Kinetics of Indomethacin Degradation II: Presence of Alkali plus Surfactant

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Abstract □ The kinetics of indomethacin were studied in the presence of the surfactants ethoxylated lanolin, polysorbate 80, and cetrimonium bromide under alkaline conditions at 30.3°. The degradation followed apparent first-order kinetics. Plots of k_{obs} *versus* surfactant concentrations were curved with negative slopes for nonionic surfactants; but with the ionic surfactant, the plots showed a marked positive change in k_{obs} as the surfactant concentration passed through the critical micelle concentration. Literature model systems adequately explained the data for nonionic surfactants but not for the ionic surfactant. A new set of equations was derived for each case using electrochemical potentials. The experimental data for all three surfactants fit the derived equations quite well.

Keyphrases □ Indomethacin—degradation kinetics in presence of ionic and nonionic surfactants in alkaline solutions □ Degradation kinetics—indomethacin in presence of ionic and nonionic surfactants in alkaline solutions □ Kinetics, degradation—indomethacin in presence of ionic and nonionic surfactants in alkaline solutions □ Surfactants, ionic and nonionic—effect on degradation kinetics of indomethacin in alkaline solutions □ Anti-inflammatory agents—indomethacin, degradation kinetics in presence of ionic and nonionic surfactants in alkaline solutions

In Part I of this series (1), indomethacin degradation in the presence of alkali was studied over a temperature range of 20.1–40.7°. The rate constant–hydroxyl-ion concentration profile was linear with a positive slope. Activation energies and other related parameters were calculated from Arrhenius-type plots. This report describes the effect of some added surfactants on the kinetics of degradation at one temperature.

EXPERIMENTAL

Materials—All materials were analytical grade, including indomethacin[1], sodium hydroxide, and sodium chloride. In addition, three surfactants were used: ethoxylated lanolin[2], polysorbate 80[3], and cetrimonium bromide[4]. The ethoxylated lanolin was obtained from lanolin containing 25 ethylene oxide units. Double-distilled water from an all-glass still was used throughout the study.

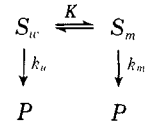
Kinetic Studies—Stock solutions of 2 mg of indomethacin/ml, 0.1 *M* sodium hydroxide, and 1% (w/v) surfactant were prepared. In all runs, 2 ml of stock indomethacin was used plus a fixed volume of sodium hydroxide (2, 3, or 5 ml) in an increasing concentration of surfactant up to 1%. All runs were made at 30.3° as described previously (1).

RESULTS AND DISCUSSION

Results for indomethacin degradation in the presence of the nonionic surfactants, ethoxylated lanolin and polysorbate 80, and in the presence of the ionic surfactant, cetrimonium bromide, are shown in Figs. 1 and 2, respectively.

Plots of k_{obs} *versus* surfactant concentration were usually curved, as with the nonionic surfactants (Fig. 1). However, with cetrimonium bromide (Fig. 2), there was a marked change in k_{obs} as the surfactant concentration passed through the critical micelle concentration (CMC). The CMC's of ethoxylated lanolin, polysorbate 80, and cetrimonium bromide are 7.15×10^{-5}, 8.20×10^{-4} (2), and 2.09×10^{-3} (% w/w), respectively, at 37°.

[1] Lot L-590, 229-00A108, Merck Sharp & Dohme, Rahway, N.J.
[2] Solulan 25, lot 108F, 4251, American Cholesterol Products Inc.
[3] Lot 1026-1740, Sigma Chemical Co.
[4] Imperial Chemical Industries Ltd.



Scheme I

Model systems explaining chemical changes in the presence of micelle-forming surfactants usually have postulated that micelles formed in aqueous systems form a second phase into which reactants partition so that the ratio of concentrations in the micelle and water phases is a constant and that this equilibrium is maintained during chemical change. Rates of change are considered to be first order, as is usually observed in practice (Scheme I).

In this scheme, S_w and S_m represent the substrate in water and the micelle, respectively; C_w and C_m are corresponding concentrations of the substrate, which decomposes to product P both in water and in the micelle; k_w and k_m are rate constants for degradation of the substrate in water and the micelle, respectively; and the partition coefficient, K, is a ratio of C_m and C_w. Scheme I has led to several equations relating observed rates to postulated contributing rates (3–5). The following equation is essentially that of Winterborn *et al.* (4):

$$k_{obs} = \frac{k_w - k_w V + k_m K V}{1 - V + KV} \qquad \text{(Eq. 1)}$$

where k_{obs} is the observed rate constant related to the total amount of the substrate in the total volume; and V is the volume fraction of the micellar phase, which is given by the ratio of the volume of the micellar phase in milliliters, V_m, to the total volume in milliliters, V_T.

