	590	2.5	11,172	0.4	5,009	4.00
normalize data	269	4.2	7,879	1.1	3,309	5.04
	114	4.1	4,530	0.9	2,069	6.35
	59	4.5	4,530	0.9	2,069	6.35
	32	6.1	1,723	2.6	1,499	10.08
	20	10.1	1,388	6.1	1,695	12.70
	16	15.9	1,087	13.8	1,911	16.00
	9	22.5	769	24.6	1,693	20.20
	5	19.2	344	30.1	1,077	25.40
	2	7.3	70	15.1	297	32.00
	1	0.9	5	2.1	23	40.30
	1	0.1	0	0.8	4	50.80
	0	0.7	1	0.5	1	64.00
Total	2592	(99.9)	48,806	(99.9)	29,966	

The dissolution curves in Fig. 3 for Lot A show that all of the suspending agents retarded prednisolone acetate dissolution, with carboxymethylcellulose sodium having a lesser effect than hydroxypropyl methylcellulose.

Due to dilution with the large volume of the dissolution medium, the high viscosity of the suspension medium seemed to exert little or no effect on dissolution. Carboxymethylcellulose sodium was the most viscous suspension medium, and it showed the least dissolution retardation. The 0.5% carboxymethylcellulose sodium solutions showed a viscosity of 140 cps. Hydroxypropyl methylcellulose 50 cps, which showed the greatest dissolution retardation effect, was the least viscous suspending medium, with a 0.5% solution having a viscosity of 2.7 cps. Hydroxypropyl methylcellulose 4000 cps had a viscosity of 19.2 cps in a 0.5% solution. Figure 3 shows that the 0.1 and 0.5% solutions of hydroxypropyl methylcellulose 4000 cps had approximately the same retarding effects on prednisolone acetate; at least in this original concentration range, retardation was concentration independent.

The addition of a suspending agent was thought to contribute to particle-size increases, resulting in the dissolution inhibition. To determine the contribution of the suspending agents to the particle-size increase, a 0.5% hydroxypropyl methylcellulose 4000 cps solution was sized utilizing the electronic counter[2] population accessory. Prednisolone acetate suspensions with and without 0.5% hydroxypropyl methylcellulose 4000 cps and 0.5% solutions of hydroxypropyl methylcellulose 4000 cps were tested in the same manner. Exactly one-third of a milliliter of each solution or suspension was added to 170 ml of presaturated and filtered electrolyte solution in a 250-ml conical beaker. The percentages of relative volume and particle count for each channel were recorded (Table II).

As can be seen from Table II, the total and overall counts for the simple 0.5% hydroxypropyl methylcellulose solution after dilution were insignificant when compared to the counts for the prednisolone acetate suspension containing the same amount of suspending agent. Total counts for the 0.5% hydroxypropyl methylcellulose solution were approximately 2600, compared to 30,000 total counts for the prednisolone acetate suspension containing 0.5% hydroxypropyl methylcellulose. There were too few particles in the hydroxypropyl methylcellulose solution to calculate the percent total volume. Therefore, the presence of 0.5% hydroxypropyl methylcellulose does not in itself add counts or size to the suspension.

A comparison of the suspension containing no suspending agent and the suspension containing 0.5% hydroxypropyl methylcellulose 4000 cps was interesting. The total counts for the plain prednisolone acetate suspension (~48,000 counts) were much greater. Nevertheless, the number of counts in the larger channels (≥16 μm) was greater for the hydroxypropyl methylcellulose-containing suspension, especially in the 25.4- and 32-μm channels. The increased counts in the large channels account for the shift in the particle means for the relative volume distributions.

For particles less than 10.08 μm, the pure prednisolone acetate suspension had approximately twice the counts when compared with the suspension containing a suspending agent. This finding also was reflected in the relative volume percentage. The suspension without a suspending agent had ~17% of its total volume with particle sizes less than 10.08 μm, whereas the suspension containing a suspending agent had only 4% of its volume below 10.08 μm in particle size. These data substantiate the

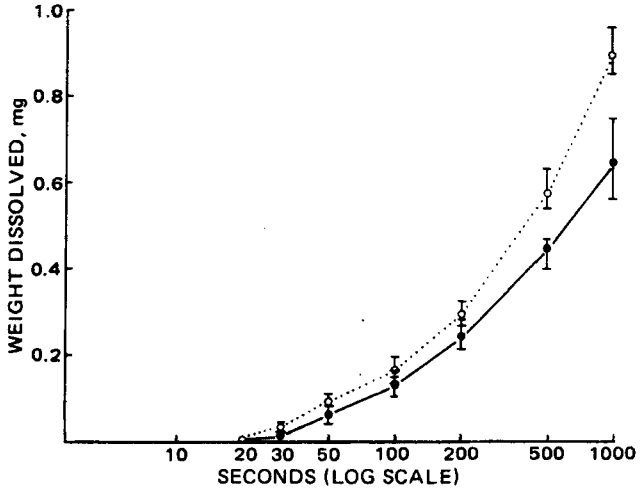

Figure 4—*Dissolution profiles for suspensions of Lot B prednisolone acetate (●) and Lot B prednisolone acetate containing 0.5% carboxymethylcellulose sodium (○).*

