Importance of Molecular Aggregation in the Development of a Topical Local Anesthetic

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The surface active properties of a new, topical, local anesthetic formulation containing 60% tetracaine free base and 40% tetracaine acid salt (w/w) in 40% propylene glycol and 60% saline (v/v) were investigated. The surface tension, electrical conductivity, and apparent pH were monitored as functions of concentration to detect the presence of micellar aggregates in solution. Micelles were detected in solvents containing 20% to 60% propylene glycol. The use of pH as a method for detecting micelles yields lower critical micelle concentrations than either surface tension or electrical conductivity. Quasi-elastic light scattering was used to determine the size of tetracaine micelles in solution. The largest micelles were detected in the aqueous solution. Micelle size decreased rapidly and no micelles were detected at 80% propylene glycol or above.

Introduction

Despite the proliferation of new topical drug treatments, an effective, topical, local-anesthetic formulation has not yet been developed. The reason is not a lack of interest, for many researchers have been working on this problem for years.[1-10] Rather, the fundamental stumbling block is the inability to transport sufficient anesthetic through the skin to create an effective nerve block in a reasonable time.

A new, topical, local anesthetic formulation has been developed that is at least comparable to the current state of the art in transdermal local anesthesia. The formulation consists of a mixture of tetracine free base and tetracaine acid salt in a vehicle of 40% propylene glycol and 60% saline. The development, characterization, and optimization of this formulation have been detailed elsewhere.[11,12] The anomalous solubility behavior of a 60% tetracaine free base, 40% acid salt (w/w) mixture[11] suggested possible micellization in light of the known surface-active nature of such local anesthetics.[13] Micellization, if it occurred, could influence the diffusion of the anesthetic through skin and change the effective concentration in the formulation. Significant numbers of molecular aggregates could also increase the lifetime of a transdermal formulation by increasing its drug content. Consequently, there was a need to determine whether this mixed system (free base, acid salt, propylene glycol, saline) contained micelles, under what circumstances, and their size.

Materials and Methods

Materials. Two forms of tetracaine were used in the diffusion experiments, tetracaine free base (hydrophobic ester) and tetracaine hydrochloride (hydrophilic salt of the free base). Both forms of tetracaine (Sigma) were used as received. Tetracaine base penetrates the neuron more effectively but has very low aqueous solubility.[14] Tetracaine salt, however, is quite soluble in aqueous solutions (>200 g/L or 0.67 M).[11] Tetracaine salt is also much more stable than the free base, which must be kept refrigerated and dry. Since tetracaine hydrochloride is thermally more stable, it can be sterilized and still remain effective. For transdermal diffusion however, sterility is not so great a concern and tetracaine base becomes more attractive.

To compromise between the favorable diffusion characteristics of the base form and the high aqueous solubility of the salt form, a mixture of the base and salt forms was used. Such a mixture takes advantage of the tetracaine salt–tetracaine free base equilibrium (Figure 1).[15]

The solvents used in surface studies were propylene glycol (Fisher, USP), deionized, distilled water, and saline (0.9% (w/w) or 0.15 M). The saline was prepared from deionized, distilled water and NaCl (Fisher, Biological Grade).

Methods. *Surface Tension.* All surface tension measurements were made on a Rosano surface tensiometer, Model LG, with a Wilhelmy plate (1 cm × 3 cm × 0.5 mm). This instrument was calibrated using water with a known surface tension of 72.4 mN/m. The uncertainty of these measurements is approximately 0.2 mN/m. Significant micellization was detected by a discontinuity in the slope of surface tension (γ_C) or surface pressure ($\pi_C = \gamma_0 - \gamma_C$) versus concentration ($C$), where γ_0 is the surface tension of solvent. The concentration at which this discontinuity occurred (onset of significant micellization) was assumed to be the critical micelle concentration (cmc).