By rearrangement of Eq. 1:

$$k_{obs} = k_m + \frac{(1 - V)}{K}\frac{(k_w - k_{obs})}{V} \qquad \text{(Eq. 2)}$$

Since the highest concentration of surfactant used was 1%, the largest value of V is approximately 0.01. Thus, Eq. 2 simplifies to:

$$k_{obs} = k_m + \frac{1}{K}\frac{(k_w - k_{obs})}{V} \qquad \text{(Eq. 3)}$$

From Eq. 3, plots of k_{obs} *versus* $(k_w - k_{obs})/V$ should be linear with the intercept at zero surfactant concentration equal to k_m and the slope equal to $1/K$ (Figs. 3 and 4). Surfactant solutions (C_s) were prepared on a weight per volume basis. Conversion of C_s to V requires a knowledge of micelle phase density. Since it was not known, the density was arbitrarily taken as 1 g/cm³. Any error should not affect graph linearity.

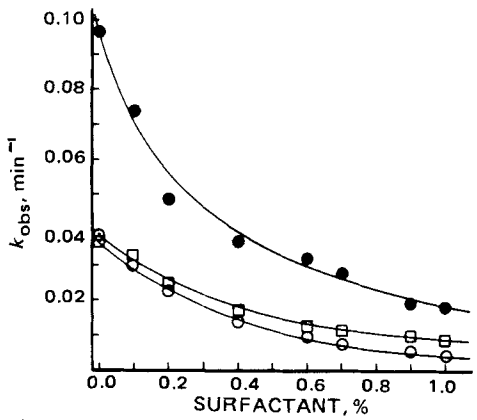


Figure 1—*Observed rate constant,* k_{obs}, versus *varying concentrations of surfactants in alkaline aqueous solutions at 30.3°. Key:* ○, *ethoxylated lanolin in 0.002* M *hydroxide-ion concentration;* ●, *ethoxylated lanolin in 0.005* M *hydroxide-ion concentration; and* □, *polysorbate 80 in 0.002* M *hydroxide-ion concentration.*

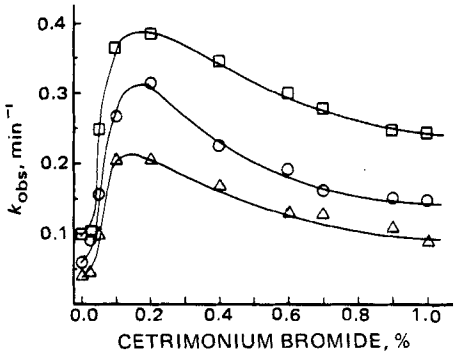


Figure 2—*Observed rate constant,* k_{obs}, versus *varying concentrations of cetrimonium bromide in alkaline aqueous solutions at 30.3°. Key (hydroxide-ion concentration):* △, *0.002* M; ○, *0.003* M; *and* □, *0.005* M.

Figure 3 shows that plots based on Eq. 4 were satisfactorily linear for the nonionic surfactants, but the plots show gross curvature for cetrimonium bromide (Fig. 4). Correcting for the fraction of surfactant not micellized (by subtracting the CMC from the total concentration) made no appreciable difference.

Values of k_m and K derived from the intercepts and slopes of Figs. 3 and 4 are given in Table I. Values of K, which depend upon the value used for micelle density, are unlikely to be grossly in error through use of the value 1 g/cm^3, and the choice permits comparisons to be made. The values of k_m and K derived for cetrimonium bromide are of low reliability, being obtained from the first portion of the curves, but are included for comparison. Some Eq. 3 values of k_m in Table I are negative, which is physically impossible. Such values may reflect experimental error or a model that does not account for all factors.

Divergence from model behavior may arise from false assumptions in model development or from neglect of some relevant factor. One assumption that should be questioned is the maintenance of an equilibrium between the substrate in the water phase and in the micelle. Studies of transfer rates in such cases (6) show that equilibria are rapidly established and that this factor is not likely to be relevant. The effects of micellar and substrate charges on the phase equilibrium are neglected in the model used. Even with the nonionic surfactants, indomethacin in the presence of sodium hydroxide is ionized, so solubilized molecules will confer a charge on the micelle.

