Effect of Formulation Factors on Penetration of Hydrocortisone through Mouse Skin

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Abstract □ The effect of formulation factors on the steady-state flux of hydrocortisone through mouse skin was evaluated. The flux of hydrocortisone from solutions containing propylene glycol as a cosolvent varied inversely with the propylene glycol concentration. Solutions containing 2-propanol gave flux values higher than those obtained from propylene glycol solutions and independent of the 2-propanol concentration. Addition of polysorbate 80 to 2-propanol–water solutions produced an increase in flux at low surfactant concentrations that reached an apparent limiting value at higher concentrations. The penetration flux was the same from solutions and gels. The role of vehicle–skin interactions in penetration is emphasized.

Keyphrases □ Hydrocortisone—penetration through mouse skin, effect of formulation factors □ Penetration—hydrocortisone through mouse skin, effect of formulation factors □ Glucocorticoids—hydrocortisone, penetration through mouse skin, effect of formulation factors

The effect of formulation additives on drug permeation through skin has been investigated (1–3). The penetration rate of a topical agent may be influenced by drug–vehicle, drug–skin, and vehicle–skin interactions. In the clinical assessment of a topical agent, the vehicle may significantly affect drug release and skin penetration, thereby altering biological activity (4–7). If drug–skin and vehicle–skin interactions can be ignored, optimal penetration takes place when the drug concentration in the vehicle is equal to its solubility (6).

The release characteristics of a topical vehicle have been studied by determining the partition coefficient of the drug between the vehicle and a suitable organic solvent (4, 6). However, to study the effect of vehicles on the barrier function of the stratum corneum, permeation experiments using skin as a membrane are more meaningful. The use of *in vitro* diffusion models to screen topical vehicles to optimize drug penetration is convenient. Furthermore, there is evidence that results obtained with excised human skin in a diffusion cell correlate with those obtained *in vivo* (8).

This study investigated the effect of vehicle composition on percutaneous absorption of hydrocortisone, using excised mouse skin as a model membrane. Mouse skin was chosen for permeation studies for two reasons: the rank order of permeation of several drugs through skin of various animals and human skin was the same, and the permeation rates were always slowest in the human. Hydrocortisone penetrates human skin very slowly (10), requiring about 2 weeks to approach a steady state.

EXPERIMENTAL

Materials—Hydrocortisone[1] was used as received. 2-Propanol[2] and chloroform[2] were spectral grade. Propylene glycol[3], 1-octanol[2], sodium chloride[2], and chlorobutanol[4] were reagent grade. The surfactant used in some formulations was polysorbate 80[5]. Hydroxyethylcellulose[6] was employed as a gelling agent. All materials were used as supplied. Water was double distilled in an all-glass still.

Preparation of Solutions—Accurately weighed amounts of hydrocortisone were dissolved in the desired volume of propylene glycol or 2-propanol, and distilled water was added to volume. Solutions containing surfactant were prepared by adding an aliquot of an aqueous solution of the surfactant to the previously dissolved hydrocortisone. The solutions were stored in a refrigerator for about 36 hr and assayed for hydrocortisone before use.

Preparation of Gels—An aliquot of the hydrocortisone solution was transferred to a glass-stoppered conical flask and allowed to cool in the refrigerator. An appropriate amount of hydroxyethylcellulose was sprinkled on the surface of the cooled solutions, and the polymer was dissolved by intermittent shaking of the flask. The clear gels were stored in the refrigerator for 48 hr before use.

Preparation of Membranes—Excised abdominal skin of 15–20-week-old male albino mice, 20–25 g, was used. The skin was shaved on the epidermal side. Tissues on the dermal side up to and including the blood vessels were removed. The skin was washed with distilled water and mounted in the diffusion cell.

Apparatus—An *in vitro* cell similar to the one described by Weiss and Sciarrone (11) was used. The cell was fitted with an air condenser to minimize evaporation of the hydrocortisone solution or gel. The temperature of the water flowing in the closed circulatory system was kept at 24.5 ± 0.1°. The receptor phase consisted of normal saline solution containing 0.25% chlorobutanol.