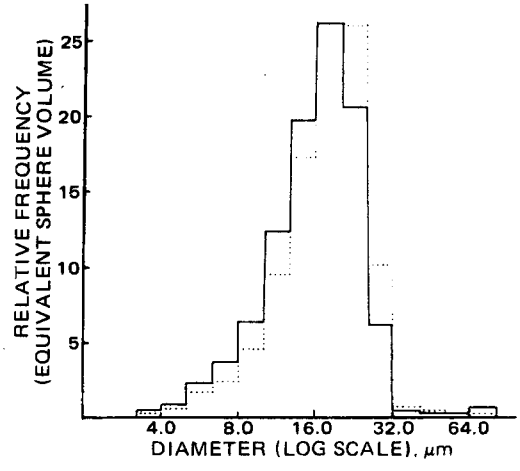

Figure 5—*Particle-size distribution of suspensions of Lot B prednisolone acetate (—) and Lot B prednisolone acetate containing 0.5% carboxymethylcellulose sodium (·····).*

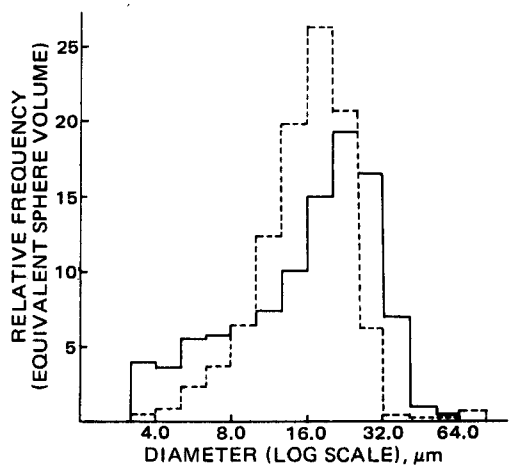

Figure 6—*Particle-size distributions of suspensions of Lot A (—) and Lot B (- - -) prednisolone acetate.*

idea that the suspending agent contributes to a particle distribution shift which, as will be demonstrated further, results in longer dissolution times for the steroid suspensions.

Lot A prednisolone acetate was exhausted at the end of these experiments. A new lot, Lot B, was obtained from the same supplier, and several experiments were repeated. The experimental conditions for Lot B were identical with those for Lot A.

The dissolution profiles for Lot B prednisolone acetate with and without 0.5% carboxymethylcellulose sodium are shown in Fig. 4. The 0.5% carboxymethylcellulose sodium did not inhibit dissolution. This finding is not consistent with previous dissolution data for Lot A prednisolone acetate, where carboxymethylcellulose sodium significantly inhibited steroid dissolution (Fig. 1). In view of this inconsistency, both lots were investigated further by differential scanning calorimetry, zeta-potential measurement, and particle-size distribution based on volume.

Pure prednisolone acetate from each lot (A and B) was studied with a differential scanning calorimeter at a heating rate of 40°/min. The thermograms showed no significant differences. Thus, the crystalline forms of each lot apparently were the same and polymorphic forms were not the cause of the dissolution differences.

The electromobilities of Lots A and B as determined with a zeta-meter were identical, indicating that the surface nature of the two lots was the same.

The size distributions based on volume for Lot B prednisolone acetate with and without 0.5% carboxymethylcellulose sodium are shown in Fig. 5. The presence of carboxymethylcellulose sodium caused a shift to the right on the volume distribution curve. The magnitude of the shift was much less pronounced, particularly in the area of fine particles, when compared with similar data for Lot A (Fig. 2). A further comparison of particle-size characteristics is shown in Fig. 6 where the particle-size distribution for Lots A and B prednisolone acetate are presented. These

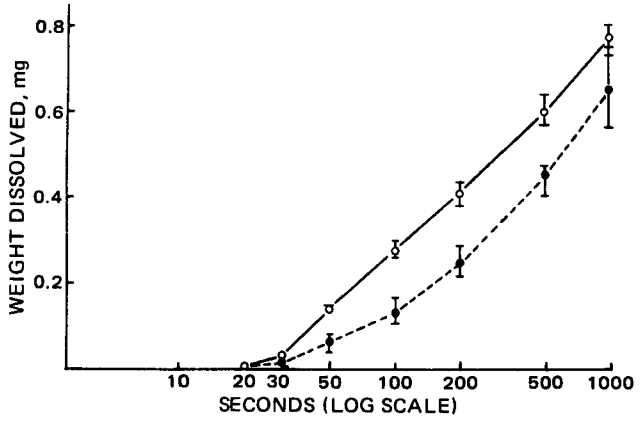

Figure 7—*Dissolution profiles for suspensions of Lot A (○) and Lot B (●) prednisolone acetate.*

