all tablets decreased with their binding agent content (Fig. 10). Although this finding is similar to a previous one where povidone was substituted for gelatin (5), it is also clear that the nature of the binding agent has a profound effect on the dissolution characteristics of a formulation.

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Micellar Distribution Equilibria: Ultracentrifugal Study of Apparent Partition Coefficients

JUNG Y. PARK * and EDWARD G. RIPPIE *

Abstract □ Ultracentrifugation was used for the partial isolation of polysorbate 80 micelles in aqueous media to determine the apparent partition coefficients of various drug species between water and the micellar pseudophase. The ratio of solute concentration in the micelles to that in water was measured for procaine, salicylic acid, sulfapyridine, sulfisoxazole, and sodium 2-naphthalenesulfonate over ranges of pH, surfactant concentration, drug concentration, and micelle sedimentation. Apparent partition coefficients for the systems investigated were independent of both drug concentration and surfactant concentration, indicating that the mode(s) of surfactant–drug interaction are essentially invariant over the ranges of systematic variables studied. The method provides a relatively simple and rapid means of quantitatively evaluating drug–surfactant interactions above the CMC, when surfactant and solute can be assayed in mixtures without interference.

Keyphrases □ Micellar distribution—ultracentrifugal study of apparent partition coefficients of various drugs between water and micellar pseudophase □ Partition coefficients, apparent—various drugs between water and micellar pseudophase, ultracentrifugal study □ Ultracentrifugation—determination of apparent partition coefficients of various drugs between water and micellar pseudophase □ Solute–micelle interactions—ultracentrifugal study of apparent partition coefficients of various drugs between water and micellar pseudophase

Much experimental evidence relating to the mechanisms of interactions between secondary solutes and surfactant micelles is obtained from measurements of the magnitude of solute–micelle interactions and their dependence on variables such as concentration, ionic strength, temperature, and pH. Experimental techniques often introduce inherent systematic errors. Methods such as dialysis, gel filtration, and ultrafiltration, which are based on the mechanical or physicochemical isolation of the micellar pseudophase from the aqueous phase (1, 2), require the use of semipermeable membranes, selectively permeable gels, or other materials that may interact with various components and perturb the system beyond the desired separation.

The micellar isolation or separation process also may result in the destruction of the micellar structure of the surfactant system by concentrating the surfactant until it separates as a true second phase. Solute–micelle interactions also were studied extensively through the solubilization resulting from such interactions (3–6). While this latter method overcomes the problems previously mentioned, it is limited to a single thermodynamic activity for a given solute.

The analytical ultracentrifuge was used to determine the molecular weight, size, and molecular interactions of micelles (7, 8) and to determine the partition coefficients of drugs between liquid and liquid crystalline phases (9) that have been caused to separate. Ultracentrifugation offers the capability of either complete or partial micellar isolation without membranes or other added components within a reasonably short experiment time. The present paper investigates the feasibility of determining solute–micelle interactions, over a range of both solute and surfactant concentrations, using a moderate partial micelle separation by ultracentrifugation. Previously studied systems of aqueous polysorbate 80 solutions containing

Table I—pH Values and Mean Concentrations of Solute and Polysorbate 80 in Solutions Studied

Solute	Concentration of Solute, % (w/w)	Concentration of Polysorbate 80, % (w/w)	pH
Procaine	0.1, 0.01	1, 2, 4	7.7, 7.9, 8.2, 8.7, 9.5
Salicylic acid	0.1, 0.01	1, 2, 4	2.0, 2.2, 2.6, 3.6, 3.8
Sulfapyridine	0.01, 0.001	1, 2, 4	7.7, 7.9, 8.2, 8.7, 9.5
Sulfisoxazole	0.01, 0.001	1, 2, 4	4.7, 4.9, 5.2, 5.7, 6.5
Sodium naphthalenesulfonate	0.2, 0.02, 0.002	1, 2, 4	7.0

procaine, salicylic acid, sulfapyridine, sulfisoxazole, and sodium 2-naphthalenesulfonate were selected.

