Pharmaceutical Research, Vol. 4, No. 4, 1987

Report

Temperature and pH Dependence of Fluocinolone Acetonide Degradation in a Topical Cream Formulation

Richard A. Kenley,[1,2] Maryann Ott Lee,[1] Lakshmi Sukumar,[1] and Michael F. Powell[1]

Received October 23, 1986; accepted April 21, 1987

We investigated the degradation of fluocinolone acetonide (FA) incorporated into an oil-in-water cream base. The study examined the influence of temperature (23 to 80°C) and cream pH (pH 2.3 to 6) on FA degradation rates. FA degradation followed pseudo-first-order kinetics and adhered to the Arrhenius expression over the entire temperature range investigated. At all temperatures, the pH strongly influenced the observed degradation rate constant (k_{obs}) values, with rate minima observed near pH 4. The FA log(degradation rate)–pH profiles were consistent with a reaction mechanism requiring drug hydrolysis catalyzed by hydroxide and hydrogen ions. Taking into account both the temperature and the pH dependence of FA degradation permits calculating k_{obs} values from the following equation:

$$k_{obs} = \exp\{22.5 - (17{,}200/RT)\} + \exp\{38.7 - (22{,}200/RT)\} \times [H^+] + \exp\{49.5 - (21{,}100/RT)\} \times [OH^-]$$

where the three bracketed terms represent Arrhenius expressions for neutral, acid-catalyzed, and base-catalyzed hydrolysis reactions. FA degradation in the cream base parallels the degradation of a related steroid (triamcinolone acetonide) in an aqueous alcohol solution. The equivalence between FA and triamcinolone acetonide kinetics in the different reaction media suggests that in the cream base, FA degradation is limited to an aqueous phase largely unperturbed by the presence of nonaqueous constituents that comprise the cream formulation.

KEY WORDS: fluocinolone acetonide stability; pH dependence; Arrhenius parameters; steroid formulation; acid–base catalysis.

INTRODUCTION

The widespread clinical application enjoyed by corticosteroids has evoked considerable interest in the kinetics and mechanisms of steroid chemical degradation in reaction media that model pharmaceutical dosage forms. Relevant investigations include hydrocortisone (1–5), prednisolone (6,7), cloprednol (8), fluocortolone (9), betamethasone (10), dexamethasone (10), methylprednisolone (11), and triamcinolone acetonide (12,13) reactivity in aqueous alcohol solutions and fluorandrenolide (14), hydrocortisone (15), budenoside (16), prednisolone (17), and hydrocortisone butyrate (18) degradation in topical formulations.

The cited studies permit some general conclusions to be made regarding the chemical reactivity of steroids that feature a dihydroxyacetone group at C17. Thus, it is generally recognized that:

(1) degradation occurs primarily at the C17 side chain;
(2) transition metal, hydroxide, and hydrogen ions catalyze the degradation; and
(3) both oxidative and hydrolytic reaction pathways are available.

The extensive existing information not withstanding, some important questions regarding steroid degradation remain unanswered. First, the literature fails to demonstrate both the temperature dependence of steroid degradation and individual rate constants for reactivity. Some authors, for example, report Arrhenius parameters for observed pseudo-first-order rate constants but do not determine bimolecular rate constants for steroid reaction with water, acid, and base. Other workers cite bimolecular rate constants for steroid reactivity but not Arrhenius parameters. However, the effect of temperature on bimolecular rate constants is important fundamental information necessary for predicting steroid reactivity under defined reaction conditions.

Second, it is currently unknown whether the log(rate)–pH profiles typically observed for steroid degradation in aqueous solutions accurately extrapolate to actual dosage forms. Such knowledge would have both fundamental and practical significance. In the former regard, there is general interest in demonstrating the degree to which simple aqueous systems accurately model complicated semisolid topical dosage forms. From a practical perspective, the influence of medium acidity and alkalinity on steroid degradation could define a pH range for optimal drug stability and maximal drug-product shelf life.

Accordingly, we have probed in detail the effects of pH

[1] Syntex Research, 3401 Hillview, Palo Alto, California 94304.
[2] To whom correspondence should be addressed at Travenol Laboratories, 6301 Lincoln Avenue, Morton Grove, Illinois 60053.

0724-8741/87/0800-0342$05.00/0

and temperature on fluocinolone acetonide (FA) degradation in an oil-in-water-type topical cream.