Conductivity. Critical micelle concentrations (cmc's) can be detected by conductivity as well as by surface tension. The interpretation of the conductivity (κ) versus concentration is analogous to surface tension (the discontinuity in slope indicates a change in the solution structure). Conductivity measurements were made using a YSI conductivity bridge, Model 31, with a YSI

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(15) Mixing a drug and its HCl salt in solution is equivalent to adding HCl acid to a preparation containing only free base (or adding NaOH to a preparation containing only the salt form).[14]

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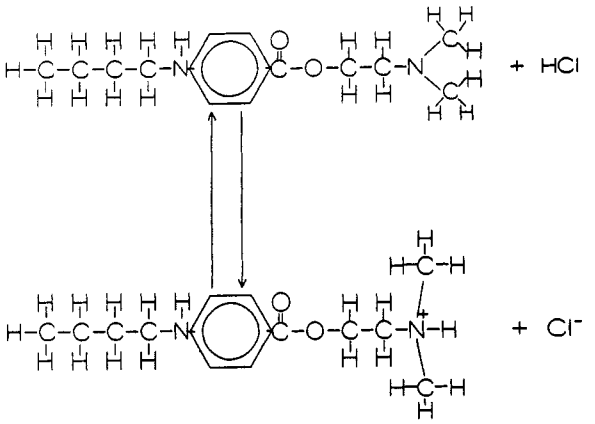

Figure 1. Tetracaine free base, tetracaine acid salt equilibrium.

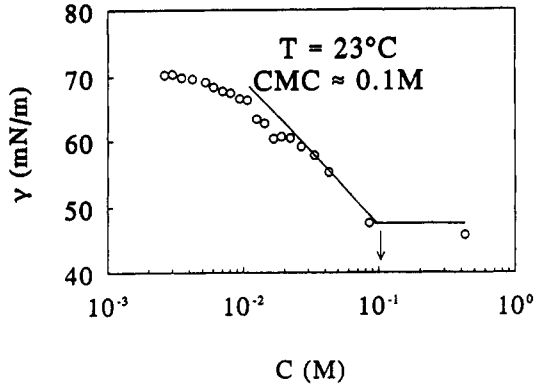

Figure 2. Surface tension of aqueous tetracaine acid salt.

3043 electrode (cell constant = 1/cm). The instrument can measure conductivities from about 0.5 $\mu\Omega^{-1}$ to 2 Ω^{-1} (resistance from 0.5 Ω to 2 MΩ). Conductivities from this instrument have an uncertainty of approximately 0.2 $\mu\Omega^{-1}$.

Titration. The acid–base behavior of tetracaine-containing formulations was explored by simple titration. The apparent pH of the tetracaine formulation was monitored as a function of concentration. Again, a discontinuity in the slope indicated the onset of significant micellization.

Quasi-elastic Light Scattering. A Brookhaven quasi-elastic light scattering (QELS) system was used to determine the size of tetracaine micelles in saline, propylene glycol, and two intermediate solvents (50% and 80% propylene glycol). To determine the micelle diameter using QELS, the viscosity and refractive index of the solvent were estimated. This was accomplished by linear interpolation based on the fraction of propylene glycol. The refractive index of propylene glycol was obtained from ref 16. The viscosity of propylene glycol was calculated using a correlation.[17]

Results and Discussion

Surface Tension of Tetracaine Formulations. A surface tension versus concentration plot for tetracaine hydrochloride in water is presented in Figure 2. The surface tension decreases rapidly with increasing drug concentration initially but eventually flattens out as more drug is added. Such a strong effect of concentration on surface tension indicates that tetracaine hydrochloride is surface active (accumulates preferentially at the surface) and the flattening of the curve at higher concentrations indicates the presence of appreciable numbers of micelles at a cmc of approximately 0.1 M. This agrees almost identically with the previously published value of 0.13 M.[13]

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(17) Reid, R. C.; Prausnitz, J. M.; Sherwood, T. K. *The Properties of Liquids and Gases*, 3rd ed.; McGraw-Hill: New York, 1977; Chapter 9.

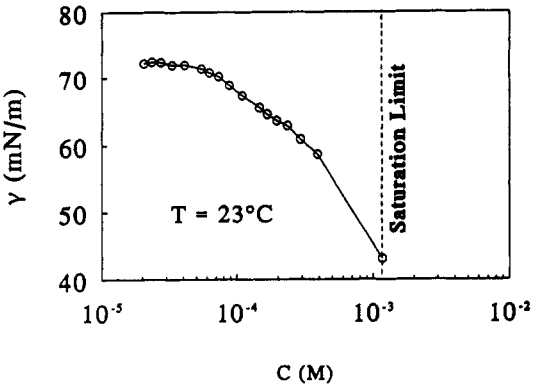

Figure 3. Surface tension of aqueous tetracaine free base.