For solubilized ions and charged micelles, an equilibrium between the phases depends on electrochemical potentials, not simply on chemical potentials. Thus, at equilibrium:

$$\bar{\mu}_{iw} = \bar{\mu}_{im} \qquad \text{(Eq. 4)}$$

where $\bar{\mu}_{iw}$ and $\bar{\mu}_{im}$ are electrochemical potentials of ion i in water and the micelle, respectively. The bulk of the water phase is arbitrarily assigned a zero potential, while the micelle has potential ϕ_m. When using molar concentrations, C_i, as approximations for activities and Z_i for ion valency, the following equation can be written (7):

$$\mu^\circ_{iw} + RT \ln C_{iw} = \mu^\circ_{im} + RT \ln C_{im} + Z_i F \phi_m \qquad \text{(Eq. 5)}$$

where F is the Faraday constant, R is the gas constant, and the μ°_i terms refer to standard ordinary chemical potentials. Upon rearrangement, Eq. 5 yields:

$$\frac{C_{im}}{C_{iw}} = \exp\left[-\left(\frac{1}{RT}\right)(\Delta\mu^\circ_i + Z_i F \phi_m)\right] \qquad \text{(Eq. 6)}$$

$$\frac{C_{im}}{C_{iw}} = K' \exp\left(-Z_i F \phi_m / RT\right) \qquad \text{(Eq. 7)}$$

The effect of charge and surfactant concentration on the right-hand side of Eq. 7 may be approximated on the basis of simplified colloid theory. Such a treatment (see *Appendix*) leads to expressions for $Z_i F \phi_m$ as follows.

For nonionic surfactants:

$$Z_i F \phi_m = \frac{K_1 K_2 n C_m}{A_m \sqrt{\Sigma C_{iw}}} \qquad \text{(Eq. 8)}$$

For the ionic surfactants:

$$Z_i F \phi_m = -\frac{K_1 n (1 - K_2 C_m)}{A_m \sqrt{(\Sigma C_{iw} + C_{is})}} \qquad \text{(Eq. 9)}$$

where n is the micelle aggregation number, A_m is the surface area of the micelle, ΣC_{iw} is the counterion concentration not derived from surfactant ions, C_{is} is the counterion concentration derived from the surfactant, and K_1 and K_2 (see *Appendix*) are constants.

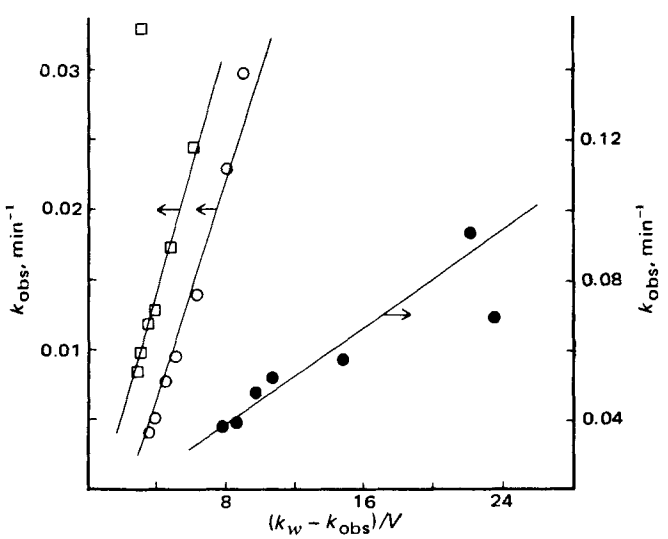


Figure 3—*Relationship between observed rate constants and function of the volume fraction of micelles. Key:* ○, *ethoxylated lanolin in 0.002* M *hydroxide-ion concentration;* ●, *ethoxylated lanolin in 0.005* M *hydroxide-ion concentration; and* □, *polysorbate 80 in 0.002* M *hydroxide-ion concentration.*

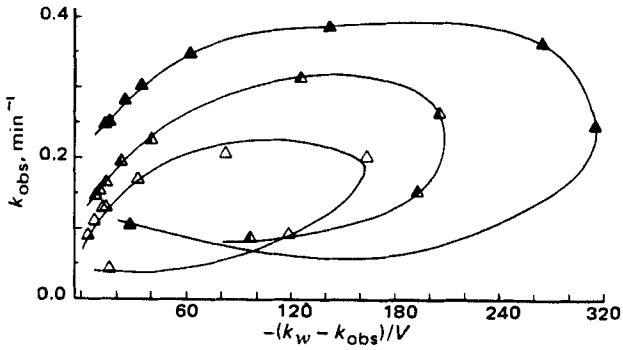


Figure 4—*Relationship between observed rate constants and function of volume fraction of cetrimonium bromide micelles. Key (hydroxide-ion concentration):* △, *0.002* M; ▲, *0.003* M; *and* ▲, *0.005* M.