EXPERIMENTAL

Materials. Fluocinolone acetonide (FA) and norethindrone were USP grade. FA was incorporated (0.01%, w/w) in a modified Synalar (19) cream base containing citric acid (0.01%, w/w) buffer and disodium EDTA (0.01%, w/w).

Methods. Creams were made at various pH's by adjusting the aqueous phase of the cream base with HCl or NaOH. Cream samples (3 g per sample) were added to scintillation vials and maintained for timed intervals at a constant (±2°C) temperature. At each time point, duplicate samples were removed and assayed according to the procedure described below.

pH Determination. Cream samples were diluted 1:4 with distilled water and the pH of the resulting mixture was recorded at ambient temperature with a Fisher Model 420 pH meter. The results reported below are therefore apparent pH values.

Analysis. The following components comprised the high-performance liquid chromatography (HPLC) system: Altex Model 110A pump, Schoeffel Model 740 spectrophotometric detector, Spectra-Physics Model 4000 data system, Waters Model 710A autosampler and injector, Altex Ultrasphere ODS (5 μm), 250 × 4.6-mm reverse-phase analytical column, and 70 × 2.1-mm guard column with Whatman CO:Pell ODS packing.

The mobile phase was a 60:40 mixture of 1% (v/v) aqueous glacial acetic acid and acetonitrile. The flow rate was 1.0 ml/min, giving approximately 2000-psi backpressure at ambient temperature. With a 40-μl injection (120 ng of FA injected) and a 254-nm detection wavelength, the detector sensitivity range was set to 0.02 AUFS. FA eluted at 8.7 min, and norethindrone at 15.4 min. Run times were typically 25 min..

Sample and Standard Preparation. FA reference standard stock solutions were prepared by adding 10, 15, and 20 mg of FA into separate 250-ml volumetric flasks and diluting to volume with acetonitrile. Internal standard (norethindrone) stock solutions were similarly prepared by diluting 37 mg into 250 ml of acetonitrile. Calibration standard solutions were prepared by transferring 5 ml of FA stock solution and 2 ml of norethindrone stock solutions into separate 100-ml flasks, adding 45 ml of acetonitrile, and diluting to volume with water.

Sample preparation required transferring an accurately weighed 3-g cream sample to a 250-ml conical flask, followed by adding 2 ml of internal standard stock solution and 50 ml of acetonitrile. Heating the flask over a steam bath dissolved the sample in approximately 8 min. Next, approximately 50 ml of water was added to the flask, followed by manual agitation and centrifugal sedimentation to precipitate excipients. Aliquots of the resulting supernatant were directly injected onto the HPLC system.

Method Performance. The analytical method satisfied all the normal statistical performance criteria (linearity, recovery, and precision). The method was shown to be specific for FA in the presence of its degradation products by comparing chromatograms of standard solutions, spiked placebos, partially degraded samples, and placebos spiked with authentic samples of known or potential degradation products. For brevity, chromatograms are not shown. Additionally, we assayed partially degraded cream samples in parallel with the above method and with a second HPLC method using a different mobile phase and column. Both assay methods gave equivalent results for fluocinolone acetonide concentration and further confirmed the specificity of the above method.

RESULTS AND DISCUSSION

Kinetic Order and Degradation Products

We examined FA degradation in 11 topical creams of pH 2 to 6, at 23, 40, 50, and 80°C. FA disappearance followed pseudo-first-order kinetics according to Eq. (1):

$$\ln([FA]_t/[FA]_O) = -(k_{obs}) \times t \quad (1)$$

where k_{obs} is the observed degradation rate constant. Typically, cream samples maintained at elevated temperatures were followed for one to two half-lives. Although the limited FA conversion at 23°C (5 to 15% degradation in 12 months) masked any clear distinction between zero-order and first-order kinetics, we assumed pseudo-first-order degradation kinetics at all temperatures. Figure 1 shows representative data for FA degradation at three different pH/temperature combinations.

We estimated the degradation product yields only semiquantitatively. The FA C-21 aldehyde and C-17 acid (etianic acid analogue) appeared in most chromatograms, however, these apparently represent only a minor fraction of the total FA lost in any sample. Other degradation products appeared with (or shortly after) the solvent artifact, indicating polar or ionized functional groups; these were probably secondary degradation products and were not identified. The degradation products of FA in a similar cream formulation are reported elsewhere (20).