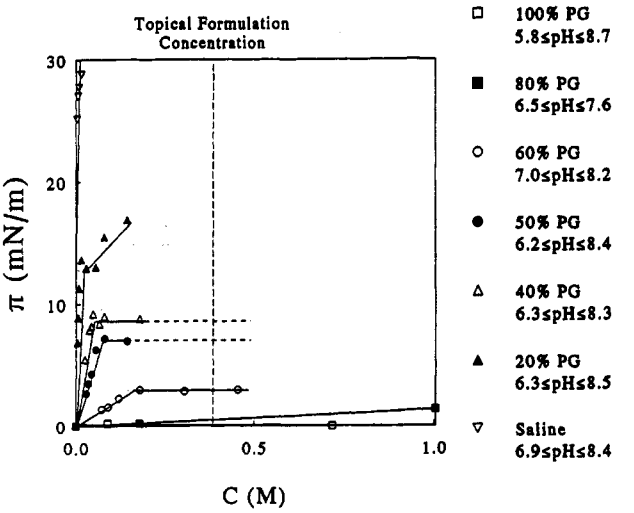

Figure 4. Surface pressure of 60% tetracaine free base and 40% tetracaine acid salt (w/w) in solvents of propylene glycol and saline.

Similar measurements were made for tetracaine base in water (Figure 3). The surface tension of the tetracaine base solution decreases more rapidly than for tetracaine hydrochloride, indicating that it is more surface active. The surface tension drops to about 40 mN/m before the aqueous solubility of tetracaine base is exceeded. Tetracaine base shows higher surface activity than the HCl salt, which appears to be linked to its lower solubility. The surface tension data indicate that tetracaine base does not form appreciable numbers of micelles like the HCl salt but precipitates out of solution as solid crystals.

To determine whether micelles formed in mixed solvents of propylgne glycol and saline, similar measurements were performed with a 40% tetracaine acid salt, 60% free base (w/w) solute. The normal surface tension of the solvent ranges from 72.4 mN/m for water to about 30 mN/m for pure propylene glycol. In order to more clearly illustrate the effect of added solute, the surface tension (γ_C) has been converted to surface pressure (π_C); where $\pi_C = \gamma_0 - \gamma_C$ (γ_0 refers to $C = 0$ or no solute). Figure 4 shows the surface pressure of tetracaine in propylene glycol–saline solvents. Surface pressure rises from 0 in the pure solvent to some maximum value which depends on the solute–solvent interaction. To determine a cmc, the location of the change in slope must be identified. Table I summarizes the cmc from surface pressure versus concentration measurements. As the fraction of propylene glycol increases, the cmc of tetracaine increases. The increase in cmc may be caused by an increase in molecular drug solubility. As the molecular solubility increases, the tendency to form micelles decreases. The 20% saline–

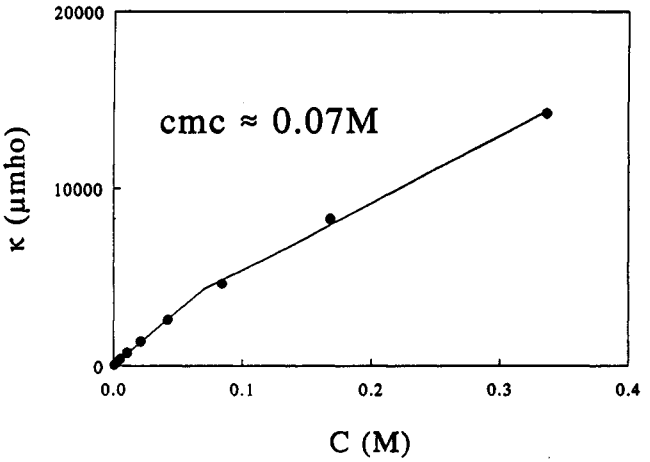

Figure 5. Electrical conductivity of aqueous tetracaine acid salt.

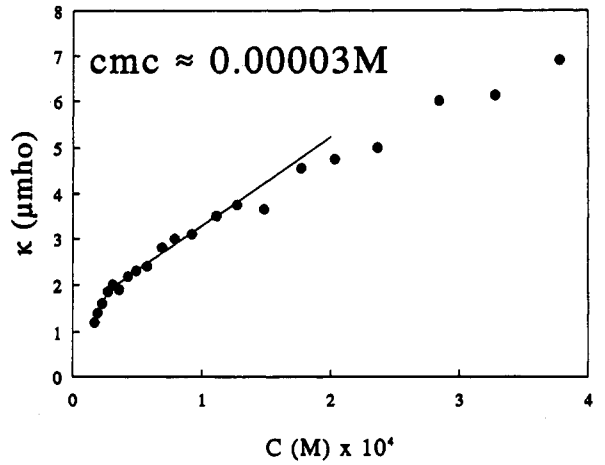

Figure 6. Electrical conductivity of aqueous tetracaine free base.

