radioactive acetate. This procedure should cause the dilution of radioactive malonyl-CoA formed from radioactive acetate. (Also, the added malonate might alter the acetate-malonate equilibrium to prevent incorporation of radioactive label into malonyl-CoA.) If no labeled acetate were converted to malonyl-CoA, 100% of the rubrofusarin molecule's radioactivity would be in the acetate of the terminal starting unit.

Flooding the cultures with nonradioactive malonate increased the amount of radioactivity of the carbon in Position 2 of the ring system of rubrofusarin; this supports the view that acetate serves as the terminal starting unit in the formation of a polyketide chain. Since the increase in percent incorporation was 47.5% instead of the theoretical 100%, apparently, some radioactive acetate was converted to malonyl-CoA, decreasing the percentage value of the experimental results. Addition of nonlabeled malonate, however, caused a significant increase in radioactivity of carbon in Position 2 of rubrofusarin by 133% over the results of the acetate only experiment.

Schmidt degradation of acetate recovered from the Kuhn-Roth oxidation of radioactive rubrofusarin revealed no randomization of radioactive label in the terminal starting acetate unit. Absence of such randomization indicated that the original radioactive acetate had not been metabolized to another compound before incorporation into rubrofusarin

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Effect of Salts on the Surface/Interfacial Tension and Critical Micelle Concentration of Surfactants

LUCY S. C. WAN and PHILIP K. C. POON

Abstract □ All the salts used produced shifts of the CMC to lower concentrations and reduced the surface/interfacial tensions of air-surfactant solution and liquid paraffin-surfactant solution systems, respectively. No appreciable difference was observed when air was substituted for liquid paraffin as the upper phase, indicating that the hydrocarbon layer exerted no pressure effects. It appeared that shifts of CMC were related to the valency of the gegenion, a divalent gegenion would produce a shift much greater than a monovalent gegenion. Cationic gegenions were more effective in lowering the CMC of sodium lauryl sulfate than similar anions while anionic gegenions were effective with cetrimide and cetylpyridinium chloride. The CMC of cetomacrogol 1000 was practically unaffected by the addition of salt and the extent of interfacial tension reduction with respect to salt concentration was small when compared to corresponding systems containing ionic surfactants.

Keyphrases □ Surface-interfacial tension—salts effect □ Critical micelle concentration, surfactants—salts effect □ Interfacial, surface tension determinations—surfactant, salt-surfactant solutions

Anionic surfactants have been shown to be influenced by the type, magnitude of charge, and degree of hydration of the gegenions (1, 2), whereas cationic surfactants appear to be influenced only by the ionic size or degree of hydration of the gegenion (1, 3, 4). Nonionic surfactants although not charged may form hydroxonium ions with weak cationic properties (5) and their CMC's are affected by anions and their degree of hydration (5–10). It has been found that electrolytes promote adsorption and aggregation processes, possibly by decreasing the electrokinetic repulsion (5, 11–15) which can also be due to the screening of the double layer. Salts have been shown to suppress the dissociation of the surfactant monomer and cause a decrease in the desorption rate (16, 17). Greshfeld (18) attributes the decrease in surface tension to the formation of a monolayer of nondissociated monomers. Tartar (16, 19) postulates that addition of electrolyte would reduce the thickness of the ionic atmosphere enveloping the surfactant monomer.

Nonionic surfactants are characterized by their undissociation and higher degree of hydration. The latter property is indicated by an increase in the CMC with increase in the hydrophobic chain length (20, 21) and by the large positive values of the heat and entropy of micellization in the presence of salts as compared with ionic surfactants in the same concentration of salt (22). This communication reports the change in CMC and the further reduction of surface/interfacial tension of an air-liquid system and a liquid-liquid system where one phase is a surfactant solution, in the presence of added salts. The information obtained may be useful for the preparation of solubilized and emulsified products.

EXPERIMENTAL

Materials—The following salts were used: ammonium chloride BP, ammonium bromide BPC, ammonium sulfate,[1] lithium chloride,[1] lithium sulfate,[2] magnesium chloride,[3] magnesium sulfate

[1] E. Merck, Darmstadt, Germany.
[2] A. R. Grade, British Drug House Ltd.
[3] May and Baker, Dagenham, England.