Temperature and pH Effects

Table I lists k_{obs} values determined by linear least-squares regression analysis of data plots according to Eq. (1). The table omits rate constants found to be not statistically significantly different from zero (confidence limits overlapping zero).

At all pH values, the FA degradation rate constants increased with increasing temperatures. Arrhenius behavior held for all the creams tested over the entire 23 to 80°C temperature range as evidenced by data plots according to Eq. (2):

$$\ln(k_{obs}) = \ln(A) - E_a/RT \quad (2)$$

where E_a is the Arrhenius activation energy (cal/mol), R is the gas constant, T is the absolute temperature, and A is the preexponential factor (months^{-1}). Table II lists E_a values, A factors, and least-squares correlation coefficient (r) values for the Arrhenius plots at each pH. Figure 2 plots representative data according to Eq. (2) for creams made with pH 2.3, 3.1, and 3.7.

At all temperatures, k_{obs} demonstrated a strong dependence on pH, with rate constants increasing at decreasing pH's in the region pH 2.3 to 3.7 and increasing at increasing

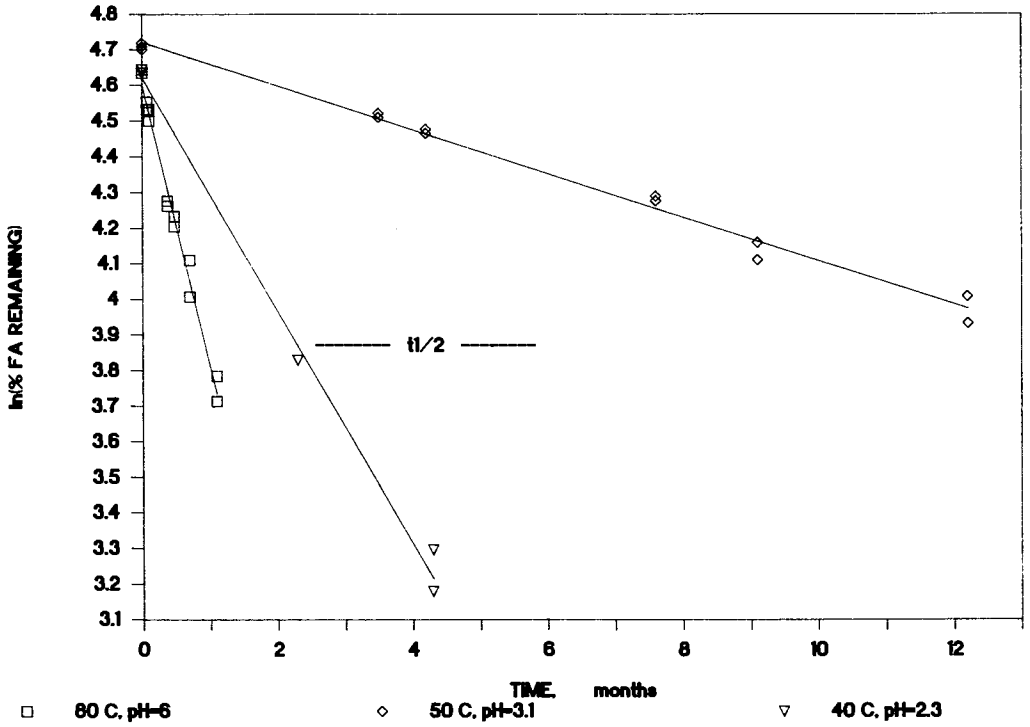

Fig. 1. Pseudo-first-order kinetic plots of fluocinolone acetonide degradation in cream samples at 80°C and pH 6.0, 50°C and pH 3.1, and 40°C and pH 2.3. The horizontal dashed line represents the half-life, i.e., the time to 50% fluocinolone acetonide remaining.

pH's in the region pH 4.1 to 6. The k_{obs} values showed a minimum near pH 4. A mechanism consistent with the observed dependence of k_{obs} on pH involves specific acid catalysis of FA degradation at low pH's and specific base catalysis at pH's above 4. The subsequent section examines FA degradation catalysis in greater detail.