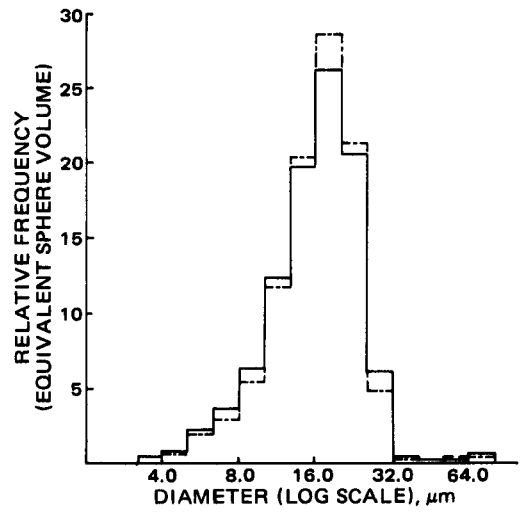

Figure 8—*Particle-size distributions of suspensions of Lot B prednisolone acetate (—) and Lot B prednisolone acetate containing 0.5% hydroxypropyl methylcellulose 4000 cps (- · -).*

data show that Lot A contained considerably more fine particles than did Lot B. In fact, 20% of the population for Lot A based on volume was ≤10 μm, whereas only 10% by volume of Lot B was in the same size range. Thus, the dissolution rate for Lot A was expected to exceed that for Lot B because of increased surface area. This rationalization was substantiated by comparing the dissolution profiles for Lots A and B. The dissolution data are shown in Fig. 7 where the initial dissolution rate for Lot A exceeded that for Lot B.

It is apparent that Lot A prednisolone acetate dissolved at a more rapid rate than Lot B (Fig. 7) because of particle-size differences (Fig. 6). Therefore, it is believed that carboxymethylcellulose sodium interacts with prednisolone acetate in some manner and that this interaction may have a twofold effect: (*a*) it might reduce the number of fine particles (≤10 μm) in suspension (dominant in Lot A), and (*b*) it might enhance the dissolution of larger prednisolone acetate particles (dominant in Lot B). With Lot A, where many fine particles exist, the removal of fine particles predominates and an apparent dissolution reduction occurs. With Lot B, there are fewer fine particles initially and an apparent prednisolone acetate dissolution increase occurs (Fig. 4) when carboxymethylcellulose sodium is present.

Unlike carboxymethylcellulose sodium, hydroxypropyl methylcellulose inhibited the dissolution of Lot B prednisolone acetate. This inhibition

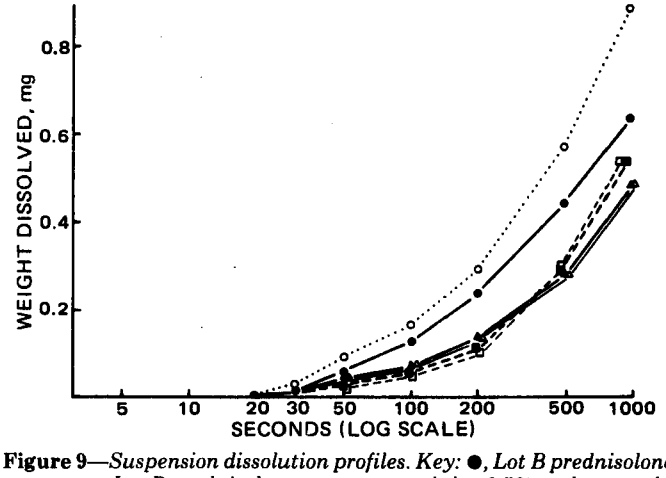

Figure 9—*Suspension dissolution profiles. Key: ●, Lot B prednisolone acetate; ○, Lot B prednisolone acetate containing 0.5% carboxymethylcellulose sodium; ▲, Lot B prednisolone acetate containing 0.5% hydroxypropyl methylcellulose 4000 cps; △, Lot B prednisolone acetate containing 0.5% hydroxypropyl methylcellulose 50 cps; ■, Lot B prednisolone acetate containing 0.1% hydroxypropyl methylcellulose 4000 cps; and □, Lot B prednisolone acetate containing 0.1% hydroxypropyl methylcellulose 50 cps; n = 3.*

was unrelated to the particle size. The size distributions for suspensions of Lot B prednisolone acetate with and without the presence of hydroxypropyl methylcellulose were almost identical (Fig. 8).

