was believed to have been formed after elimination of hydrobromide from this displacement intermediate.

Samour *et al.* (12) reported that some alkoxymethyl derivatives of I were effective anticonvulsants. Therefore, it was of interest to test some alkyl derivatives for anticonvulsant activity. Three compounds representing three different chemical structures were studied. Results of the biological testings showed that VI, XIV, and XVII, when suspended in 10% acacia and given orally, exhibited no activity against pentylenetetrazol-induced seizures in mice at a dose of 1 g./kg. According to the acute toxicity studies, these compounds appeared to be relatively nontoxic. None of the animals died as a result of oral administration at a 2-g./kg. dose level, which was the maximum amount of the compound that could be suspended in an appropriate volume of 10% acacia.

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Kinetics and Factors Affecting Stability of Methylprednisolone in Aqueous Formulation

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Abstract ☐ An investigation was made of the factors affecting the rate of degradation of methylprednisolone solubilized by polysorbate 80 in an aqueous formulation containing polar additives and a sequestering agent as a function of temperature at pH 4.6 and 5.1. The presence of polar additives decreased the apparent solubility of methylprednisolone in one formulation at pH 4.6 and 25°. However, since methylprednisolone was in solution, but not within the polyoxyethylene exterior of the polysorbate 80 micelles, it degraded at a faster rate. By selecting the proper concentration of polysorbate 80 and adjusting to the same pH of 4.6 in another formulation, the autoxidative degradation rate of the primary alcoholic group at C-21 was reduced to approximately half even in the presence of oxygen. The increase in stability was also evident from the increase of the apparent activation energy from 18.2 to 23.1 kcal./mole. The mechanism of solubilization and stabilization based on hydrogen bonding and inclusion into the polyoxyethylene exterior of the polysorbate 80 micelles is proposed.

Keyphrases ☐ Methylprednisolone aqueous formulations—effect of polysorbate 80 solubilization on stability, mechanism, kinetics ☐ Polysorbate 80—effect on methylprednisolone stability in aqueous formulations, mechanism of solubilization ☐ Solubilization, methylprednisolone—effect of polysorbate 80 on stability in aqueous formulations, mechanism, kinetics ☐ Stabilization of methylprednisolone in aqueous formulations—polysorbate 80 solubilization

In the pharmaceutical field, the phenomenon of micellar solubilization of drugs in aqueous solutions of surfactants is used not only for solubilizing the drug but also for protecting against degradative processes such as hydrolysis and autoxidation. The stabilization of esters against alkaline hydrolysis in aqueous solutions containing nonionic, cationic, and anionic surfactants has been reported (1–3). Vitamin A alcohol solubilized in an aqueous nonionic surfactant solution was reported (4) to be more stable to autoxidation than vitamin A solubilized in cottonseed oil. Similarly, vitamin A alcohol solubilized in aqueous nonionic surfactant solution containing 30% (w/v) glycerin was more stable to autoxidation than vitamin A solubilized in arachis oil (5).

Nonionic surfactant polysorbate 80 (polyoxyethylene 20 sorbitan monooleate) increased the solubility and the stability of methylprednisolone in aqueous solutions prepared by heating between 40° and the decomposition point (6). However, the quantitative data regarding the extent of stabilization of methylprednisolone were not presented (6).

This article deals with the factors affecting the chemical stability of methylprednisolone solubilized by