Table I. Observed Pseudo-First-Order Rate Constant (k_{obs}) Values[a] for Fluocinolone Acetonide Degradation in Cream Samples

Cream pH	100 × k_{obs} (months^{-1}) and 95% confidence interval (CI) at 80°C k_{obs}	CI	50°C k_{obs}	CI	40°C k_{obs}	CI	23°C k_{obs}	CI
2.3	680	290	57.8	2.4	32.7	3.5	0.735	0.27
3.1	56.9	4.9	6.13	0.4	3.69	0.88	0.279	0.10
3.7	14.6	2.3	1.46	0.2	0.735	0.10	0.106	0.08
4.1	17.0	3.7	1.50	0.3	ND[b]	ND	—[c]	—
4.2	21.8	6.4	1.70	0.2	0.350	0.08	—	—
4.3	23.4	4.6	1.63	0.7	0.540	0.13	0.140	0.11
4.3	16.9	4.3	1.64	0.4	0.300	0.18	—	—
4.4	20.8	8.4	3.24	0.4	0.500	0.08	—	—
4.6	27.6	1.9	2.34	0.2	1.24	0.08	0.263	0.13
5.6	ND	ND	7.70	3.9	5.57	1.6	ND	ND
6.0	78.8	5.8	11.1	0.8	6.35	0.49	0.643	0.35

[a] Calculated by least-squares linear regression according to Eq. (1).
[b] Not determined.
[c] Rate constant not statistically significantly different from zero.

Acid and Base Catalysis of FA Degradation

To describe the pH dependence of FA degradation, we can express k_{obs} values as in Eq. (3):

$$k_{obs} = k_O + k_{H^+}[H^+] + k_{OH^-}[OH^-] \quad (3)$$

where k_O is the pseudo-first-order rate constant for spontaneous reaction and k_{H^+} and k_{OH^-} are bimolecular rate constants for acid- and base-catalyzed FA degradation, respec-

Table II. Activation Parameters Calculated[a] for Fluocinolone Acetonide Degradation in Cream Samples

Cream pH	cal/mol E_a	95% CI	ln(A) (months^{-1})	r
2.3	17,000	20,000	26.1	0.996
3.1	18,800	9,800	26.4	0.985
3.7	17,700	3,500	23.4	0.998
4.1	ND[b]	ND	ND	ND
4.2	21,900	34,000	29.8	0.993
4.3	18,900	4,700	25.4	0.996
4.3	21,198	45,000	28.6	0.986
4.4	19,141	64,000	25.9	0.967
4.6	16,900	2,900	22.7	0.998
5.6	ND	ND	ND	ND
6.0	18,600	13,000	26.6	0.976

[a] Calculated according to Eq. (2) using k_{obs} values taken from Table I.
[b] Not determined. Only two k_{obs} values available.