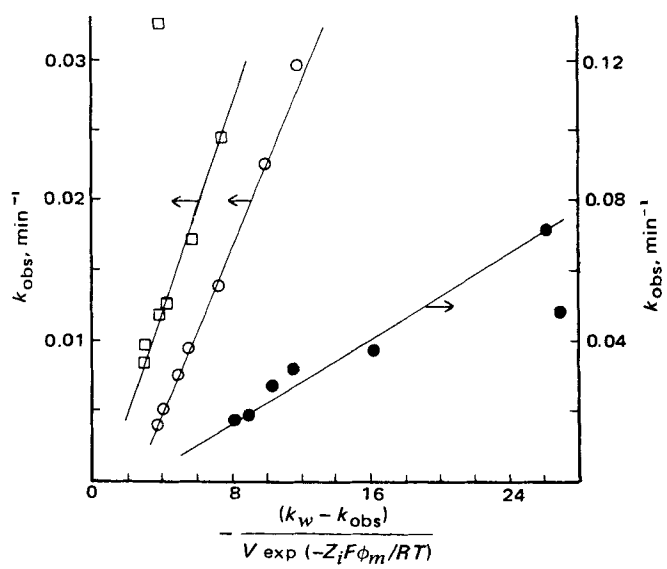


Figure 5—*Relationship between observed rate constants and the function of the micellar potential. Key:* ○, *ethoxylated lanolin in 0.002* M *hydroxide-ion concentration;* ●, *ethoxylated lanolin in 0.005* M *hydroxide-ion concentration; and* □, *polysorbate 80 in 0.002* M *hydroxide-ion concentration.*

Table I—Rate Constants (Minutes^{-1}) for Scheme I based on Eqs. 3 and 11 for Various Surfactants in Varying Concentrations of Hydroxide Ion

Surfactant	Hydroxide Ion, M	k_w	From Eq. 3 k_m	From Eq. 3 K (10^{-2})	From Eq. 11 k_m	From Eq. 11 K' (10^{-2})
Ethoxylated lanolin	0.002	0.039	−0.013	2.2	−0.007	2.9
	0.005	0.096	−0.012	2.7	−0.008	3.2
Polysorbate 80	0.002	0.036	−0.004	2.2	−0.001	3.0
Cetrimonium bromide	0.002	0.040	0.080	3.6	0.044	0.2
	0.003	0.061	0.120	3.5	0.071	0.2
	0.005	0.100	0.205	3.5	0.160	0.3

Table II—Observed First-Order Rate Constants for the Degradation of Indomethacin in Alkaline Solutions in the Presence of Varying Concentrations of Ethoxylated Lanolin at 30.3°

Surfactant Concentration, % (w/v)	Volume Fraction[a] of Micellar Phase (V)	Concentration[b] of Indomethacin in Micelles (C_m), M	$\frac{\exp(-Z_iF\phi_m)}{RT}$	k_{obs}, min^{-1}
	In Presence of 0.002 M NaOH			
0	0	0	—	3.85×10^{-2} [c]
0.1	0.001	0.027	0.77	2.96×10^{-2}
0.2	0.002	0.022	0.81	2.27×10^{-2}
0.4	0.004	0.016	0.86	1.39×10^{-2}
0.6	0.006	0.012	0.98	9.50×10^{-3}
0.7	0.007	0.011	0.90	7.64×10^{-3}
0.9	0.009	0.009	0.92	5.13×10^{-3}
1.0	0.010	0.008	0.92	4.06×10^{-3}
	In Presence of 0.005 M NaOH			
0	0	0	—	9.63×10^{-2} [c]
0.1	0.001	0.029	0.84	7.37×10^{-2}
0.2	0.002	0.023	0.87	4.88×10^{-2}
0.4	0.004	0.016	0.91	3.65×10^{-2}
0.6	0.006	0.012	0.93	3.21×10^{-2}
0.7	0.007	0.011	0.94	2.77×10^{-2}
0.9	0.009	0.009	0.95	1.90×10^{-2}
1.0	0.010	0.008	0.95	1.82×10^{-2}

[a] Assuming micellar density = 1. [b] From Eq. 10. [c] k_w.

The "uncharged" partition coefficient, K of Eq. 7, is equivalent to exp $(-\Delta\mu°/RT)$ and is a constant, so it does not change. The value of C_{im}/C_{iw} of Eq. 7 will be decreased with nonionic surfactants because of the sign of $Z_iF\phi_m$ and will be increased with the ionic surfactant because C_m in Eq. 8 depends on C_s (see *Appendix*) and is given by:

$$C_m = \frac{K'C_T \exp(-Z_iF\phi_m/RT)}{1 - V + KV \exp(-Z_iF\phi_m/RT)} \qquad \text{(Eq. 10)}$$

where the value of $Z_iF\phi_m$ is obtained from either Eq. 8 or 9, depending upon whether the surfactant is nonionic or ionic. Because ϕ_m contains C_m, an iterative calculator program was used for evaluation of C_m from Eq. 10. It is also necessary to assume a value for K to solve Eq. 10. Tables II–IV show values for $\exp(-Z_iF\phi_m/RT)$.