Table I—Compositions (Milligrams per Milliliter) of Various Formulations Studied

Ingredients	Lot A	Lot B	Log C	Lot D-1	Lot D-2	Lot D-3	Lot E
Polysorbate 80, purified	35.8	35.8	35.8	41.0	41.0	41.0	37.0
Disodium edetate	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Methylprednisolone	0.55	0.55	0.575	0.575	0.575	0.575	0.525
Neomycin base	22.00	22.00	22.00	22.00	22.00	22.00	22.00
Lincomycin base	22.00	22.00	22.00	22.00	22.00	22.00	22.00
Hydrochloric acid	*q.s. ad.*[a] pH 5.0	*q.s. ad.* pH 5.0	*q.s. ad.* pH 4.6	*q.s. ad.* pH 4.6	*q.s. ad.* pH 4.6	*q.s. ad.* pH 4.6	*q.s. ad.* pH 4.6
30% sodium hydroxide solution	*q.s. ad.* pH 5.0	*q.s. ad.* pH 5.0	*q.s. ad.* pH 4.6	*q.s. ad.* pH 4.6	*q.s. ad.* pH 4.6	*q.s. ad.* pH 4.6	*q.s. ad.* pH 4.6
Purified water	*q.s. ad.*	*q.s. ad.*	*q.s. ad.*	*q.s. ad.*	*q.s. ad.*	*q.s. ad.*	*q.s. ad.*

[a] Adjust to final volume.

polysorbate 80 in aqueous formulations and also the mechanism of solubilization and stabilization. Since the other drug components present were extremely stable, their stability data are not reported.

EXPERIMENTAL

Materials—The following were used: polysorbate 80 USP, purified[1]; disodium edetate USP[2]; methylprednisolone NF, sterile micronized; neomycin sulfate powder USP; lincomycin hydrochloride monohydrate[3]; hydrochloric acid, analytical reagent grade; 30% sodium hydroxide solution; and purified water USP. Since polysorbate 80 USP contained 7.2–14.8% of polyoxyethylene glycol, it was purified by the procedure of Malkemus and Swan (7). The presence or absence of polyoxyethylene glycol in the surfactant was determined by the method of Ginn *et al.* (8). Other ingredients were used as received.

Preparation of Formulations—The formulations shown in Table I were prepared using Procedure B of Johnson (6). Polysorbate 80 and disodium edetate were dissolved in 90% of the purified water.The solutions were then heated to 58–63°, and methylprednisolone was added and dissolved by stirring for 90 min. or until dissolved. Solutions were allowed to cool to 30° or below, and neomycin sulfate and lincomycin hydrochloride monohydrate were added and dissolved by continuous stirring. The solutions were allowed to stand for at least 2 hr., the pH was adjusted (initial pH approximately 6.9), and purified water was added to adjust the final volume. The solutions were then filtered through a 0.45-μ cellulose filter[4] and packaged. These packaged samples were stored in a constant-temperature oil bath, dry heat oven, or metal cabinets in a room equilibrated at 25°. Samples were removed periodically and assayed according to the general procedure for methylprednisolone by blue tetrazolium using a semiautomated analyzer. Methylprednisolone, having a primary α-keto group adjacent to a primary hydroxyl group, reacts with blue tetrazolium in the presence of tetramethylammonium hydroxide to form a colored solution. The blue tetrazolium is reduced and the absorbance of the color complex is measured spectrophotometrically in comparison with a standard solution. A decrease in potency, as determined by this method, indicates the degradation of the side chain at the C-17 position.

Solubility Profile: Polysorbate 80–Water Solutions—*Equilibration*—Polysorbate 80–water solutions containing excess methylprednisolone were equilibrated at 25° for 5 days in 100-ml. vials in a constant-temperature water bath, equipped with an oscillating-type shaker[5]. The vials were then allowed to stand for 2 days at 4°, and the contents were filtered through a 0.45-μ cellulose filter[4] and assayed.

Assay Procedure—The aqueous polysorbate 80-methylprednisolone solutions were diluted 1:25 with 95% 3A alcohol. Polysorbate 80 solution diluted in the same manner as the sample was used as a blank in the assay to eliminate error due to the absorbance of the surfactant. The absorption maximum at 243 nm. was used to calculate the steroid concentration. An absorptivity (1%, 1 cm.) of

398.11 was used. The presence of polysorbate 80 at the dilutions used did not significantly change the absorptivity value from that obtained with solution in 95% alcohol. Since the samples were analyzed immediately after dilution, the degradation of the side chain at the C-17 position was considered to be insignificant.