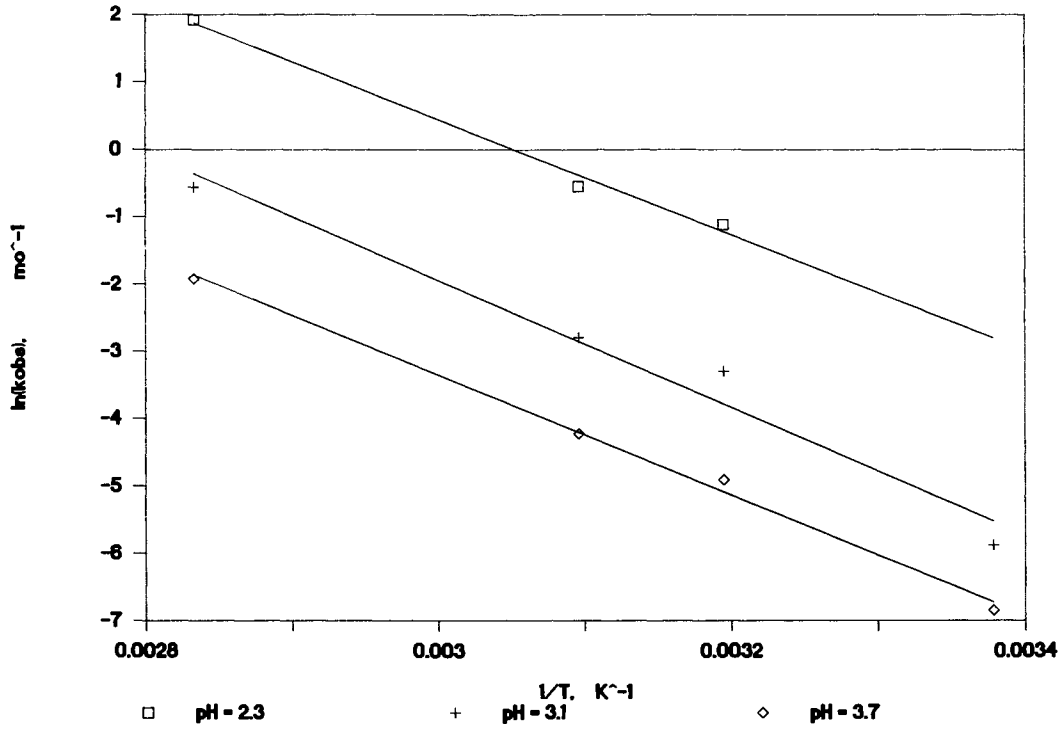

Fig. 2. Arrhenius plot of observed pseudo-first-order degradation rate constant (k_{obs}) values versus reciprocal absolute temperature for fluocinolone acetonide in creams made at pH 2.3, 3.1, and 3.7.

tively. The k_O values may represent neutral hydrolysis, an oxidation reaction, or more likely, a sum of the two.

Table III summarizes k_O, k_{H+}, and k_{OH-}, values calculated at four temperatures by nonlinear least-squares regression analysis of k_{obs} values according to Eq. (3). The data in Table III also provide Arrhenius parameters for the individual rate constants, k_O, k_{H+}, and k_{OH-}. Correlation of ln(rate constant) versus reciprocal absolute temperature data according to Eq. (2) gave the E_a and A factors summarized in Table IV. For each of the three rate constants evaluated, the fit to the Arrhenius expression was good ($r > 0.96$) over the 23 to 80°C temperature range. The good correlation is especially impressive considering the uncertainties surrounding k_{obs} values at 23°C and the fact that the temperature range investigated extends above and below the congealing point (approximately 55°C) of the cream base.

Table III. Rate Constants for Neutral (k_0), Acid-Catalyzed (k_{H+}), and Base-Catalyzed (k_{OH-}) Fluocinolone Acetonide Hydrolysis[a]

Rate	Temperature (°C)			
	80	50	40	23
k_{H+} (M^{-1} − months^{-1})	759	72.5	44.7	1.32
SD	298	23	14	0.74
$k_{OH-} \times 10^{-6}$ (M^{-1} − months^{-1})	168	17.0	13.6	0.372
SD	72	5.8	4.53	0.27
$k_0 \times 100$ (months^{-1})	13.3	1.16	0.079	0.117
SD	5.2	1.2	0.18	0.03

[a] Calculated by nonlinear least-squares regression analysis of k_{obs}, [H$^+$], and [OH$^-$] data according to Eq. (3).

To illustrate the degree to which Eq. (3) and the Arrhenius parameters given in Table IV accurately represent FA degradation kinetics, we can compare observed degradation rate constants with values calculated at any temperature and pH value. Rewriting Eq. (3) in terms of activation parameters for k_O, k_{H+}, and k_{OH-} gives Eq. (4):

$$k_{obs} = \exp\{22.5 - (17{,}200/RT)\} + \exp\{38.7 - (22{,}200/RT)\} \times [H^+] + \exp\{49.5 - (21{,}100/RT)\} \times [OH^-] \quad (4)$$

Table IV provides the preexponential factors and activation energies. Figure 3 compares the directly determined k_{obs} (Table I) values with values calculated according to Eq. (4). From Fig. 3, it is evident that the calculated and directly determined k_{obs} values compare very well.

Figure 3 also compares observed versus calculated k_{obs} values for the degradation of a related steroid, triamcinolone acetonide, in an aqueous solution at 50°C. The following section further elaborates the comparisons of FA cream degradation with literature data for triamcinolone acetonide.

Table IV. Arrhenius Parameters[a] for Neutral (k_0), Acid-Catalyzed (k_{H+}), and Base-Catalyzed (k_{OH-}) Hydrolysis of Fluocinolone Acetonide

Rate	E_a	95% CI	ln(A)	r
k_{H+} ($1/M$ − months)	22,200	17,400	38.7	0.968
k_{OH-} ($1/M$ − months)	21,100	19,000	49.5	0.960
k_0[b] (months^{-1})	17,200	8,300	22.5	0.999

[a] Calculated according to Eq. (2) using data in Table III.
[b] This calculation excluded the 40°C point for k_0 in Table III.