Donor samples, 1 ml, were placed on the epidermal side of the membrane. Samples of the receptor phase, 25 ml, were removed at intervals and assayed for hydrocortisone. Saline containing chlorobutanol was immediately added to the receptor phase to maintain a constant volume. The donor phase was not stirred, but a magnetic stirrer was used in the receptor phase. The stirring rate was 60 rpm.

Assay—An extraction procedure was used. A 10-ml aliquot of a sample containing hydrocortisone was pipetted into a 15-ml centrifuge tube. Chloroform–octanol (3:1), 5 ml, was added, and the tube was shaken mechanically for 15 min and then centrifuged. The aqueous phase constituted the top layer in the tube. Any organic phase floating on the top was removed by aspiration.

The aqueous phase absorbance was read[7] at 242 nm with water as the blank. The difference in absorbance, ΔA, of the aqueous layer before, A_b, and after, A_e, extraction was proportional to the hydrocortisone concentration. A ΔA *versus* concentration plot was linear, passing through the origin. Chlorobutanol and sodium chloride did not interfere. Compounds that entered the receptor phase from the skin membranes had no effect on the accuracy of the assay.

Solubility Studies—An excess of hydrocortisone was weighed and transferred to a 25-ml volumetric flask, and the cosolvent was pipetted into the flask. Where surfactant was included, the required amount was then added as an aqueous solution. The system was then diluted to volume with distilled water. The flasks were allowed to stand in a water bath thermostatically controlled at 24.5 ± 0.1°. The solutions were hand shaken at intervals, filtered, and analyzed daily for several days.

A second technique was used as a check. Aliquots of 25 ml of the preconstituted solvents were pipetted into 50-ml volumetric flasks containing 0.3 g of hydrocortisone. The flasks were then placed in a shaker thermostatically controlled at 24.5 ± 0.1°. The solutions were analyzed at 48 and 96 hr after filtration. Both methods gave essentially equivalent results.

[1] Merck Sharp and Dohme, West Point, Pa.
[2] Fisher Scientific Co., Fair Lawn, N.J.
[3] Ruger Chemical Co., Irvington, N.J.
[4] City Chemical Co., New York, N.Y.
[5] Atlas Chemical Industries, Wilmington, Del.
[6] Natrosol 250M, Hercules Inc., Wilmington, Del.
[7] Beckman spectrophotometer.

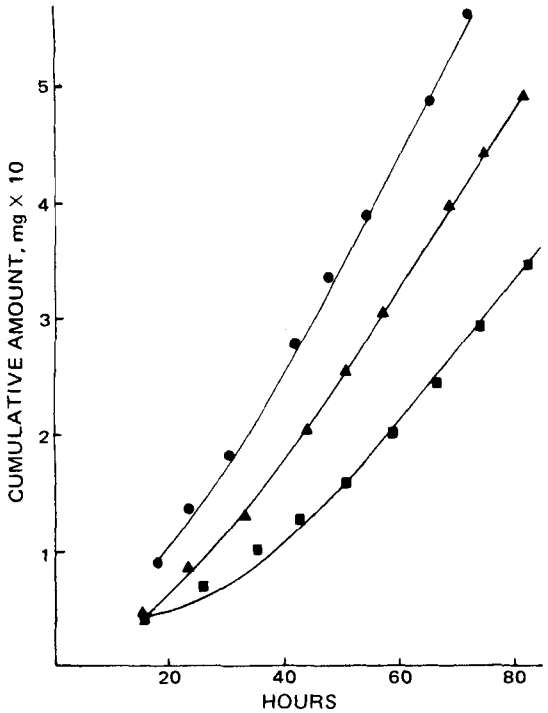

Figure 1—*Hydrocortisone penetration through mouse skin from a 0.2% solution in 40% (v/v) 2-propanol and water. Each curve represents skin from a different animal.*

Surface Tension—Surface tension values were determined by the Wilhelmy plate method (12).