Solubility Profile: Polysorbate 80–Water Formulations—*Preparation of Formulations*—Polysorbate 80–water formulations containing various amounts of polysorbate 80 were prepared following Procedure B of Johnson (6) and adjusted to pH 4.5 and 5.0. These formulations were similar to Lot A of Table I, except that they did not contain methylprednisolone and the amounts of polysorbate 80 were the same as in the polysorbate 80-water solutions.

Equilibration—The same method of equilibration followed for polysorbate 80-water solutions was used.

Assay Procedure—Methylprednisolone was determined according to the general procedure for steroids by blue tetrazolium.

RESULTS AND DISCUSSION

The rate of autoxidation reactions in solution depends upon the pH, thermal energy of UV light, oxygen concentration, and concentration of heavy metal ions. Since the degradation of prednisolone (9) in aqueous buffer solutions containing disodium edetate was less at pH 4 than at pH 5.0, these formulations (Table I) were made to contain disodium edetate and the pH was adjusted between 4.5 and 5.0. A pH of 4.5 was selected as the lower limit because polysorbate 80 hydrolyzed at pH values lower than 4.0 and at high temperatures, producing an irreversible colloidal milky precipitate.

The primary degradation pathway for methylprednisolone in these formulations appears to be autoxidation of the primary alcoholic grouping at C-21. This is consistent with the observation that, in alkaline aqueous solutions and in the presence of air, prednisolone degraded to a steroidal acid as a major component (10). Thus, the pH of the formulations decreased. The photolytic degradation of methylprednisolone observed (11) in aqueous solutions of tyloxapol (oxyethylated tertiary octylphenol formaldehyde polymer)[6] was absent. This was confirmed using TLC and assaying the methanol eluate spectrophotometrically at 242 nm. for the 22-month old samples of Lots A and B stored at room temperature. The concentration of methylprednisolone in both lots was unchanged from the initial value. Also, the photolytic degradation of the formulations packaged in ampuls and stored in an oil bath would be at a minimum, since the oil baths were located in a room where the UV light exposure was negligible.

The autoxidation of methylprednisolone followed an apparent first-order process under all experimental conditions. Typical first-order plots of the thermal degradation of methylprednisolone are shown in Figs. 1 and 2.

The effects of various factors on the apparent first-order thermal degradation of methylprednisolone in aqueous formulations are shown in Table II. The apparent first-order rate constants were calculated from the slopes of log percent residual concentration–time data by means of a least-squares regression analysis.

Effect of Oxygen Concentration—The concentration of oxygen present does affect the rate of degradation of methylprednisolone. The magnitude of the apparent first-order rate constant at room temperature for Lot B, packaged in syringes (Roehr) sealed in aluminum foil with no head space, was 1.5 times smaller than that

[1] Tween 80, Atlas Chemical Ind., Wilmington, Del.
[2] Dow.
[3] Upjohn.
[4] Millipore filter type HA with clarifying pads, Millipore Corp., Bedford, Mass.
[5] Temperature control water bath shaker, model 2156, Research Specialists, Berkeley, Calif.

[6] Triton WR 1339, Rohm & Haas Co., Philadelphia, Pa.