DATA TREATMENT

Various separate and distinct modes of solute–micelle interaction occur, including adsorption onto the micelle surface, penetration into the palisade layer of nonionic micelles, and dissolution in the fluid hydrocarbon central core. No attempt is made here to imply or otherwise define the particular mechanisms operating, but the solute–micelle association is treated as a partition phenomenon having an associated apparent partition coefficient. The appropriateness of this simple pseudophase model was discussed (1), and it serves as a useful approximation if contributions to micellar free energy from changes in solvent are small and if the micelles are essentially noninteracting. That these conditions are met is evidenced by the independence of the apparent partition coefficients on surfactant concentration. Further assumptions and approximations implicit in the pseudophase model were discussed by Mukerjee (10).

Consider an aqueous micellar solution of a nonionic surfactant containing a second solute partitioned between the water and the micellar pseudophase. After a suitable period of ultracentrifugation, a higher concentration of the more dense micelles exists in the lower (outer) portion of the centrifuge tube as opposed to the upper portion. By assuming that there is no change in the system other than the induced micellar concentration gradient, a resultant concentration gradient of the associated partitioning solute can be predicted.

If, following centrifugation, the contents of the centrifuge tube are separated into upper and lower portions, which are subsequently mixed individually to ensure their homogeneity, the following expressions can be written for X and Y, which are solute concentrations in grams per milliliter in the upper and lower portions, respectively:

$$X = (U/\rho)[(HA) + (A^-)] + (1 - U/\rho)[[HA] + [A^-]] \quad \text{(Eq. 1)}$$

$$Y = (L/\rho)[(HA) + (A^-)] + (1 - L/\rho)[[HA] + [A^-]] \quad \text{(Eq. 2)}$$

where U and L represent surfactant concentrations in grams per milliliter in the upper and lower portions, respectively, and ρ is the micellar density in grams per milliliter. The equations are derived for weakly acidic solutes whose free acid, HA, and conjugate base, A^-, concentrations in grams per milliliter are denoted by ( ) and [ ] in the micellar and aqueous pseudophases, respectively. The first terms on the right in Eqs. 1 and 2 represent the micelle associated solute in 1 ml of aqueous micellar solution, whereas the second terms represent the aqueous solute in the same 1 ml of solution. These expressions can be written in terms of the apparent acid dissociation constant of the solute, K_a, and the solute's apparent partition coefficients, $K_1 = (HA)/[HA]$ and $K_2 = (A^-)/[A^-]$, in its free acid and conjugate base forms:

$$[H^+]\rho X = U[HA][K_1[H^+] + K_2K_a] + (\rho - U)[HA][[H^+] + K_a] \quad \text{(Eq. 3)}$$

$$[H^+]\rho Y = L[HA][K_1[H^+] + K_2K_a] + (\rho - L)[HA][[H^+] + K_a] \quad \text{(Eq. 4)}$$

Combining Eqs. 3 and 4 to eliminate [HA] yields the following expression relating K_1 and K_2 to experimentally measurable quantities:

$$[H^+]K_1 + K_2K_a = ([H^+] + K_a)\left[1 - \frac{\rho(X - Y)}{(XL - YU)}\right] \quad \text{(Eq. 5)}$$

A plot of the expression on the right of Eq. 5 *versus* $[H^+]$ yields a straight line with slope K_1 and intercept K_2K_a. For weakly basic drugs, the following definitions apply to Eq. 5: $K_a = K_w/K_b$, $K_1 = (BH^+)/[BH^+]$, and $K_2 = (B)/[B]$.