RESULTS AND DISCUSSION

Figure 1 shows typical penetration curves obtained using different skin membranes with the same hydrocortisone formulation. The volume of the donor solution remained constant during the experiments. The penetration rate was slow at first but gradually increased until it became constant; it remained constant until about 50% of the hydrocortisone was transferred to the receptor phase. If the volume of the donor phase was changed from 1.0 to 0.5 ml, there was no significant change in the steady-state penetration rates.

To evaluate the effect of formulation on hydrocortisone penetration, the steady-state rate for each experiment was determined and divided by the exposed area of the membrane to yield the penetration flux. Each experiment was performed at least in triplicate. The values of penetration flux were averaged, and the mean was utilized as an index of percutaneous absorption for that formulation.

During the steady-state phase, the transdermal route is believed to represent the major pathway for permeation through skin (13). Other routes involving penetration through hair follicles, sweat ducts, and sebaceous glands also may contribute, but these routes are most important before steady state is reached. Unless there is restricted diffusion through the living epidermal and dermal cells because of poor water solubility, the stratum corneum represents the most important barrier to penetration among the skin layers. Passage of drug across the stratum corneum is, therefore, the rate-limiting step in permeation through the skin at steady state. Two equations were suggested (4) to describe the steady-state penetration flux, J, under these circumstances:

$$J = \frac{K_m C_v D}{L} \qquad \text{(Eq. 1)}$$

where K_m is the partition coefficient between the stratum corneum and the vehicle, C_v is the drug concentration in the vehicle, D is the diffusion coefficient of the drug through the stratum corneum, and L is the effective barrier thickness. An equivalent expression is:

$$J = \frac{a_v D}{\gamma_s L} \qquad \text{(Eq. 2)}$$

where a_v is the activity of the drug in the vehicle and γ_s is the activity coefficient of the drug in the skin barrier. The value of γ_s is usually taken to be constant, so changes in penetration are attributed to alterations in the activity of the drug in the vehicle.

Table I—Penetration Flux of Hydrocortisone from 0.2% Aqueous Solutions Containing Propylene Glycol

Propylene Glycol, % (v/v)	Mean Flux ± *SD*, μg/hr/cm²	Hydrocortisone Solubility, mg/ml
25	0.119 ± 0.007	1.79
40	0.089 ± 0.006	2.20
60	0.078 ± 0.003	3.08

The influence of propylene glycol concentration on hydrocortisone penetration from aqueous solutions is summarized in Table I. The hydrocortisone concentration was kept constant at 0.2%. When the donor solution contained 60% propylene glycol, the penetration rate of hydrocortisone progressively increased after about 70 hr. This change in penetration rate from the constant values observed up to 70 hr was probably due to membrane deterioration (7). The mean flux from 60% propylene glycol solutions (Table I) was calculated from data obtained prior to this upturn in penetration. The solution containing 25% propylene glycol was slightly supersaturated but showed no visible evidence of precipitation after storage for several months.

The values in Table I indicate that hydrocortisone flux decreased with increasing concentrations of propylene glycol. Raising the propylene glycol concentration in the vehicle increased hydrocortisone solubility, implying an enhanced affinity of the drug for the vehicle. This solubility increase resulted in a reduction in K_m, the partition coefficient (Eq. 1), and thereby reduced the penetration flux. The pattern of behavior obtained with hydrocortisone in propylene glycol–water mixtures through mouse skin was similar to that observed (6) for fluocinonide through human skin.

Table II shows the effect of increasing 2-propanol concentrations on the flux of hydrocortisone penetration from aqueous solutions. The values were higher than those obtained from any propylene glycol solution. There was no evidence of membrane damage. Hydrocortisone solubility in the solutions was increased considerably at the higher 2-propanol concentrations. In fact, 2-propanol was a more effective cosolvent than propylene glycol. Nevertheless, in contrast to the propylene glycol solutions, increasing the 2-propanol concentration did not reduce the penetration flux of hydrocortisone significantly.