The dissolution profiles for Lot B prednisolone acetate suspensions are summarized in Fig. 9. The presence of hydroxypropyl methylcellulose in all concentrations and at all viscosities seemed to inhibit the dissolution of Lot B. This finding is similar to the results found in experiments with Lot A. As with Lot A, the degree of inhibition did not appear to depend on the original viscosity or concentration of hydroxypropyl methylcellulose used. The 0.1 and 0.5% hydroxypropyl methylcellulose 4000 cps and the 0.1 and 0.5% hydroxypropyl methylcellulose 50 cps all inhibited Lot B prednisolone acetate to approximately the same degree.

The agents studied are common suspending agents, and brand-to-brand variations in the dissolution of commercial prednisolone acetate products exist (5). Particle-size differences are not the only factors to consider when studying the variation of drug dissolution profiles. Other factors such as the method of manufacture (milling and order of milling and mixing) and aging may alter these results.

In conclusion, the common ionic suspending agent carboxymethylcellulose sodium acted in two ways. It seemed to alter the particle-size distribution by reducing the percentage of fine prednisolone acetate particles in suspension, and it enhanced prednisolone acetate dissolution. With Lot A prednisolone acetate, which contained a larger percentage of fine particles, the reduction of these fine particles was sufficient to exceed the concurrent dissolution rate increase. Therefore, the net result was decreased prednisolone acetate dissolution. But with Lot B, where the proportion of fine particles was small and there was no significant change in particle-size distribution, the dissolution enhancement properties of carboxymethylcellulose sodium predominated. Thus, the effects of carboxymethylcellulose sodium on prednisolone acetate dissolution were lot-to-lot dependent, with the main variable being the particle distribution of the powder.

Dissolution inhibition of the two lots by nonionic hydroxypropyl methylcellulose, which also can alter particle-size distribution, was independent of lot-to-lot variation. This inhibition might be due to the formation of a viscous diffusion layer similar to that described for methylcellulose (4). Since hydroxypropyl methylcellulose is used in many commercial prednisolone acetate suspensions, this finding should be considered by formulation scientists.

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Corticosteroid Determination in Skin Preparations by a Reaction Rate Method

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Received March 19, 1979, from the **Laboratory of Analytical Chemistry, University of Athens, Athens, Greece,* and the ‡*School of Chemical Sciences, University of Illinois at Urbana-Champaign, Urbana, IL 61801.* Accepted for publication June 5, 1979.

Abstract □ A reaction rate method for the determination of betamethasone, betamethasone valerate, triamcinolone acetonide, and fluocinolone acetonide is described. The method is based on a modification of the widely accepted blue tetrazolium reaction. Analysis times of 30–70 sec are required. Relative standard deviations of 0.3–1.9% are obtained, and the analytical working curves are linear. Analysis of pharmaceutical skin preparations by the new method gave results that correlated well with the time-consuming standard equilibrium method. Analysis of betamethasone and betamethasone valerate mixtures by measuring absorbance values at two different times was performed also.

Keyphrases □ Corticosteroids—analysis, skin preparations, reaction rate method □ Betamethasone—analysis, skin preparations, reaction rate method □ Betamethasone valerate—analysis, skin preparations, reaction rate method □ Triamcinolone acetonide—analysis, skin preparations, reaction rate method □ Fluocinolone acetonide—analysis, skin preparations, reaction rate method □ Skin preparations—analysis, corticosteroids, reaction rate method

A widely used spectrophotometric method for the determination of corticosteroid purity and the potency of dosage forms containing such steroids is based on the blue tetrazolium reaction (1). Blue tetrazolium (I), 3,3′-(3,3′-dimethoxy[1,1′-biphenyl]-4,4′-diyl)bis[2,5-diphenyl-2*H*-tetrazolium] dichloride, in an alcoholic solution of a strong base oxidizes the α-ketol group on the C_{17} side chain and is reduced quantitatively to red formazan, which is measured spectrophotometrically at 525 nm. This absorbance, measured a specified time after mixing of the sample with I and the base, is compared to the absorbances of a blank and of a standard solution to quantitate the steroid concentration in the sample. This procedure is the basis for the official NF (2) and USP (3) method.

BACKGROUND

Extensive investigations (4–13) of the reaction conditions established that the analytical procedure is subject to many variables such as temperature; solvent; concentrations of base, water, and tetrazolium; steric configuration of the corticosteroid molecule; light; and air oxygen. The effect of these variables is minimized by analyzing reagent blanks, standards, and samples concurrently. In addition to these problems, which decrease the precision and the accuracy of the official equilibrium method, long measurement times of 30–470 min are necessary for maximum absorbance (10).

Under the conditions usually employed in the assay, where the steroid

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