Table I. Critical Micelle Concentrations of Tetracaine (60% Free Base, 40% Acid Salt (w/w)) in Propylene Glycol and Saline As Measured by Surface Pressure

% propylene glycol	cmc (M)	pH range (apparent)
0	(no cmc)	6.85–8.37
20	0.02	6.30–8.51
40	0.04	6.26–8.29
50	0.07	6.17–8.43
60	0.15	7.03–8.41
80	(no cmc)	6.46–7.55
100	(no cmc)	5.80–8.65

80% propylene glycol and 100% propylene glycol systems do not show micelle formation. Micelles will cease to form readily as molecular solubility increases or as the micelles become unstable. The decrease in overall solubility of the tetracaine mixture[18] (60% free base, 40% acid salt (w/w)) from 80% to 100% propylene glycol (v/v)[11] may, therefore, be a result of fewer micelles.

Conductivity. The conductivity of tetracaine salt and base versus concentration has also been measured. The results of these measurements are in Figures 5 and 6. The conductivity of these aqueous solutions rises with drug concentration for both forms (acid salt and free base). By analysis similar to that for surface tension versus concentration, the cmc can be obtained by identifying a change in slope between two linear portions.[19] Through this method, the cmc of aqueous tetracaine salt is found to be

(18) Overall solubility refers to the cumulative solubility of tetracaine acid salt and tetracaine free base (total tetracaine concentration).

(19) Vold, R. D.; Vold, M. J. *Colloid and Interfacial Chemistry*; Addison-Wesley: Reading, PA, 1983; p 590.

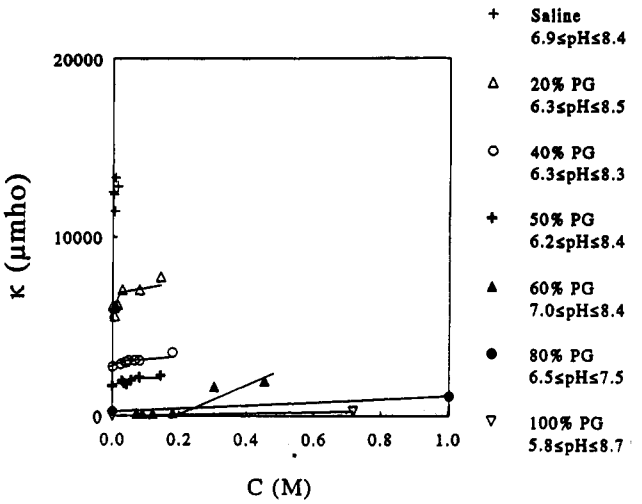

Figure 7. Electrical conductivity of 60% tetracaine free base and 40% tetracaine acid salt (w/w) in solvents of propylene glycol and saline.

Table II. Critical Micelle Concentration of Tetracaine (60% Free Base, 40% Acid Salt (w/w)) in Propylene Glycol and Saline As Measured by Conductivity

% propylene glycol	cmc (M)	pH range (apparent)
0	(no cmc)	6.85–8.37
20	0.03	6.30–8.51
40	0.04	6.26–8.29
50	0.06	6.17–8.43
60	0.18	7.03–8.41
80	(no cmc)	6.46–7.55
100	(no cmc)	5.80–8.65

0.07 M, which is in general agreement with that from surface tension measurements given the resolution of the data (Figure 2).

The graph of conductivity versus concentration for tetracaine base (Figure 6) indicates that there is an association product at very low concentration (3×10^{-5} M). This behavior, unlike that suggested by the surface tension versus concentration graph (Figure 3), further elucidates the uncertainty of cmc values measured by different means.

The conductivity versus concentration behavior for mixtures of tetracaine acid salt (40% (w/w)) and tetracaine free base (60% (w/w)) was also measured in propylene glycol–saline solvents (Figure 7 and Table II). Comparing cmc values from surface tension (Table I) and conductivity (Table II) shows that the values are in general agreement.