Application of Eq. 5 requires that the stoichiometric concentrations of both the surfactant and the partitioning solute be determined. The concentration of micellar surfactant was taken to be equal to its stoichiometric concentration since the critical micelle concentration (CMC) of polysorbate 80 is approximately $1.0–6.2 \times 10^{-3}$% (w/v) (3, 11–14) and can be neglected at the surfactant concentrations studied. The density, ρ, of the polysorbate 80 micelles was taken as 1.11 (11), and the K_a values (moles per liter) employed are: procaine, 1.40×10^{-9}; salicylic acid, 1.06×10^{-3}; sulfapyridine, 3.60×10^{-9}; and sulfisoxazole, 7.60×10^{-6}. Slopes and intercepts of data plotted in accordance with Eq. 5 were obtained by the method of least squares, and their standard deviations were estimated from an analysis of variance of the experimental data points.

EXPERIMENTAL

Centrifugation—Aliquots, 10 ml, of aqueous solutions containing the desired concentrations of polysorbate 80[1] and the secondary solutes were centrifuged at 25° for 10 hr in an ultracentrifuge[2] at 40,000 rpm in cellulose nitrate tubes. The tubes were then cut in half with a tube slicer, and the upper and lower samples were assayed to determine the polysorbate 80 and partitioning solute concentrations. The period of centrifugation was selected to give an approximately 40% separation of micelles and was a fraction of the time required to reach sedimentation equilibrium. This degree of separation was sufficient to provide suitable sensitivity for the method and yet not great enough to alter micellar properties significantly.

The solutes investigated were procaine hydrochloride[3], salicylic acid[4], sulfapyridine[5], sulfisoxazole[5], and sodium 2-naphthalenesulfonate[6]. All other chemicals were reagent grade and were used as received without further purification. Solutions were prepared by dissolving measured quantities of polysorbate 80 and the various drugs in double-distilled water, diluting to 90% of volume, adjusting the pH to the desired values with either hydrochloric acid or sodium hydroxide solutions, and diluting to volume. The pH's were checked in all cases following centrifugation and did not change.

Polysorbate 80 Assay—Polysorbate 80 was assayed in the presence of the solubilized drugs by the method of Cucakovich (15), based on the formation of a complex between the amylose fraction of potato starch and polysorbate 80. That portion of the starch added in excess and not complexed with polysorbate combined quantitatively with iodine to form a blue amylose–iodine complex. The blue color was determined spectrophotometrically[7] at 680 nm, and the procedure gave good results without interference by the solubilized drugs present.

Secondary Solute Assays—Following centrifugation of the micellar polysorbate 80–drug solutions, samples of the upper and lower fractions were diluted with 95% ethyl alcohol to appropriate concentrations of drug and assayed spectrophotometrically[7] for drug content. The presence of polysorbate 80 did not interfere with the assays conducted at the following wavelength maxima: procaine, 288 nm; salicylic acid, 298 nm; and sulfapyridine and sulfisoxazole, 260 nm.

The assay for sodium 2-naphthalenesulfonate was performed fluorometrically[8] at an excitation wavelength of 280 nm and an emission wavelength of 340 nm following dilution of the centrifuged samples with water to a concentration of approximately 1 μg/ml at pH 7. No interference by polysorbate 80 was observed, and the same calibration curve was used with all surfactant concentrations.

RESULTS AND DISCUSSION

Distribution measurements were made at several concentrations of the surfactant and of the various drugs at a number of pH values. Table I summarizes the scope of these experimental variables. The good linearity of data plotted in accordance with Eq. 5 is apparent from Fig. 1, for 0.01% solutions of procaine and sulfapyridine in 1% polysorbate 80, and is typical of all systems studied.

[1] Polyoxyethylene (20) sorbitan monooleate, Tween 80, Atlas Chemical Industries, Wilmington, Del.
[2] Model L2-65B, Beckman Instruments, Palo Alto, Calif.
[3] Matheson, Coleman and Bell, Milwaukee, Wis.
[4] Fisher Scientific Co., Pittsburgh, Pa.
[5] Sigma Chemical Co., St. Louis, Mo.
[6] Eastman Chemical Co., Rochester, N.Y.
[7] Hitachi model 139 spectrophotometer.
[8] Aminco-Bowman spectrophotofluorometer.