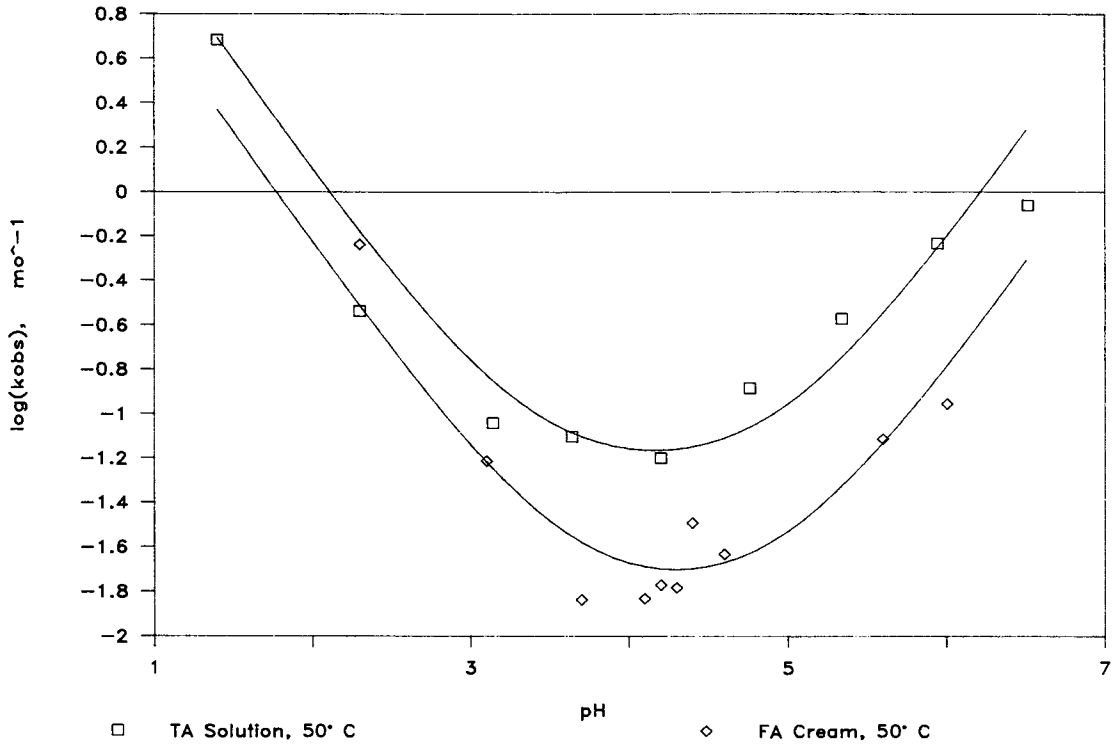

Fig. 3. pH–log (rate) profile for fluocinolone acetonide degradation in cream at 50°C and for triamcinolone acetonide degradation in aqueous solution at 50°C. Symbols represent directly determined k_{obs} values, and solid lines represent k_{obs} values calculated according to Eq. (4).

Literature Comparisons

Timmins and Gray (12) examined the pH dependence of triamcinolone acetonide (TA) degradation in aqueous methanol solutions at 50°C. TA represents a good model for FA degradation because both molecules are corticosteroids that share the dihydroxyacetone functional group at C-17 and the cyclic ketal bridging at C-16 and C-17. TA and FA differ only in the substitution pattern at C-6 (TA is unsubstituted, FA is fluoro-substituted). Figure 3 shows our data for observed FA degradation rate constants at 50°C (see Table I) versus pH and the pseudo-first-order rate constants for TA degradation at the same temperature. The solid line shown for FA is a best fit using Eq. (4), and the line for TA is calculated from Eq. (3) using rate constants reported by Timmins and Gray.[3]

From Fig. 3, it is clear that TA solutions and FA creams share similar log(rate)–pH profiles over the range pH 2 to 6. In both cases, specific acid and specific base catalysis are evident, and rate minima occur near pH 4.