The conductivity of propylene glycol–saline mixtures decreases as propylene glycol content increases. This is a result of fewer ions in solution as water is replaced by propylene glycol. Propylene glycol does not dissociate appreciably in solution, so it is less capable of solvating ions or conducting electricity.

pH. Measuring the apparent pH as a function of concentration can also be used to determine the cmc. The pH versus concentration behavior for tetracaine (40% acid salt, 60% free base (w/w)) in solvents of propylene glycol and saline is illustrated in the following sequence of graphs (Figures 8–13). As drug is added to solution, the pH rises monotonically for all systems except pure propylene glycol. For some systems, the apparent pH reaches a maximum and begins to fall. This change in slope indicates a change in the structure of the solution. This change in structure can be viewed as the onset of significant micellization; the

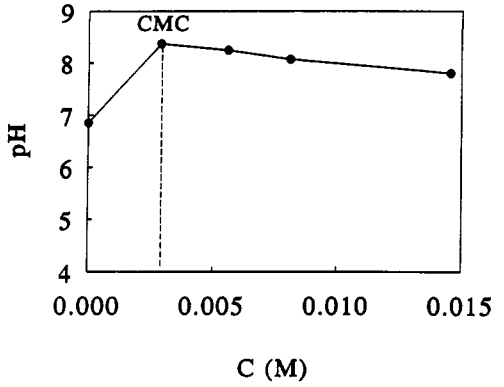

Figure 8. pH of 60% tetracaine free base and 40% tetracaine acid salt (w/w) in saline.

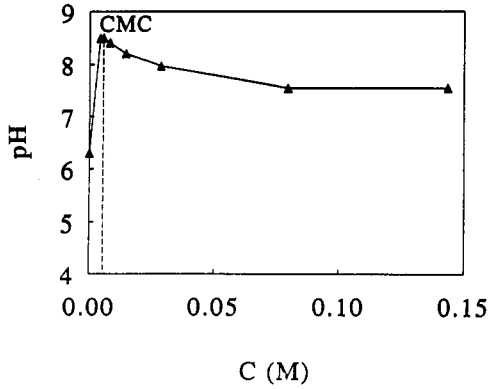

Figure 9. Apparent pH of 60% tetracaine free base and 40% tetracaine acid salt (w/w) in 20% propylene glycol and 80% saline (v/v).

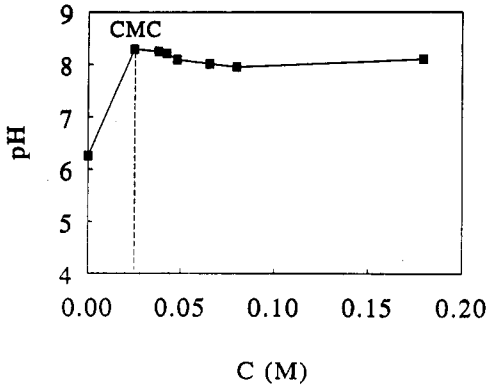

Figure 10. Apparent pH of 60% tetracaine free base and 40% tetracaine acid salt (w/w) in 40% propylene glycol and 60% saline (v/v).

concentration at which it occurs can be viewed as the cmc.[20] On the basis of these assumptions, Table III lists the cmc's of these solutions as measured by apparent pH versus concentration. With the exception of 20% propylene glycol, these values agree almost as well with those of Tables I and II as the latter do with each other (this method is the most conservative as it suggests a cmc for 0% propylene glycol and gives lower cmc's for the other solvents).

Quasi-elastic Light Scattering. Many features of micellar behavior can be deduced through surface pressure, conductivity, and even pH versus concentration measure-

(20) Definitive analysis of Figure 12 (80% propylene glycol) is not possible as no maximum was detected. This may be a result of insufficient data, but the graph is included for the sake of completeness.

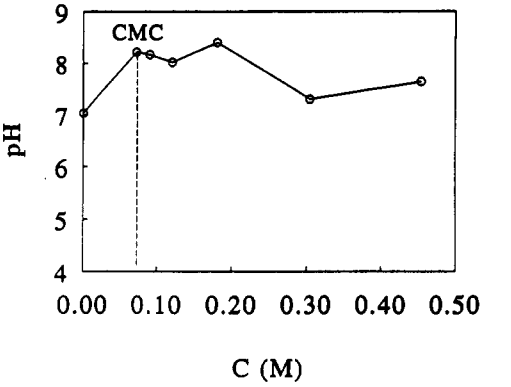

Figure 11. Apparent pH of 60% tetracaine free base and 40% tetracaine acid salt (w/w) in 60% propylene glycol and 40% saline (v/v).