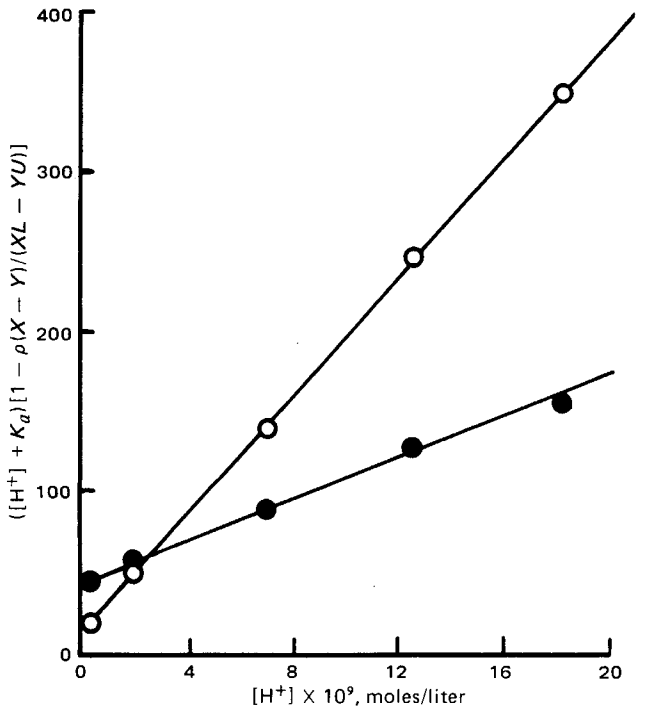

Figure 1—*Relationship of solute distribution data to hydrogen-ion concentration for 0.01% (w/w) procaine and sulfapyridine in 1% (w/w) polysorbate 80 plotted according to Eq. 5. Key: ●, procaine; and ○, sulfapyridine.*

Apparent partition coefficients for the weakly acidic and basic drugs and their conjugate forms are listed in Table II together with their estimated standard deviations and the corresponding linear correlation coefficients, r, of the various plots. No significant difference was found between the apparent partition coefficients of any given drug or its conjugate acid or base over the range of drug or surfactant concentrations studied. This observation is consistent with the assumption, made in deriving Eq. 5, that the nature of the micelles would not be measurably altered by the concentration differences induced by centrifugation.

Apparent partition coefficients of the free drugs reported here are approximately 50–60% of those previously reported or estimated from the literature (5, 6, 16). Also, micellar association with procaine ion (5) and with salicylate ion (2, 6) occurred, in contrast to previous reports. However, these findings should be considered in light of the rather large drug concentration differences and temperature and ionic strength differences used in these methods.

Implicit in the present method is the assumption that ultracentrifugation does not significantly alter the chemical potential of molecular solutes at various levels within the centrifuge tube. For incompressible solutions of neutral molecules, the chemical potential at equilibrium, u_i, is given as a function of angular velocity, ω, radial distance, r, and molecular weight, M_i (17), as:

$$d\bar{u}_i/dr = du_i/dr - M_i\omega^2 r = 0 \qquad \text{(Eq. 6)}$$

The magnitude of centrifugal effects is small for low molecular weight solutes. Furthermore, the apparent average molecular weight of association colloids can be assumed to be a function only of the monomer activity; therefore, the apparent average molecular weight *versus* monomer concentration curve is independent of the rotor speeds and solution depths at which various monomer concentrations occur.