The "charged" partition coefficient, K' exp $(-Z_iF\phi_m/RT)$ of Eq. 7, corresponds to partition coefficient K of Eqs. 1–3 and should replace it.

Table III—Observed First-Order Rate Constants for the Degradation of Indomethacin in 0.002M NaOH in the Presence of Varying Concentrations of Polysorbate 80 at 30.3°

Surfactant Concentration, % (w/v)	Volume Fraction[a] of Micellar Phase (V)	Concentration[b] of Indomethacin in Micelles (C_m), M	$\frac{\exp(-Z_iF\phi_m)}{RT}$	k_{obs}, min^{-1}
0	0	0	—	3.56×10^{-2} [c]
0.1	0.001	0.027	0.77	3.29×10^{-2}
0.2	0.002	0.022	0.81	2.44×10^{-2}
0.4	0.004	0.016	0.86	1.71×10^{-2}
0.6	0.006	0.012	0.89	1.26×10^{-2}
0.7	0.007	0.011	0.90	1.18×10^{-2}
0.9	0.009	0.009	0.92	9.74×10^{-3}
1.0	0.010	0.008	0.92	8.38×10^{-3}

[a] Assuming micellar density = 1. [b] From Eq. 10. [c] k_w.

Table IV—Observed First-Order Rate Constants for the Degradation of Indomethacin in Alkaline Solutions in the Presence of Varying Concentrations of Cetrimonium Bromide at 30.3°

Surfactant Concentration, % (w/v)	Volume Fraction[a] of Micellar Phase (V)	Concentration[b] of Indomethacin in Micelles (C_m), M	$\frac{\exp(-Z_iF\phi_m)}{RT}$	k_{obs}, min^{-1}
	In Presence of 0.002 M NaOH			
0	0	0		4.01×10^{-2} [c]
0.025	0.00025	0.21	210	4.39×10^{-2}
0.05	0.0005	0.12	140	9.90×10^{-2}
0.1	0.001	0.058	69	2.04×10^{-1}
0.2	0.002	0.027	30	2.04×10^{-1}
0.4	0.004	0.013	13	1.69×10^{-1}
0.6	0.006	0.0096	8.8	1.28×10^{-1}
0.7	0.007	0.007	7.6	1.28×10^{-1}
0.9	0.009	0.006	6.1	1.08×10^{-1}
1.0	0.01	0.005	5.6	8.89×10^{-2}
	In Presence of 0.003 M NaOH			
0	0	0		6.13×10^{-2}
0.025	0.00025	0.14	110	8.45×10^{-2} [c]
0.05	0.0005	0.088	79	1.58×10^{-1}
0.1	0.001	0.049	47	2.67×10^{-1}
0.2	0.002	0.025	24	3.15×10^{-1}
0.4	0.004	0.012	12	2.24×10^{-1}
0.6	0.006	0.008	8.3	1.93×10^{-1}
0.7	0.007	0.007	7.2	1.62×10^{-1}
0.9	0.009	0.006	5.9	1.51×10^{-1}
1.0	0.01	0.005	5.4	1.48×10^{-1}
	In Presence of 0.005 M NaOH			
0	0	0		1.00×10^{-1}
0.025	0.00025	0.075	46	1.07×10^{-1} [c]
0.05	0.0005	0.056	38	2.48×10^{-1}
0.1	0.001	0.036	28	3.65×10^{-1}
0.2	0.002	0.021	18	3.85×10^{-1}
0.4	0.004	0.011	10	3.47×10^{-1}
0.6	0.006	0.008	7.5	3.01×10^{-1}
0.7	0.007	0.007	6.7	2.77×10^{-1}
0.9	0.009	0.006	5.5	2.48×10^{-1}
1.0	0.01	0.005	5.2	2.48×10^{-1}

[a] Assuming micellar density = 1. [b] From Eq. 10. [c] k_w.

Equation 3 can now be rewritten (combining Eqs. 7 and 3) as:

$$k_{obs} = k_m + \frac{1}{K'} \frac{(k_w - k_{obs})}{V \exp(-Z_iF\phi_m/RT)} \qquad \text{(Eq. 11)}$$

Plots of k_{obs} *versus* $(k_w - k_{obs})/V \exp(-Z_iF\phi_m/RT)$ were satisfactorily linear and much improved (Figs. 5 and 6) over plots of k_{obs} *versus* $(k_w - k_{obs})/V$ for cetrimonium bromide results (compare Figs. 4 and 6). The