Because TA degradation in an aqueous methanol solution very closely resembles FA degradation in a cream formulation, it seems likely that a common reaction pathway is operative in both cases. Specifically, the reaction rate similarities indicate that FA degradation in the cream samples is confined to an aqueous environment that is largely unperturbed by the nonaqueous constituents of the cream base.

CONCLUSIONS

Our work with FA creams provides the first quantitative demonstration of temperature effects on bimolecular rate constants for acid- and base-catalyzed steroid hydrolysis in a pharmaceutical formulation. Furthermore, the demonstrated parallels between FA degradation in a topical cream and TA hydrolysis in an aqueous alcohol solution establish the generality of our results. The parameters shown in Eq. (4) permit estimation of the FA reactivity as a function of pH and temperature and the estimated reactivity should extend to structurally related steroids in solutions or in semisolid pharmaceutical formulations.

Comparing FA degradation in the topical cream with literature data for triamcinolone acetonide hydrolysis in aqueous methanol solutions reveals strikingly similar degradation kinetics over the range pH 2 to 6. The close equivalence between FA and TA reactivity provides strong evidence for a common reaction pathway in the cream and in the aqueous alcohol solution. It appears that FA degradation in cream samples occurs in an aqueous phase or compartment that is substantially unperturbed by nonaqueous ingredients that comprise the cream formulation.

[3] The observed degradation rate constants reported by Timmins and Gray (12) for the pH region 4 to 6 were treated according to Eq. (3) to provide TA k_{OH^-} values for comparison with the k_{OH^-} values reported herein. Timmins and Gray report a different value for k_{OH^-}, a value based on alkaline hydrolysis in the region pH 8 to 10. Because additional chemistry occurs in the alkaline pH region, the k_{OH^-} values that we calculated for the region pH 4 to 6 are preferred for the purposes of comparison.

REFERENCES

1. J. Hansen and H. Bundgaard. *Arch. Pharm. Chem. Sci. Ed.* 7:135–146 (1979).

2. J. Hansen and H. Bundgaard. *Arch. Pharm. Chem. Sci. Ed.* 8:5–14 (1980).
3. J. Hansen and H. Bundgaard. *Arch. Pharm. Chem. Sci. Ed.* 8:91–99 (1980).
4. J. Hansen and H. Bundgaard. *Int. J. Pharm.* 6:307–319 (1980).
5. I. Pitman, T. Higuchi, M. Alton, and R. Wilet. *J. Pharm. Sci.* 61:919–923 (1972).
6. D. E. Guttman and P. D. Meister. *J. Am. Pharm. Assoc.* 47:773–778 (1958).
7. T. O. Oesterling and D. E. Guttman. *J. Pharm. Sci.* 53:1189–1192 (1964).
8. D. M. Johnson. *J. Org. Chem.* 47:198–201 (1982).
9. D. Dekker and J. Buijs. *Int. J. Pharm.* 5:195–206 (1980).
10. D. Dekker and J. H. Beijnen. *Acta Pharm. Suec.* 18:185–192 (1981).
11. M. I. Amin and J. T. Bryan. *J. Pharm. Sci.* 62:1768–1771 (1973).
12. P. Timmins and E. A. Gray. *J. Pharm. Pharmacol.* 35:175–177 (1983).
13. V. D. Gupta. *J. Pharm. Sci.* 72:1453–1456 (1983).
14. R. Pearlman, B. S. Rutherford, K. M. Pozsgai, and C. A. Hirsch. *Int. J. Pharm.* 18:53–66 (1984).
15. V. D. Gupta. *J. Pharm. Sci.* 67:299–302 (1978).
16. G. Roth, P. Stromberg, A. Thalen, and L. I. Wickstrom. In S. Gorog (ed.), *Anal. Chem. Symp. Ser., Vol. 10. Adv. Steroid Anal.*, Elsevier, New York, 1982, pp. 389–394.
17. S. A. H. Khalil, S. A. El-Fattah, and L. M. Mortada. *Drug Dev. Ind. Pharm.* 10:771–787 (1984).
18. Y. W. Yip, A. L. W. Po, and W. J. Irwin. *J. Pharm. Sci.* 72:776–781 (1983).
19. U.S. Pharmacopeial Convention. *U.S. Pharmacopeia XXI*, Rockville, Md., 1985, p. 434–435.
20. M. F. Powell, A. Magill, and A. Becker. *Pharm. Res.* 3:360–363 (1986).