Effect of Temperature Upon Solubilization by a Series of Nonionic Surfactants

By K. J. HUMPHREYS and C. T. RHODES

The solubilization of benzoic acid by four *n*-alkylpolyoxyethylene surfactants as a function of temperature has been examined by the dialysis and solubility techniques. In addition to their use in formulation work, it is shown that evaluation of the equilibrium constants controlling the solubilization process can be of use in elucidating micellar properties. The calculation of the thermodynamic parameters controlling the solubilization process is discussed and some difficulties indicated.

MICELLAR SOLUBILIZATION has been studied extensively in recent decades. To the pharmacist the topic is of particular interest because many pharmaceutical formulations utilize surfactants to enhance the solubility of sparingly soluble components.

One of the most important and illuminating ways of treating micellar solutions of surfactants is to regard the micelles as forming a definite phase which is at the same time dispersed throughout the system in submicroscopic particles of definite size, the micelles. The concentration of surfactant in the aqueous or continuous phase may be equated with the CMC. In systems where the CMC is very low, *e.g.*, aqueous solutions of many nonionic surfactants, the system is divisible into two essentially single-component phases, disregarding any solvent water bound to the micelles. If a third component, termed the "cosolute," is added to the system, it will be distributed between the 2 phases. When the distribution is in favor of the micellar pseudophase, the phenomenon is referred to as solubilization (1). When such surfactant solutions are brought into equilibrium with pure solid or liquid cosolutes, the system takes up appreciably more than an equivalent amount of pure water. Micellar solubilization is characterized by the enhanced solubility of cosolute only being apparent above the CMC of the ternary system. If the CMC itself is low, then the initial region of zero solubilization is not readily detectable.

In any L_1, isotropic aqueous liquid, surfactant system an equilibrium exists between micellar and nonmicellar cosolute.

$$(S_w) \overset{K}{\rightleftharpoons} (S_m) \qquad \text{(Eq. 1)}$$

where (S_w) and (S_m) represent the equilibrium activities of the nonmicellar and micellar species of cosolute and K is the equilibrium constant.

There is considerable evidence which suggests that the pharmacological activity of a drug in micellar systems is a function of (S_w), the micellar material being regarded as an inactive reserve of drug (1, 2). Two techniques are available for evaluating (S_w) and (S_m), equilibrium dialysis (3) and potentiometry (4). Unfortunately, comparatively little data have been collected using these methods. The results of most studies have been obtained by use of the conventional solubility technique which, of itself, does not allow differentiation between (S_w) and (S_m) except at saturation values. However, it is apparent from the results of those systems for which information about (S_w) and (S_m) is available that micellar solubilization can be accomplished by more than one mechanism (5). It is important to realize that, using the two-phase approach, since the micelles occupy a part of the total volume of the system, the over-all concentration of free cosolute may not necessarily be equated with the actual free concentration in the aqueous phase. However, considerable theoretical difficulties arise when attempts are made to estimate the volume of the micellar pseudophase. The phase could be considered to consist of (*a*) the hydrocarbon core of the micelle, (*b*) the whole micelle, or (*c*) the whole micelle plus bound and trapped water. At present insufficient evidence is available for a rational choice to be made.

The effect of temperature upon the properties of binary surfactant systems has been studied by many workers. Such measurements are particularly useful in that they allow evaluation of the thermodynamic parameters controlling the equilibrium in such systems (6, 7). Fewer studies of the effect of temperature upon equilibria in ternary surfactant systems have been reported, and little work has been done on the evaluation of the thermodynamic parameters controlling such equilibria. In the present

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paper some studies of the effect of temperature upon the solubilization of benzoic acid by a series of *n*-alkylpolyoxyethylene surfactants are reported and their practical and theoretical significance discussed.

EXPERIMENTAL

Materials—Analar benzoic acid,[1] four *n*-alkylpolyoxyethylene surfactants[2] all of the general formula C_nOE_mOH where n is the number of carbon atoms in the alkyl chain and m the number of polyoxyethylene groups. The mean molecular weights of these compounds have been previously determined by nuclear magnetic resonance spectroscopy (8).