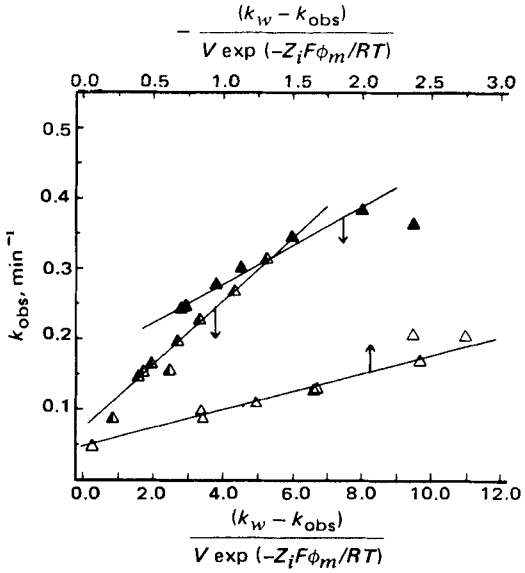


Figure 6—*Relationship between observed rate constants and function of cetrimonium bromide micellar potential. Key (hydroxide-ion concentration):* △, *0.002* M; ▲, *0.003* M; *and* ▲, *0.005* M.

intercept gave the value of k_m, and the slope was equal to $1/K'$. A repeat of the calculation process using the K' values obtained from these plots only slightly altered the values of the constants (Table I).

Values of k_m obtained in this way are near zero for nonionic surfactants, consistent with the absence of a rate increase in the presence of surfactants for indomethacin decomposition. Values of k_m obtained for the ionic surfactant are larger than k_u values but not greatly so.

Negligibly low micellar rates for nonionic surfactants are a result of the near zero k_m values. The rate constant k_m is a composite quantity, containing $k_m{}^0[OH^-]$, $k_m{}^0$ being the value at unit hydroxide-ion concentration. The micellar concentration of hydroxide ion must be near zero through repulsion of this ion by the negative charge conferred on the nonionic surfactant micelles through solubilization of ionized indomethacin.

Values of the partition coefficient K obtained for nonionic surfactants are considerably larger than those obtained for the ionic surfactant. This unexpected result may be due to the simplifications and assumptions made in the derivation. For C_m and k_m also, the simplifications and assumptions mean that no great reliance can be placed on their numerical values. Nevertheless, the improvements in plot linearity, especially for the ionic surfactant, support the general approach and stimulate further work to evaluate unknown quantities and to test the hypothesis more rigorously.

APPENDIX

Assumptions—Several assumptions were made to permit calculation:

1. The micelle phase density was 1 g/ml in all cases.
2. The surfactant molecular weights were: ethoxylated lanolin, 1500; polysorbate 80, 1300; and cetrimonium bromide, 360 (on the basis of the approximate composition).
3. The initial K' values chosen were 300 for nonionic surfactants and 200 for cetrimonium bromide [literature values vary from 20 to 5000 (4, 8, 9)].
4. The CMC value of a surfactant was a negligible proportion of the total surfactant concentration.
5. Indomethacin was completely ionized.
6. Micelles are spherical. The volume of a micelle, V_{m1}, is given by:

$$V_{m1} = \frac{4\pi r^3}{3} \qquad \text{(Eq. A1)}$$

where r is the radius of the micelle. The volume of a micelle also can be obtained as:

$$V_{m1} = \frac{Mn}{N\rho_m} \qquad \text{(Eq. A2)}$$

where M is the molecular weight of the surfactant, n is the aggregation number, N is Avogadro's number, and ρ_m is the density of a micelle. Equations A1 and A2 yield:

$$r = \left(\frac{3nM}{4\pi N\rho_m}\right)^{1/3} \qquad \text{(Eq. A3)}$$

Since the surface area of a sphere is given by $4\pi r^2$, the surface area of a micelle, A_m, is then given by:

$$A_m = 4\pi\left[\frac{3nM}{4\pi N\rho_m}\right]^{2/3} \qquad \text{(Eq. A4)}$$

7. The micelle aggregation numbers are 100 for nonionic surfactants and 50 for cetrimide (9).
8. Hydroxide ions are not specifically adsorbed.
9. As a result of counterion adsorption into the stern layer, the effective value of $Z_iF\phi_m$ is reduced to one-fifth of the value given by Eq. A13 for nonionic surfacts and to one-tenth of the value given by Eq. A14 for the more highly charged cetrimonium bromide micelles (10).