A Donnan effect may occur when a concentration gradient is induced in a charged species in a solution where no such gradient is induced in other diffusible ions. While this effect is commonly observed when a polyelectrolyte in solution is excluded from a second solution by a semipermeable membrane, it also results from the centrifugal induction of a concentration gradient of micelles containing associated charged species. The expression:

$$K_2 = \frac{\rho([A^-]_u^2 - [A^-]_l^2)}{L[A^-]_u^2 - U[A^-]_l^2} \qquad \text{(Eq. 7)}$$

holds for the apparent partition coefficient of a negatively charged species, A^-, between water and nonionic surfactant micelles where subscripts u and l denote upper and lower fractions of the centrifuged solution. Equation 7 requires that $[A^-]_u < [A^-]_l$, a condition not assumed in the derivation of Eq. 5. Neglect of the Donnan effect will diminish the magnitude of the calculated value of K_2.

The effect of a swamping electrolyte on the apparent partition coefficient, K, of sodium 2-naphthalenesulfonate between water and polysorbate 80 micelles was determined by the addition of potassium chloride to the micellar solutions prior to centrifugation. Results of measurements at several concentration combinations of polysorbate and naphthalenesulfonate are presented in Table III. Calculations of K were made with the use of:

$$K = 1 - \frac{\rho(X - Y)}{(XL - YU)} \qquad \text{(Eq. 8)}$$

which is a limiting form of Eq. 5 and applies where only charged solute species exist. Results compare favorably with those reported using dialysis (2). No attempts were made to adjust K_2 values for Donnan effects, since such calculations would require an exact knowledge of the micellar concentration profile within the centrifuge tube.

Table II—Apparent Partition Coefficients of Solutes between Polysorbate 80 Micelles and Water

Solute, % (w/w)	Polysorbate 80, % (w/w)	$K_1 \pm SD$	$K_2 \pm SD$	r
		Procaine		
0.1	1	7.0 ± 0.2	29.2 ± 1.5	0.9988
0.1	2	7.0 ± 0.1	28.9 ± 0.7	0.9997
0.1	4	7.0 ± 0.1	28.8 ± 0.9	0.9995
0.01	1	6.4 ± 0.3	30.2 ± 2.2	0.9969
0.01	2	6.6 ± 0.3	29.2 ± 1.9	0.9977
0.01	4	6.9 ± 0.2	28.9 ± 1.1	0.9993
		Salicylic Acid		
0.1	1	57.3 ± 2.9	24.0 ± 14.7	0.9924
0.1	2	57.6 ± 2.7	23.6 ± 13.9	0.9967
0.1	4	57.6 ± 2.8	24.0 ± 14.3	0.9964
0.01	1	56.3 ± 3.8	21.6 ± 19.5	0.9932
0.01	2	56.4 ± 3.2	26.6 ± 16.4	0.9952
0.01	4	56.4 ± 2.5	33.8 ± 12.6	0.9972
		Sulfapyridine		
0.01	1	18.5 ± 0.4	4.0 ± 1.2	0.9992
0.01	2	18.6 ± 0.5	3.8 ± 1.4	0.9991
0.01	4	19.0 ± 0.6	3.2 ± 1.8	0.9984
0.001	1	18.9 ± 0.6	2.8 ± 1.9	0.9983
0.001	2	18.8 ± 0.6	3.6 ± 1.7	0.9985
0.001	4	18.8 ± 0.6	3.6 ± 1.7	0.9986
		Sulfisoxazole		
0.01	1	79.0 ± 2.0	14.5 ± 2.7	0.9991
0.01	2	79.4 ± 2.3	13.8 ± 3.2	0.9987
0.01	4	79.2 ± 2.2	14.3 ± 3.0	0.9989
0.001	1	78.5 ± 2.0	15.0 ± 2.7	0.9991
0.001	2	77.8 ± 2.1	15.2 ± 2.9	0.9989
0.001	4	78.9 ± 1.7	15.6 ± 2.3	0.9993

Table III—Apparent Partition Coefficients of Sodium 2-Naphthalenesulfonate between Polysorbate 80 Micelles and Water[a]

Sulfonate, % (w/w)	Polysorbate 80, % (w/w)	Potassium Chloride, moles/liter	K
0.2	1	0	4.1
0.2	1	0.1	8.8
0.2	1	0.2	13.9
0.2	2	0	4.6
0.2	2	0.1	8.5
0.2	2	0.2	13.4
0.2	4	0	4.6
0.2	4	0.1	8.6
0.2	4	0.2	13.5
0.02	2	0	4.3
0.02	2	0.1	8.8
0.02	2	0.2	13.1
0.002	2	0	4.4
0.002	2	0.1	8.7
0.002	2	0.2	12.8

[a] All solutions were adjusted to pH 7.