Methods—Dialysis studies were performed using dialysis cells similar in design to those described by Patel and Foss (9). The cells were thermostatically controlled to ±0.1° of the required temperature. Each cell, compartment capacity 10 ml., contained a glass bead to ensure continuous mixing of contents. Nylon membranes[3] were shown by preliminary tests to be impermeable to the surfactants but to allow diffusion of the benzoic acid. The solvent used in the dialysis and solubility studies was 0.01 *N* hydrochloric acid, to depress ionization of the benzoic acid. The cells were shaken for 4 days, a time found to be sufficient for equilibrium to be reached. Samples from both sides of the membrane were removed by pipet, calibrated gravimetrically at the appropriate temperature, and after suitable dilution, the benzoic acid concentrations were determined by spectrophotometric assay at 273 mm. using a Uvispek.[4] The molar absorptivity of benzoic acid, 966, compares closely to the literature value of 970 (10).

Solubility determinations were made by shaking excess benzoic acid in various concentrations of surfactant solution for 4 days. Samples for assay were filtered through No. 3 filter sticks, and the benzoic acid content determined as described above.

Determinations of weight/ml. were made by use of pycnometers, calibrated gravimetrically at the appropriate temperature. The apparatus was thermostatically controlled to within ±0.1° of the required value.

RESULTS AND DISCUSSION

Figure 1 is typical of the results obtained from the dialysis studies. HA_m represents the amount of benzoic acid solubilized by the surfactant and $[HA_w]$ the concentration of nonbound acid. The linear nature of such plots indicates that the solubilization process in such systems is governed by a distribution coefficient, correlation coefficients calculated for all systems being above 0.95. Thus for these systems the solubilization mechanism is most readily interpreted in terms of distribution between an aqueous continuous phase and a micellar pseudophase.

$$K_d = [HA_m]/[HA_w] \qquad \text{(Eq. 2)}$$

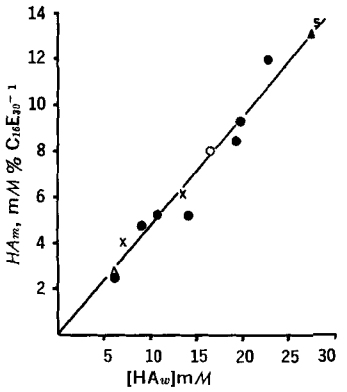

Fig. 1—Dialysis results for the interaction between benzoic acid and an n-*alkyl polyoxyethylene surfactant, $C_{16}E_{30}$, at 25°. Key: ▲, solubility point; ○, 1% w/v $C_{16}E_{30}$; ●, 2% w/v $C_{16}E_{30}$; ×, 3% w/v $C_{16}E_{30}$; △, 4% w/v $C_{16}E_{30}$.*

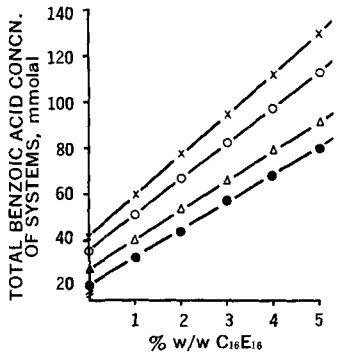

Fig. 2—Effect of temperature on the solubilization of benzoic acid by aqueous solutions of an n-*alkyl polyoxyethylene surfactant, $C_{16}E_{16}$. Key: ×, 37°; ○, 31°; △, 25°; ●, 18°.*

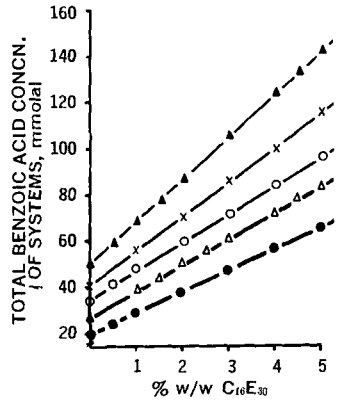

Fig. 3—Effect of temperature on the solubilization of benzoic acid by aqueous solutions of $C_{16}E_{30}$. Key: ▲, 45°; ×, 37°; ○, 31°; △, 25°; ●, 18°.