According to Eq. 2, the penetration flux is proportional to drug activity in the donor solution. At saturation, hydrocortisone activity is considered to be unity; at other concentrations, activity cannot be calculated exactly from the available data, but the ratio of hydrocortisone concentration to its solubility in the vehicle has been suggested as a useful approximation (5). The data from Tables I and II are plotted in Fig. 2 to show the relationship between the penetration flux of hydrocortisone and the ratio of concentration to solubility in aqueous vehicles containing propylene glycol and 2-propanol. Hydrocortisone penetration from propylene glycol–water solutions behaved in accordance with Eq. 2. As the ratio of concentration to solubility decreased, so did the flux.

On the other hand, Eq. 2 does not seem to fit the data for the 2-propanol–water mixtures. The penetration flux was practically independent of the concentration–solubility ratio over a wide range of values. Moreover, the flux at an activity of unity was quite different, depending on the solvent system. However, if γ_s can be considered constant, Eq. 2 states that the flux should be independent of the solvent composition at the same thermodynamic activity.

This difference in flux can only be explained by considering interactions between the 2-propanol solutions and the membrane. The stratum corneum is not an inert structure. Solvent migration from the vehicle into the stratum corneum may alter the effective resistance of the barrier to penetration of other substances. This effect is not necessarily due to damage to the stratum corneum but may be caused by partial solvation of the stratum corneum by the penetrating solvent. As an example, Scheuplein and Ross (14) found that octanol increased the butanol penetration rate through excised human epidermis. When the octanol was washed out of the membrane, it regained its previous resistance to butanol.

Although the penetration rates for propylene glycol and 2-propanol

Table II—Penetration Flux of Hydrocortisone from 0.2% Aqueous Solutions Containing 2-Propanol

2-Propanol, % (v/v)	Mean Flux ± *SD*, μg/hr/cm²	Hydrocortisone Solubility, mg/ml
20	0.217 ± 0.007	1.79
40	0.214 ± 0.037	9.10
60	0.196 ± 0.036	18.2

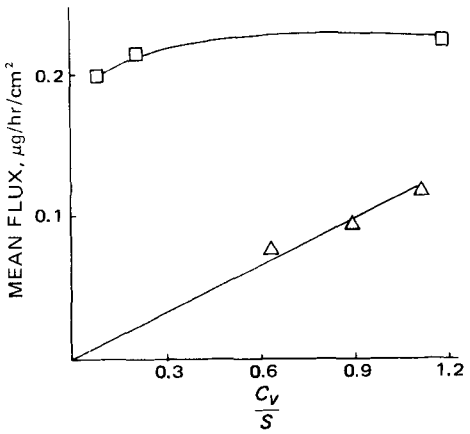

Figure 2—*Mean steady-state flux of hydrocortisone as a function of the ratio of concentration to solubility in the vehicle. Key: □, 2-propanol–water vehicle; and △, propylene glycol–water vehicle.*

through mouse skin were not measured, there is evidence that 2-propanol is likely to be a much better penetrant. Equivalent concentrations of 1-butanol penetrated human stratum corneum about 60 times faster than did 2,3-butanediol (13). Apparently, the addition of a second hydroxyl moiety slows penetration markedly. 2-Propanol probably penetrates into the stratum corneum of the mouse skin membranes and alters the barrier characteristics. It is possible that the presence of 2-propanol in the stratum corneum increases hydrocortisone partitioning from the vehicle.

Solutions containing varying concentrations of hydrocortisone in 40% 2-propanol were studied (Fig. 3). The penetration flux of hydrocortisone was proportional to the drug concentration. In other words, at a constant 2-propanol concentration, the product of K_m and D is a constant. This result shows that a linear relationship between flux and drug concentration does not necessarily rule out vehicle–skin interactions.

Addition of polysorbate 80 to aqueous solutions containing 40% 2-propanol and 0.2% hydrocortisone gave penetration rates that were higher than those for hydrocortisone solutions without surfactant. Figure 4 shows the effect of increasing polysorbate 80 concentrations on the penetration flux of hydrocortisone. The penetration rate of hydrocortisone increased rapidly at low surfactant concentrations and approached a limiting value at higher concentrations. There was no significant difference in the steady-state flux obtained at 0.51 and 1.0% polysorbate 80.