Table II—Various Factors Affecting the Apparent First-Order Thermal Degradation of Methylprednisolone in Aqueous Formulations

Lot Number and Type of Package	Methylprednisolone[a], Initial Concentration, %	Amount of Polysorbate 80, mg./ml.	Initial pH[b]	k_{app}, months^{-1} × 10^3				
				25°	40°	47°	54°	56°
Lot A; 10-ml. ampuls	106.0	35.80	5.10	—	49.81	98.37	167.4	—
Lot A; 30-ml. Type I glass vials with butyl rubber plugs	106.0	35.80	5.10	11.25	—	—	—	—
Lot B; 10-ml. syringes[c] sealed in aluminum foil, and no head space	107.0	35.80	5.15	7.09	--	—	—	—
Lot C; 10-ml. x' presit syringes[d]	112.0 113.8	35.80	4.60	9.78	--	—	—	—
Lot D-1; 10-ml. x' presit syringes[d]	116.6	41.00	4.55	4.12	21.43	—	—	—
Lot D-2; 10-ml. x' presit syringes[d]	117.6	41.00	4.60	4.59	23.25	—	—	—
Lot D-3; 10-ml. ampuls	114.8	41.00	4.60	—	—	—	—	171.1
Apparent first-order rate constant (average D) for Lots D-1, D-2, and D-3				4.36	22.34	—	—	171.1
Lot E; 10-ml. x' presit syringes[d]	104.0	37.00	4.60	4.98	—	—	—	—

[a] Methylprednisolone, 0.5 mg./ml., is equivalent to 100.0%. [b] Initial pH = ±0.05 unit. [c] Roehr Monoject barrels and plungers of high density polypropylene. Snap-on rubber plugs made from natural rubber. [d] Barrels and plungers of high density polypropylene. Snap-on rubber plugs made from natural rubber. Composition similar to Roehr Monoject syringes.

of Lot A, packaged in 30-ml. vials and having a head space (Table II). The amount of polysorbate 80 and the pH were the same in these lots.

Effect of Purified Polysorbate 80 Concentration— The amount of polysorbate 80 used in these formulations plays a vital role in stabilizing methylprednisolone, as can be seen from the higher value of the apparent first-order rate constant at room temperature for Lot C than Lot D-1 (Table II). Lot C was prepared using 3.58% (w/v) polysorbate 80 to dissolve 0.575 mg./ml. of methylprednisolone. This amount of polysorbate 80 was higher than the actual value of 3.35% (w/v) obtained from the solubility profile for methylprednisolone in polysorbate 80-water solutions (Fig. 3). Surprisingly, the solubility of methylprednisolone decreased in the presence of soluble polar additives, as seen in the solubility profile for methylprednisolone in polysorbate 80-water formulations adjusted to pH 5 (Fig. 3). Thus, Lot D-1 was made with 4.1% (w/v) polysorbate 80 to dissolve 0.575 mg./ml. methylprednisolone. The solubility profile at pH 4.5 in polysorbate 80-water formulations (not shown) had exactly the same slope as the solubility profile at pH 5.

An increase in pH decreases the micellar molecular weight and the hydration per unit mass of surfactant (12). This would be expected to reduce the solubility at pH 5. However, this was not observed since the pH range of formulations studied was very narrow.
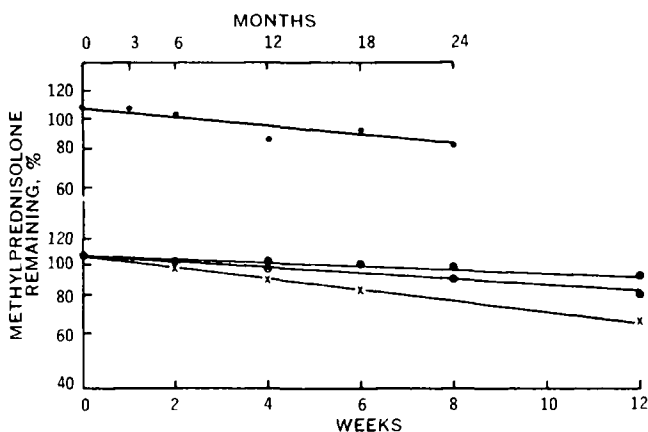
Effect of Temperature on Solubilization of Methylprednisolone—An increase in temperature increased the solubility of methylprednisolone. Furthermore, the methylprednisolone stayed in solution even after Lot D-1 was frozen, allowed to come to room temperature, filtered, and assayed. The assay results were exactly the same when compared with those for Lot D-1 stored at 4°. These results show that methylprednisolone will be in solution regardless of the temperature used during the manufacture of the formulation.