For unionized components such as benzoic acid the aqueous activity coefficient will be very close to

[1] Supplied by B. D. H. Ltd., Poole, England.
[2] Texofors, supplied by Glover's Ltd., Leeds, England.
[3] Supplied by Portex Plastics Ltd., Hythe, Kent, England.
[4] Supplied by Hilger & Watts Ltd., London, England.

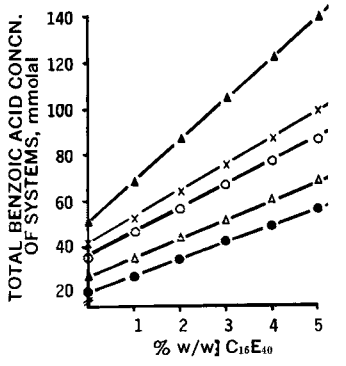

Fig. 4—Effect of temperature on the solubilization of benzoic acid by aqueous solutions of $C_{16}E_{40}$. Key: ▲, 45°; ×, 37°; ○, 31°; △, 25°; ●, 18°.

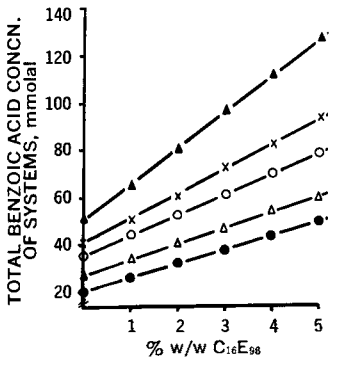

Fig. 5—Effect of temperature on the solubilization of benzoic acid by aqueous solutions of $C_{16}E_{96}$. Key: ▲, 45°; ×, 37°; ○, 31°; △, 25°; ●, 18°.

unity, and provided the activity coefficient of the micellar species also approximates to unity, the K_d values as defined in Eq. 2 will represent the thermodynamic equilibrium constant.

Figures 2 through 5 show the effect of temperature and surfactant concentration upon the solubilization of benzoic acid. In all cases the concentration of benzoic acid was calculated in molal terms by use of the appropriate weight/ml. value, though in fact the difference between molar and molal values was very small since in all cases the weight/ml. values were close to unity. The intercept values on the ordinates represent the solubility of benzoic acid in 0.01 *N* hydrochloric acid, $[HA_w^0]$. In all the systems examined the relationship between the total amount of benzoic acid present and surfactant concentration was found to be linear. This type of solubilization behavior is common, though in some systems both negative and positive changes of slope in such plots have been observed (11). Extrapolation of the graph in Figs. 2–5 to 100% w/w surfactant allows estimation of the solubility of benzoic acid in a "bulk micellar" surfactant phase, with properties exhibited by the surfactant when in 0–5% w/w aqueous solution. This value represents the solubility of benzoic acid in the micellar pseudophase. The K_d values for the various systems investigated in this work have been calculated by use of Eq. 3 and are listed in Table I.

$$K_d = [HA_m^0]/[HA_w^0] \qquad \text{(Eq. 3)}$$

This particular method of calculation of the K_d value is of considerable use since it does not involve estimation of micellar or nonmicellar phase volumes and thus avoids the difficulties described earlier. It is, of course, only applicable to systems in which it has been demonstrated that the solubilization process is governed by a form of the distribution law.

The effect of temperature upon the bactericidal activity of a preservative in a surfactant solution depends upon the temperature coefficient of the bactericide and the variation of the K_d value with temperature (12). Bean and his co-workers have emphasized that two-phase preparations containing bactericides must be formulated from a viewpoint that considers both the physical and microbiological properties of the system.