Alteration of the penetration flux from solution by nonionic surfactants may be due to: (*a*) changes in the thermodynamic activity of the drug in the vehicle; (*b*) better wetting, *i.e.*, promotion of contact between the

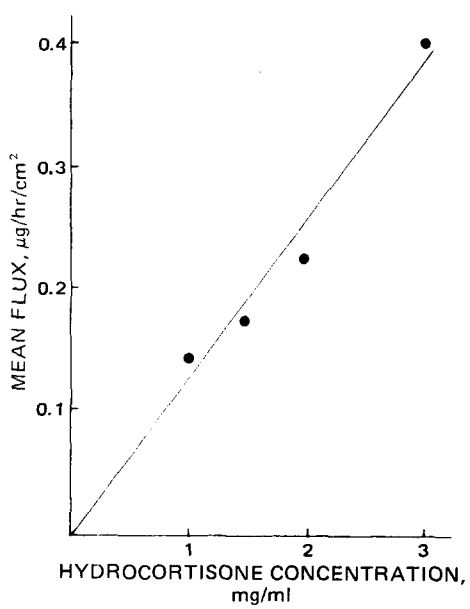

Figure 3—*Mean steady-state flux of hydrocortisone from aqueous solutions containing 40% 2-propanol.*

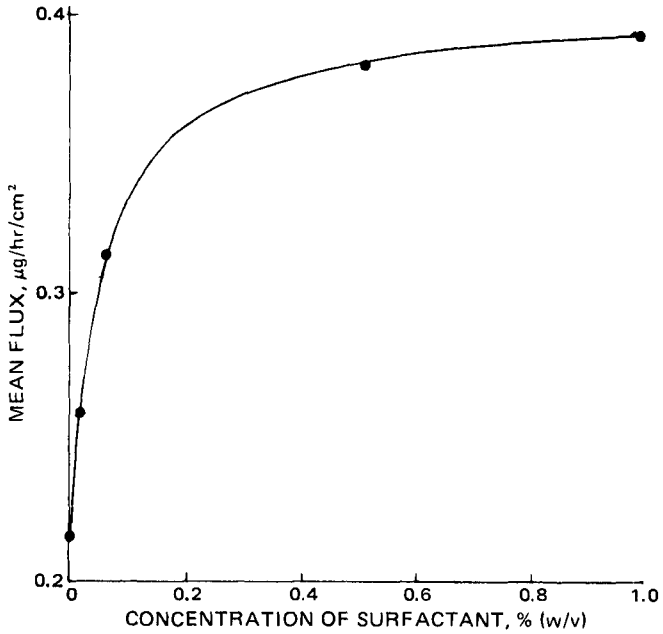

Figure 4—*Mean steady-state flux of 0.2% hydrocortisone from aqueous solutions containing 40% 2-propanol and polysorbate 80.*

solution and skin surface; and/or (*c*) surfactant–membrane interactions.

The enhanced penetration rate of hydrocortisone from solutions containing polysorbate 80 does not seem to be due to an effect of the surfactant in the thermodynamic activity of hydrocortisone in the donor system. Surfactants often form micelles in aqueous systems. Incorporation of a drug into micelles should lower its thermodynamic activity in the vehicle. However, micelle formation was suppressed at the 2-propanol concentration used in this study. Moreover, the surfactant had only a slight effect on hydrocortisone solubility, so that the ratio of hydrocortisone concentration to solubility was not markedly different in the surfactant solutions and in the solution containing no surfactant.

Wetting may be a factor in percutaneous absorption because skin is a low energy surface (15). However, in these experiments, the surface tension of the hydrocortisone solution was unchanged by the addition of increasing surfactant concentrations. All solutions had a surface tension of 26.5–26.9 dynes/cm. The critical surface tension of mouse skin has not been reported. The value for human skin is 27.5 dynes/cm (15). The relatively low values of the solution surface tensions and the lack of effect of the surfactant on surface tension rule out wetting as a significant factor in the influence of polysorbate 80 on hydrocortisone penetration from 2-propanol–water systems.