SUMMARY

The study of micelle–solute interactions by means of partial ultracentrifugal separations of the micellar components offers some possible advantages over other procedures. It permits relatively rapid separations, which are advantageous when solutes are unstable chemically, and avoids the use of membranes or other added materials for the purpose of micelle isolation. Moreover, flexibility in the choice of systems is essentially limited only by the need to assay both the micellar and secondary solute components. While the data were treated in terms of a nonspecific partitioning model in the present paper, the general approach is equally compatible with various adsorption, specific site, or other models of micelle–solute interactions.

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Physicochemical Properties of β-Lactam Antibacterials: Deuterium Solvent Isotope Effect on Penicillin G Degradation Rate

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Abstract □ To obtain kinetic evidence on the degradation mechanism of penicillin in aqueous solution, degradation rates of penicillin G in water and deuterium oxide were measured in the pH (pD) range of 4–10. The solvent isotope effect (k^{H_2O}/k^{D_2O}) of 1.53 below pH (pD) 6 supports the mechanism of water-catalyzed rearrangement of undissociated penicillin G to benzylpenicillenic acid. The spontaneous degradation at neutral pH (pD) and the hydroxide-ion-catalyzed degradation in the alkaline pH (pD) range progress with a deuterium solvent isotope effect (k^{H_2O}/k^{D_2O}) of 4.5 and 0.59, respectively. This finding indicates the mechanisms of general base-catalyzed hydrolysis by water in the neutral pH range and of nucleophilic attack of the hydroxide ion on the β-lactam in the alkaline pH range. No significant side-chain dependency was observed in the reaction of penicillins with bases. The solvent isotope studies led to the conclusion that penicillin degradation is catalyzed by a series of bases *via* general base-catalyzed and nucleophilic mechanisms, depending on their basicity.

Keyphrases □ Penicillin G—degradation in aqueous solution, deuterium solvent isotope effect, pH 4–10 □ Degradation—penicillin G in aqueous solution, deuterium solvent isotope effect, pH 4–10 □ Deuterium oxide—solvent isotope effect on penicillin G degradation in aqueous solution, pH 4–10 □ Antibacterials—penicillin G, degradation in aqueous solution, deuterium solvent isotope effect, pH 4–10

Although the stability of penicillins in aqueous solution has been studied (1–14), little work has been done on their mechanism of degradation.

Interest in the degradation of penicillin in acid and neutral solutions has been stimulated by the suggestion that its degradative product, penicillenic acid, rather than penicillin itself, may be responsible for allergic reactions[1]. During the degradation of penicillin G at a physiological pH of 7.5, the solution slowly gave rise to both benzylpenicilloic acid and benzylpenicillenic acid (15, 16). Levine (15, 16) suggested that the formation of benzylpenicilloic acid proceeded by way of benzylpenicillenic acid as an intermediate, because the latter compound was converted rapidly into the former with a half-life of 6.5 min at pH 7.5 and 37° (17).

Furthermore, some investigators (18, 19) assumed that higher sensitivity of the β-lactam ring in penicillin molecules relative to that of simple β-lactams to hydroxide-ion-catalyzed degradation can be attributed in part to the intramolecular attack of the side-chain amide on the β-lactam moiety. These two hypotheses (15–19) recently were criticized (13, 20) on the basis of kinetic observations, although the mechanism of degradation in neutral and alkaline solutions is not completely understood.

The present study was undertaken to obtain kinetic

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