Using an extension of the two-phase model of micellar systems, it has been suggested that solubilization by surfactant micelles is due to the volume of the hydrocarbon core of the micelle, and that the solubilization process is directly comparable to solution in an organic bulk phase (13). If this hypothesis is valid, it may be predicted that the $[HA_m^0]$ values for the various surfactants studied in this investigation will be linear functions of the percentage of hydrocarbon, *i.e.*, alkyl chain H.

$$H = 225 \times 100/M \qquad \text{(Eq. 4)}$$

where M is the surfactant molecular weight, and 225 is the contribution to the molecular weight made by the cetyl chain. Thus the value $[HA_m']$, as defined in Eq. 5, should for any given temperature be the same for all the surfactants studied:

$$[HA_m'] = [HA_m^0] \times 100/H \qquad \text{(Eq. 5)}$$

Further, the values of $d[HA_m']/dT$ should be constant for the various surfactants. From the plots of $[HA_m']$ against temperature shown in Fig. 6, it can be seen that not only are the $[HA_m']$ values

Table I—Solubilization Parameters as a Function of Temperature

Surfactant Formula	H	K_d Temp., °K. 291.0	298.0	304.0	310.0	318.0
$C_{16}E_{16}$	23.30	59.51	50.07	43.75	44.11	...[a]
$C_{16}E_{30}$	14.26	47.80	45.55	35.42	38.23	38.66
$C_{16}E_{40}$	11.15	37.07	32.72	28.76	29.90	37.06
$C_{16}E_{96}$	5.02	31.22	27.43	25.43	27.46	32.25

[a] No K_d value determined at this temperature for $C_{16}E_{16}$ because the cloud point temperature was exceeded.

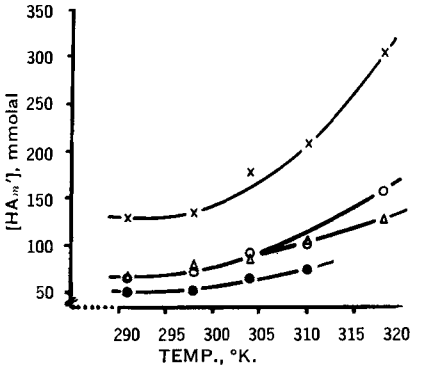

Fig. 6—Plot of [HA_m'] *as a function of temperature for four* n*-alkyl polyoxyethylene surfactants. Key:* ×, $C_{16}E_{96}$; ○, $C_{16}E_{40}$; △, $C_{16}E_{30}$; ●, $C_{16}E_{16}$.

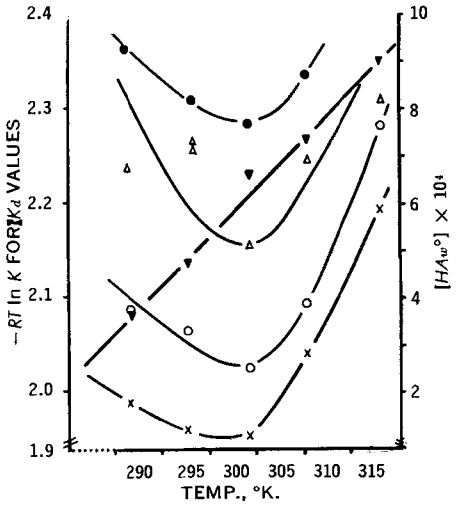

Fig. 7—Plot of −RT *ln* K *against temperature. Key:* ●, $C_{16}E_{16}$; △, $C_{16}E_{30}$; ○, $C_{16}E_{40}$; ×, $C_{16}E_{96}$; ▼, *benzoic acid, aqueous solubility.*

different for the various surfactants but also the rate of change of [HA_m'] with temperature varies. Thus the simple two-phase model appears insufficient to rationalize the solubilization phenomena in a quantitative manner. This failure may well be attributed to steric aspects of the solubilization process. Although the surfactant micelle is believed to be a loosely organized structure of transient existence, there are elements of rigidity not present in a hydrocarbon bulk phase. It has been suggested that benzoic acid and similar cosolutes are located at the junction of the hydrocarbon core and palisade layer of the micelle (14). Such an orientation provides a unique environment for the solubilized species; the hydrocarbon "tail" surrounded by the alkyl chains of the surfactant, and the carboxylic acid head group hydrogen bonded to either water molecules or polyoxyethylene groups in the palisade layer. This process cannot be envisaged entirely as a simple solution mechanism but rather as an interaction between surfactant and cosolute, *i.e.*, comicellization.