Derivation of Eqs. 8 and 9—The surface charge density, σ, of the double layer around a spherical particle is given by (11):

$$\sigma = \frac{\chi\epsilon}{4\pi}\psi_\alpha \qquad \text{(Eq. A5)}$$

where ψ_α is the electrical potential, ϵ is the dielectric potential, and the quantity $1/\chi$ is a rough measure of the thickness of the double layer, δ (12). Treating a micelle as a charged spherical colloidal particle and applying and rearranging Eq. A5 give micelle potential ϕ_m (in place of ψ_α) as:

$$\phi_m = \frac{4\pi\delta\sigma_m}{\epsilon} \qquad \text{(Eq. A6)}$$

where σ_m is the surface charge density and ϵ is the dielectric constant for water. Double layer thickness, δ, is given by (13):

$$\delta = \frac{K_3}{(|Z|\sqrt{\Sigma C_{iw} + C_{is}})} \qquad \text{(Eq. A7)}$$

where K_3 is a combination of fundamental constants and temperature [$K_1 = 4\pi K_3 eF/\epsilon = 1.44 \times 10^6$; $K_2 = 0.1VM/C_s = 10^{-3}\,M/\rho_m = 1.5$ for ethoxylated lanolin, 1.3 for polysorbate 80, and 0.36 for cetrimonium bromide; and $K_3 = \{\epsilon kT/[(8\pi e^2)(6.02 \times 10^{20})]\}^{1/2} = 4.97 \times 10^{-9}$], Z is the counterion valency, and C_{iw} and C_{is} are molar concentrations of counterions in water and the surfactant, respectively.

The surface charge density of the micelle, σ_m, can be obtained from the ratio of the total charge on all ions in the micelle surface to the micellar surface area, A_m. If Z_s is the charge on the surfactant ions (n in the micelle) and if Z_i is the charge on the number of counterions, then:

$$\sigma_m = \frac{ne}{A_m}\left(Z_s + \frac{0.001\Sigma Z_i V_m C_{im} N}{n\nu}\right) \qquad \text{(Eq. A8)}$$

where n is the micelle aggregation number, e is the electronic charge, V_m is the volume of micellar phase, ν is the number of micelles, N is Avogadro's number, and C_{im} is the molar concentration of counterions in micelles.

The number of micelles, ν, in the total volume, V_T, is given by:

$$\nu = \frac{NC_sV_T}{100Mn} \qquad \text{(Eq. A9)}$$

where M is the molecular weight of the surfactant, and C_s is the surfactant concentration. Substitution of Eq. A9 into Eq. A8 gives:

$$\sigma_m = \frac{ne}{A_m}\left(Z_s + \frac{0.1MV}{C_s}\Sigma Z_i C_{im}\right) \qquad \text{(Eq. A10)}$$

Since all ions are univalent in this work, Eq. A10 reduces to:

$$\sigma_m = \frac{ne}{A_m}(Z_s + K_2\Sigma C_{im}) \qquad \text{(Eq. A11)}$$

Combining Eqs. A6, A7, and A11 yields:

$$\phi_m = \frac{4\pi K_3 ne}{\epsilon A_m}\left(\frac{Z_s + K_2\Sigma C_{im}}{\sqrt{\Sigma C_{iw} + C_{is}}}\right) \qquad \text{(Eq. A12)}$$

Multiplying each side by Z_iF and combining constant terms yield:

$$Z_iF\phi_m = \frac{K_1 n}{A_m}\left(\frac{Z_s + K_2 C_{im}}{\sqrt{\Sigma C_{iw} + C_{is}}}\right) \qquad \text{(Eq. A13)}$$

The sign of $Z_iF\phi_m$ will be a combination of the signs of Z_i and ϕ_m. Since the solubilization of indomethacin ions is considered here, Z_i is negative. For the nonionic micelles, incorporation of indomethacin ions makes ϕ_m negative so that $Z_iF\phi_m$ is positive. For positively charged surfactant ions, solubilization alters the total charge but is unlikely to alter its sign. Therefore, for cetrimonium bromide micelles, $Z_iF\phi_m$ is negative.

Equation A13 can be put into a more useful form if the values for Z_s, C_{is}, ΣC_{im}, and ΣC_{iw} can be obtained for nonionic and ionic surfactants.

Values for Z_s—For nonionic surfactants, $Z_s = 0$. For ionic surfactants, $Z_s = +1$.

Values for C_{is}—For nonionic surfactants, $C_{is} = 0$. For ionic surfactants, $C_{is} = C_s$.

Value for ΣC_{im}—Since the only ion likely to be solubilized is that of indomethacin, $\Sigma C_{im} = C_m$.

Value for ΣC_{iw}—For nonionic or ionic surfactants, the value of C_{iw} could be found using:

$$C_T = VC_m + (1 - V)C_w \qquad \text{(Eq. A14)}$$

where C_m, C_w, and C_T are the concentrations of indomethacin in the micelle, water, and the total volume, respectively.