These considerations lead to the conclusion that the enhancement of hydrocortisone penetration by polysorbate 80 is due to a surfactant–membrane interaction.

Experimentally, polysorbate 80 did not appear to have any deleterious effect on the mouse skin membranes. The penetration plots for systems with polysorbate 80 were quite similar in shape to those obtained without surfactant. There were no changes in slope after steady state was attained, which would signal membrane deterioration. The fact that a limiting value of hydrocortisone penetration was reached is further evidence against membrane damage since such an effect would be expected to be most pronounced at higher surfactant concentrations.

Although the results of the penetration studies do not point to the exact mechanism by which polysorbate 80 exerts its effect, the limiting value of the steady-state flux at higher surfactant concentrations suggests a saturation phenomenon. The shape of Fig. 4 is similar to that of an adsorption isotherm.

Enhanced drug penetration in the presence of nonionic surfactants was reported previously (16–18). Various explanations for the effect of the surfactants were offered, but solubility or some other measure of drug activity in the donor systems was not determined and surface tensions were not measured. For these reasons, it is difficult to compare the conclusions of this study regarding surfactant effects with previous work.

Both Eqs. 1 and 2 assume that drug movement through the vehicle to the epidermal surface is a fast process relative to the transport rate across intact skin. The same assumption was used in interpreting the data

Table III—Steady-State Flux of Hydrocortisone from Solutions and Gels Containing 0.2% Hydrocortisone and 40% 2-Propanol

Polysorbate 80, % (w/v)	Formulation	Mean Flux ± SD, μg/hr/cm²
0	Solution	0.214 ± 0.037
0	Gel	0.239 ± 0.038
0.51	Solution	0.377 ± 0.008
0.51	Gel	0.367 ± 0.024

presented in this paper. From the intercept of the linear portion of the penetration plots with the abscissa, the hydrocortisone diffusion coefficient in the membrane was calculated (10) to be about 10^{-12} cm²/sec. The hydrocortisone diffusion coefficient in water is about 4.6×10^{-6} cm²/sec (19). Although the diffusion coefficient in the donor solutions was probably somewhat larger or smaller than this figure, it is clear that the hydrocortisone diffusion rate across the skin was much slower than that of the drug in the unstirred donor phase.

In some experiments, gels of practically the same composition were used in place of the hydrocortisone solutions. The gels differed from the solutions only in that they contained 1% hydroxyethylcellulose as a gelling agent. There was no significant difference in the penetration rate between the gels and the solutions (Table III). This result was true whether polysorbate 80 was included or not. The increased viscosity of the solution on the addition of the gelling agent did not influence penetration significantly. This finding might be anticipated since changes in macroscopic viscosity in a gel system usually do not lead to marked changes in the diffusion coefficient of a drug dissolved in the gel (20). Therefore, with the gels as with the solutions, hydrocortisone transport through the skin was rate limiting and the relatively minor changes in the hydrocortisone diffusion coefficient in the donor did not affect the flux.

An interesting application of this phenomenon is that preliminary formulation work involving percutaneous absorption can be conducted using simple solutions of the drug in a device similar to the cell used in this study. If the finished dosage form is to be a gel, the preliminary results should be useful indicators of how the gel will perform.

REFERENCES

(1) M. Katz and B. Poulsen, in "Handbook of Experimental Pharmacology," vol. 28, Concepts in Biochemical Pharmacology Part I, B. B. Brodie and J. R. Gillette, Eds., Springer, New York, N.Y., 1971, pp. 107–174.

(2) J. W. Hadgcraft, *Br. J. Dermatol.*, **81**, 386 (1972).

(3) B. J. Poulsen, in "Drug Design," vol. IV, E. J. Ariens, Ed., Academic, New York, N.Y., 1973, pp. 149–192.

(4) B. J. Poulsen, E. Young, V. Coquilla, and M. Katz, *J. Pharm. Sci.*, **57**, 928 (1968).