Although techniques for the calculation of the thermodynamic parameters controlling the micellization process in binary surfactant systems are well established, little attention has been directed to thermodynamic aspects of solubilization. When thermodynamic equilibrium constants for the solubilization process are available, it is possible to evaluate the free energy change of the process by use of Eq. 6:

$$\Delta G = -RT \ln K \qquad \text{(Eq. 6)}$$

Free energy changes may be resolved into two components, enthalpic and entropic values by means of Eq. 7:

$$\Delta G = \Delta H - T\Delta S \qquad \text{(Eq. 7)}$$

Thus a plot of $-RT \ln K$ against T should be linear with a slope of $-\Delta S$ and an intercept at 0°K. of ΔH. However, in order to substitute relevant ΔG values in Eq. 7, it is essential that the equilibrium being considered should exist between the same standard states. In the case of micellar solubilization it is doubtful if this criterion is fulfilled. As has been indicated previously, the uptake of solubilized material by the micelle must to some extent be a function of the micellar architecture. Since it is known that the aggregation number of surfactants, in both binary and ternary systems, varies with temperature (7), it may be concluded that micellar structure is temperature dependent and thus Eq. 7 may not be validly applied to such systems. Figure 7 shows the variation of $-RT \ln K$ as a function of T for the solubilization of benzoic acid by the four surfactants studied in this project. For comparison purposes the [HA_w°] values, solubility of benzoic acid in 0.01 N hydrochloric acid, expressed in terms of mole fraction are plotted in Fig. 7. However, plots of $-RT \ln K$, derived from the K_d values, against T show considerable curvature, all curves having a minimum at about 300°K. Though this curvature could be simply explained for equilibrium between the same standard states in terms of variation of ΔH or ΔS with temperature, it is felt that a more likely explanation is in terms of the variation of micellar structure as outlined above.

Relatively few attempts appear to have been made previously to estimate ΔH and ΔS for the micellar solubilization process from studies of the effect of temperature. Examination of the literature shows that studies of the effect of temperature upon solubilization processes made at several temperatures and over a considerable temperature range are comparatively rare. However, Bahal and Kostenbauder (15), who studied the interaction of chlorobutanol with polyvinylpyrrolidone, have shown that Eqs. 6 and 7 are obeyed by such a system.

Clearly, further studies of micellar solubilization, in particular the effect of cosolutes upon micelle size and shape, are required before a detailed theory rationalizing such interaction can be formulated.

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Keyphrases

Benzoic acid solubilization
Solubilization with *n*-alkylpolyoxyethylene surfactants—temperature effect
Equilibrium constants controlling solubilization
Micellar solubilization
Distribution coefficient
Dialysis studies
UV spectrophotometry—analysis

Synthesis and Properties of the Antileukemic Agent 5(or 4)-[3,3-Bis(2-chloroethyl)-1-triazeno]imidazole-4(or 5)-carboxamide

By Y. FULMER SHEALY, CHARLES A. KRAUTH*, LEE B. HOLUM, and WILLIAM E. FITZGIBBON

The antileukemic agent 5(or 4)-[3,3-bis(2-chloroethyl)-1-triazeno]imidazole-4(or 5)-carboxamide (II, NSC-82196) is an unstable compound that is easily convertible to an isomeric transformation product. By taking appropriate precautions, however, the triazene may be prepared in good yield, with very little of the transformation product as contaminant, and may be stored for long periods at low temperatures. The quality of specimens of II may be estimated from its infrared spectrum and that of its transformation product.

In the mouse lymphoid leukemia L1210 test system (1), 5(or 4)-[3,3-bis(2-chloroethyl)-1-triazeno]imidazole-4(or 5)-carboxamide (II) has demonstrated excellent activity. Some of the animals treated with large single doses of this compound survived until the experiments were terminated 4–8 months after treatment. From the cell-kill kinetic studies of Skipper, Schabel, and Wilcox (2, 3), it may be concluded that complete eradication of leukemic cells must have occurred in these survivors. Compound II is unstable and is readily converted to an isomeric product that is not active against leukemia L1210. In a preliminary communication (1) the authors reported, without experimental details, the preparation of II from 5-diazoimidazole-4-carboxamide (I) and bis(2-chloroethyl)amine (III). In this report complete details are recorded on the preparation of II, including improvements; and some of its properties and the precautions that must be observed during the preparation, storage, and use of this compound are described more fully.

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