Solving for C_w yields:

$$C_w = \frac{(C_T - VC_m)}{1 - V} \qquad \text{(Eq. A15)}$$

Since the highest value of V in this study is 0.01, then:

$$C_w = C_T - VC_m \qquad \text{(Eq. A16)}$$

Since:

$$\Sigma C_{iw} = C_{NaOH} + C_w \qquad \text{(Eq. A17)}$$

for ionic surfactants and:

$$\Sigma C_{iw} = C_w \qquad \text{(Eq. A18)}$$

for nonionic surfactants, substituting Eq. A16 in Eqs. A17 and A18 yields:

$$\Sigma C_{iw} = C_{NaOH} + C_T - VC_m \quad \text{(Eq. A19)}$$

for ionic surfactants and:

$$\Sigma C_{iw} = C_T - VC_m \quad \text{(Eq. A20)}$$

for nonionic surfactants.

By substituting values for Z_s, C_{is}, ΣC_{im}, and ΣC_{iw}, Eq. A13 is reduced to:

$$Z_i F\phi_m = \frac{K_1 K_2 n C_m}{A_m\sqrt{C_T - VC_m}} \quad \text{(Eq. A21)}$$

for nonionic surfactants and:

$$Z_i F\phi_m = -\frac{K_1 n(1 + K_2 C_m)}{A_m\sqrt{C_{NaOH} + C_T - VC_m + C_s}} \quad \text{(Eq. A22)}$$

for the ionic surfactant. Equations A21 and A22 are equivalent to Eqs. 8 and 9.

Evaluation of C_m (Derivation of Eq. 10)—The value for C_m can be obtained as follows. Since the only ion likely to be solubilized is that of indomethacin, then $C_{im} = C_m$ and $C_{iw} = C_w$. When solving Eq. 7 for C_w:

$$C_w = \frac{C_m}{K' \exp(-Z_i F\phi_m/RT)} \quad \text{(Eq. A23)}$$

Equation A23 is equal to Eq. A15. Solving for C_m yields Eq. 10.

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ACKNOWLEDGMENTS AND ADDRESSES

Received November 18, 1975, from the *Department of Pharmacy, University of Otago, Dunedin, New Zealand.*

Accepted for publication November 9, 1976.

Grateful acknowledgment is made to the New Zealand Medical Research Council for providing a Summer Student Scholarship to Mrs. J. E. Dawson.

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High-Pressure Liquid Chromatographic–Mass Spectrometric Determination of Δ^9-Tetrahydrocannabinol in Human Plasma following Marijuana Smoking

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Abstract □ A method was developed for analyzing Δ^9-tetrahydrocannabinol (I), a psychotomimetic constituent found in marijuana smoke. The developed method utilizes a high-pressure liquid chromatographic (HPLC) gradient elution program to separate I from the other major cannabinoids in marijuana smoke. To achieve the sensitivity required to detect I in human plasma following marijuana smoking, a mass spectrometric quantification method was developed to analyze the HPLC eluant. To 1 ml of human plasma was added a known amount of internal standard, trideuterated I. This stable isotope provided a check on extraction efficiency, a marker for UV monitoring of the HPLC effluent and subsequent collection, and a convenient mass for mass spectrometric quantification. An ion-counting techique was used in conjunction with the peak matching accessory of the mass spectrometer to provide for a rapid comparison between molecular ions of I and the internal standard. The method was linear, accurate, and reproducible over the concentration range expected for I in plasma following marijuana smoking; 2.5 ng/ml was the lower practical limit of detection. Plasma from 11 male subjects was analyzed by the method at appropriate intervals up to 24 hr after the smoking of a marijuana cigarette containing 10.8 mg of I. Results demonstrated that levels of I could be determined accurately in the plasma of marijuana smokers in the 1-hr period following smoking.

Keyphrases □ Δ^9-Tetrahydrocannabinol—high-pressure liquid chromatographic–mass spectrometric analysis, human plasma after smoking marijuana □ High-pressure liquid chromatography–mass spectrometry—analysis, Δ^9-tetrahydrocannabinol, human plasma after smoking marijuana □ Marijuana constituents—Δ^9-tetrahydrocannabinol, high-pressure liquid chromatographic–mass spectrometric analysis, human plasma after smoking marijuana □ Psychotomimetic agents—Δ^9-tetrahydrocannabinol, high-pressure liquid chromatographic–mass spectrometric analysis, human plasma after smoking marijuana

Marijuana smoking is quite prevalent in certain segments of the populace in the United States (1). Most marijuana contains four principal constituents (2): Δ^9-tetrahydrocannabinol (I), cannabidiol (II), cannabinol (III), and cannabichromene (IV). Compound I is believed to be responsible for the psychotomimetic properties of marijuana (3). Some of the physiological responses in humans were shown to change following smoking of cigarettes