Effect of pH and Temperature—As stated previously, the pH of the formulations decreased, possibly due to the steroidal acids produced by the autoxidation of methylprednisolone. However, the extent of pH decrease in Lots A and D was the same; namely, 0.15 pH unit after 24 months at 25°. Likewise, the extent of pH decrease in Lots A and D was also the same; namely, 0.25 pH unit after 6 months at 40°.

Arrhenius plots for the degradation of methylprednisolone in Lots A and D are shown in Fig. 4. The apparent activation energies calculated from the least-squares slopes of the apparent first-order rate constants versus the reciprocal of absolute temperatures for Lots A and D are shown in Table III. Also shown are the values of $t_{90\%}$, the time in months required to reach 90% of methylprednisolone concentration in Lots A, C, D, and E, which were stored at room temperature. From the data in Table III, it is apparent that the shelflife of methylprednisolone in these aqueous formu-

Figure 1 Apparent first-order plots of the thermal degradation of methylprednisolone in Lot A, containing 3.58% (w/v) purified polysorbate 80, at pH 5.1. Key: (top) •, 25°; (bottom) ●, 40°; ○, 47°; and ×, 54°.
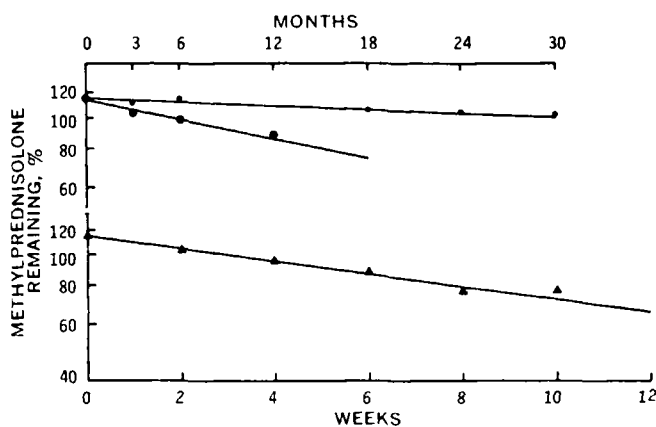

Figure 2—Apparent first-order plots of the thermal degradation of methylprednisolone in Lots D-1 and D-3, containing 4.1% (w/v) purified polysorbate 80, at pH 4.6. Key: •, 25°; ●, 40°; and ▲, 56°.
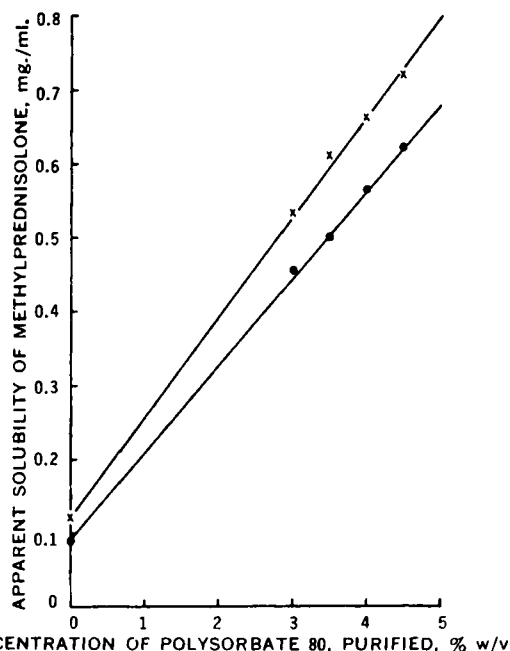
Figure 3—*Solubility profile for methylprednisolone in polysorbate 80-purified water solutions* (X) *and in polysorbate 80-purified water formulations* (●) *adjusted to pH 5.0.*

lations is increased by adjusting to a proper pH and selecting the proper concentration of the nonionic surfactant.