(5) M. R. Coldman, B. J. Poulsen, and T. Higuchi, *ibid.*, **58**, 1098 (1969).

(6) J. Ostrenga, C. Steinmetz, and B. J. Poulsen, *ibid.*, **60**, 1175 (1971).

(7) J. Ostrenga, J. Haleblian, B. J. Poulsen, B. Ferrell, N. Mueller, and S. Shastri, *J. Invest. Dermatol.*, **56**, 392 (1971).

(8) T. J. Franz, *ibid.*, **64**, 194 (1975).

(9) M. J. Bartek and J. A. LaBuddle, in "Animal Models in Dermatology," H. I. Maibach, Ed., Churchill Livingston, New York, N.Y., 1975, pp. 103–120.

(10) R. J. Scheuplein, I. H. Blank, G. J. Brunner, and D. J. Macfarlane, *J. Invest. Dermatol.*, **52**, 63 (1969).

(11) A. L. Weiss and B. J. Sciarrone, *J. Pharm. Sci.*, **58**, 980 (1969).

(12) A. W. Adamson, in "Physical Chemistry of Surfaces," 2nd ed., Interscience, New York, N.Y., 1967, p. 26.

(13) R. J. Scheuplein and I. H. Blank, *Physiol. Rev.*, **51**, 702 (1971).

(14) R. J. Scheuplein and L. Ross, *J. Soc. Cosmet. Chem.*, **21**, 853 (1970).

(15) A. Rosenberg, R. Williams, and G. Cohen, *J. Pharm. Sci.*, **62**, 920 (1973).

(16) A. J. Aguiar and M. A. Weiner, *ibid.*, **58**, 210 (1969).

(17) T. Fredriksson, *Acta Dermato-Venereol.*, **49**, 55 (1969).

(18) W. W. Shen, A. G. Danti, and F. N. Bruscato, *J. Pharm. Sci.*, **65**, 1781 (1976).

(19) J. T. Carstensen, in "Dissolution Technology," L. J. Leeson and J. T. Carstensen, Eds., APhA Academy of Pharmaceutical Sciences, Washington, D.C., 1974, p. 11.

(20) W. I. Higuchi, *Proc. Am. Assoc. Colleges Pharm. Teachers Seminar*, **1961**, p. 13.

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Rapid Blue Tetrazolium Procedure for Analysis of Corticosteroids in Pharmaceutical Preparations

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Abstract □ A rapid quantitative analysis of nine selected corticosteroids and corticosteroid esters at room temperature is described. The procedure is similar to the official blue tetrazolium reaction for corticosteroids, except that methylene chloride instead of alcohol USP is used as a solvent and the reagents are dissolved in or diluted with nonaqueous solvents. These two modifications reduce the medium polarity, which increases the reaction rate. The reactions are complete in 7–18 min, and the formazans are stable for at least 90 min. The results from 15 different pharmaceutical formulations, 12 containing hydrocortisone and three containing prednisolone acetate, show that the proposed method gives results that compare favorably with those obtained by the official blue tetrazolium, isoniazid, and phenylhydrazine procedures.

Keyphrases □ Corticosteroids, various—blue tetrazolium spectrophotometric analyses in pharmaceutical preparations □ Blue tetrazolium—reaction with various corticosteroids, spectrophotometric analyses in pharmaceutical preparations □ Spectrophotometry—analyses using blue tetrazolium reaction, various corticosteroids in pharmaceutical preparations

The blue tetrazolium reaction is widely used for the analysis of corticosteroids. USP XIX (1) and NF XIV (2) use a slightly modified procedure of Mader and Buck (3) for corticosteroid analysis. Blue tetrazolium (I), 3,3′-(3,3′-dimethoxy-4,4′-biphenylylene)bis(2,5-diphenyl-2*H*-tetrazolium chloride), oxidizes the α-keto moiety of the C_{17} side chain of corticosteroids in strongly alkaline solution (4) and is reduced quantitatively to a highly colored formazan whose concentration is measured spectrophotometrically.