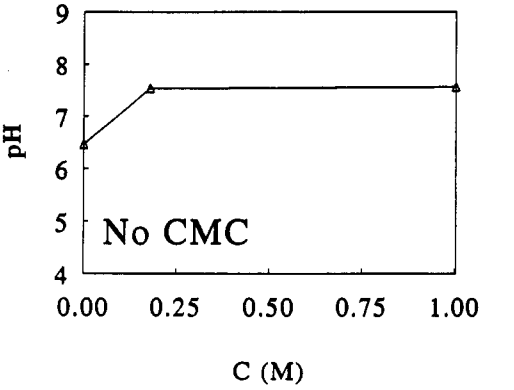

Figure 12. Apparent pH of 60% tetracaine free base and 40% tetracaine acid salt (w/w) in 80% propylene glycol and 20% saline (v/v).

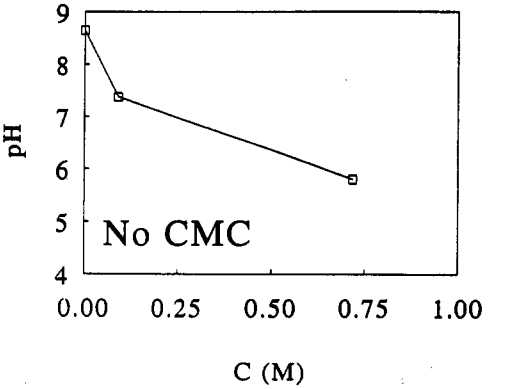

Figure 13. Apparent pH of 60% tetracaine free base and 40% tetracaine acid salt (w/w) in propylene glycol.

Table III. Critical Micelle Concentration of Tetracaine (60% Free Base, 40% Acid Salt (w/w)) in Propylene Glycol and Saline As Measured by pH

% propylene glycol	cmc (M)	pH range (apparent)
0	0.003	6.85–8.37
20	0.004	6.30–8.51
40	0.025	6.26–8.29
60	0.072	7.03–8.41
80	(no cmc)	6.46–7.55
100	(no cmc)	5.80–8.65

ments. One feature, however, cannot be determined with these methods: micelle size. Micelle size can be determined with light scattering techniques. The diameter of micelles in solutions of 40% acid salt, 60% free base (w/w) (0.12 M overall) and solvents of propylene glycol and saline were studied. The largest micelles are in

the purely aqueous system[21] and micelle size decreases rapidly as the organic fraction increases until at 80% propylene glycol no micelles are detected. Therefore, as the fraction of propylene glycol increases, the cmc increases and the micelle diameter decreases. Micelle size was found to be time dependent and equilibrium micelle diameters are not available at this time.

Conclusions

Molecular Aggregation. Like other, similar local anesthetics, tetracaine is highly surface active. Evidence of significant molecular aggregation was found by three independent methods: surface tension, specific conductivity, and pH. For all three phenomena, a discontinuity in the measured quantity versus concentration suggests the formation of significant numbers of micelles.

Using surface tension and conductivity, we found that tetracaine acid salt forms significant numbers of micelles in aqueous solution while tetracaine free base probably does not.[22] Tetracaine free base is not soluble enough to produce many molecular aggregates.

Three independent methods were used to study the molecular aggregation of 60% tetracaine free base, 40% tetracaine acid salt mixtures (w/w) in solvents of propylene glycol and saline.[23] Surface tension and conductivity measurements indicated that these tetracaine mixtures readily form micelles in solvents of 20% to 60% propylene glycol. The critical micelle concentrations from these two methods agree very well (within 33%). Critical micelle concentrations determined by pH versus concentration measurements were slightly lower although they were in general agreement with those of surface tension and conductivity. The differences in the cmc values are attributed to the broad range over which micellization begins and the way micellization affects the measured properties.

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(21) Recall that surface tension and conductivity did not detect a cmc for this system. Therefore, micellization is not strongly favored, but micelles may still form in limited numbers.

(22) The specific conductivity of aqueous tetracaine free base indicated the presence of significant numbers of micelles, but at very low concentrations.

(23) A previous paper[12] identified the solubility behavior of the drug mixture as anomalous relative to solutions of the free base and acid salt alone.