Mechanism of Solubilization and Stabilization—Several articles described the mechanism of solubilization of drugs in the presence of surfactants (3, 13, 14). In these formulations, no attempts were made to determine the exact mechanism of micellar solubilization of methylprednisolone. However, the probable mechanism seems to be hydrogen bonding of the polar groups of the steroid with the hydrated polyoxyethylene portion on the surface, as well as incorporation into the polyoxyethylene groups of the polysorbate 80 micelles. The mechanism of hydrogen bonding appears to be adsorption of water-soluble polar additives (15) in competition with methylprednisolone adsorption on the exterior surface of the nonionic micelle. The data from the solubility profile (Fig. 3) indicate that a possible mechanism may also be incorporation of methylprednisolone into the polyoxyethylene group of the polysorbate 80 micelle. Since a large excess of polar additives (neomycin
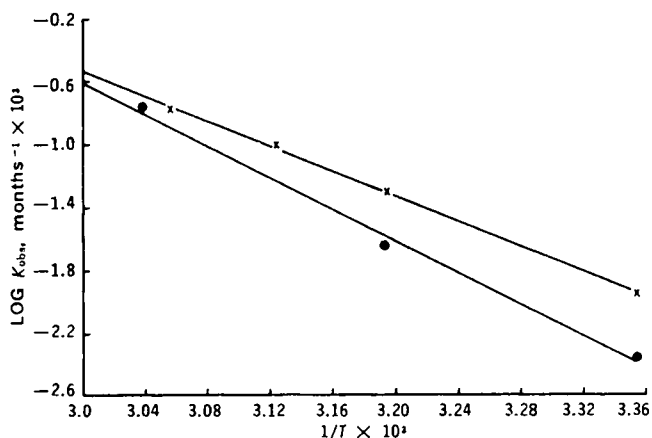

Figure 4—*Arrhenius plots for the degradation of methylprednisolone. Key:* X, *Lot A, 3.58% (w/v) purified polysorbate 80 and initial pH of 5.1; and* ●, *Lot B, 4.1% (w/v) purified polysorbate 80 and initial pH of 4.6.*

Table III—Apparent Activation Energies

Lot Number	E_a, kcal./mole	$t_{90\%}{}^a$, Months at Room Temperature
A	18.22	17.85
C	—	25.08
D[b]	23.09	56.38
E	—	30.98

[a] See Table I for initial concentration of methylprednisolone; 0.5 mg./ml. is considered as 100%. [b] See Table II.

sulfate and lincomycin hydrochloride) is present and adsorbs on the exterior surface of the polysorbate 80 micelle, there is more likelihood that methylprednisolone is solubilized into the exterior of the polysorbate 80 micelle. Thus, since a certain amount of methylprednisolone was free in the aqueous phase of Lot C, pH 4.6, it degraded approximately twice as fast as Lot D-2, pH 4.6, where, methylprednisolone was associated on the surface and into the polyoxyethylene exterior of the polysorbate 80 micelle. Because the aggregation number and the amount of water bound per unit mass of nonionic surfactant are greater at low pH values (12), oxygen attack was slower in Lot C, pH 4.6, than in Lot A, pH 5.1.

At 54–56°, the ratio of rate constants $k_{app(Lot\ A)}/k_{app(Lot\ D)}$ is closer to 1, which increases approximately to 2.6 at 25°. This can be interpreted as follows. At 54–56°, the micellar structure is disrupted in Lot D, thus preventing inclusion of the methylprednisolone into the exterior of the polysorbate 80 micelles. Consequently, the rate of degradation in Lot D is approximately the same as in Lot A. At 25°, there is negligible disruption of the micellar structure in Lot D, resulting in a lower k_{app} and thus a higher ratio of rates of degradation.

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