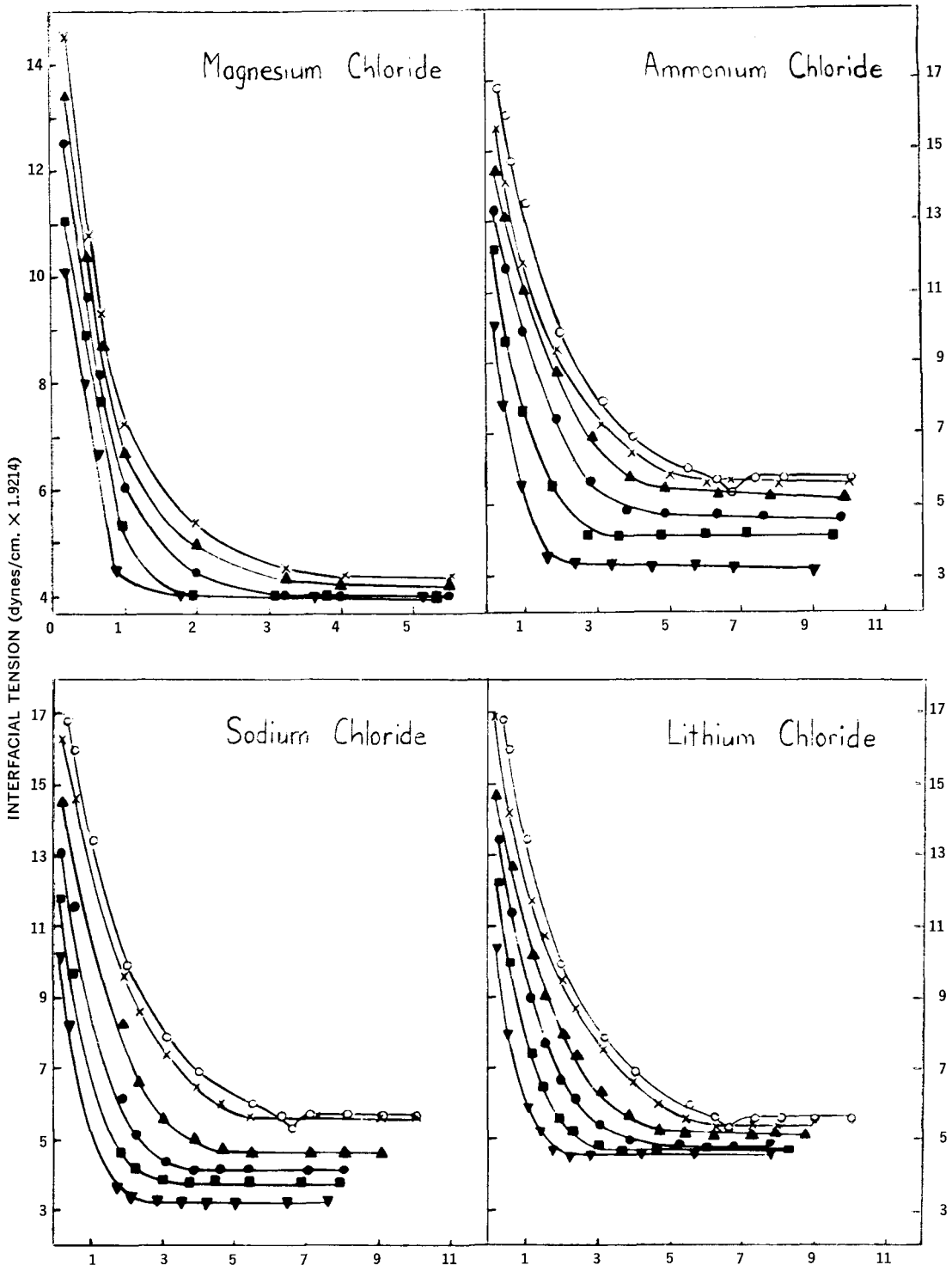

Figure 1—*Effect of various chlorides on the interfacial tension of sodium lauryl sulfate with liquid paraffin as the upper phase at 25°. Key:* ○, *no salt; magnesium chloride:* ×, *0.0100*M; ▲, *0.0206*M; ●, *0.0495*M; ■, *0.1009*M; ▼, *0.1999*M; *ammonium chloride:* ×, *0.0100*M; ▲, *0.0207*M; ●, *0.0494*M; ■, *0.1005*M; ▼, *0.1998*M; *sodium chloride:* ×, *0.0100*M; ▲, *0.0207*M; ●, *0.0494*M; ■, *0.1008*M; ▼, *0.1992*M; *lithium chloride:* ×, *0.0100*M; ▲, *0.0208*M; ●, *0.0498*M; ■, *0.1008*M; ▼, *0.1986*M.

BP, potassium chloride BP, potassium bromide BP, sodium chloride BP, sodium bromide BP, sodium sulfate BP, zinc sulfate BP, sodium citrate BP, sodium phosphate,[3] and sodium tartrate.[1] Solutions of these salts were added to the aqueous surfactant solution in the air-surfactant solution and liquid paraffin-surfactant solution systems. Sodium lauryl sulfate,[4] cetylpyridinium chloride,[1] cetrimide BP[5] (chiefly tetradecyltrimethylammonium bromide), and cetomacrogol 1000 BPC[5] were used as supplied. Their physical constants are listed in Table I. Liquid paraffin BP with a kinematic viscosity of 95.58 c.s. at 37.8° was used as the upper liquid phase.

Apparatus—Du Nouy tensiometer, Cambridge Instrument Co.

Measurement of Surface Tension of Surfactant Solutions at 25°—A series of surfactant solutions of varying concentrations was prepared and allowed to remain in the dishes for 30 min. for adsorption,

[4] Sipon Products, Ltd., London, England.

[5] Glovers (Chemicals) Ltd., Leeds, England.

Table I—Physical Constants of Surfactants

Surfactant	Melting Point,[a] °C.	CMC[b] (moles/l.) at 25°
Sodium lauryl sulfate	178–180	6.5×10^{-3}
Cetrimide BP	233–234	3.2×10^{-3}
Cetylpyridinium chloride	76.5–77.0	0.92×10^{-3}
Cetomacrogol 1000 BPC	42.5–43.0	3.0×10^{-5}

[a] Hot stage micro-melting point apparatus. [b] From surface tension measurements using the Du Nouy tensiometer.

if any, by the glass to take place, drained dry, and fresh solutions remade in the same dishes. The ring of the tensiometer was immersed in the solution and the dish covered with aluminium foil to reduce evaporation. The tensiometer was placed inside a perspex box and the room temperature maintained at 25 ± 0.5°. The surface tension was measured after 10 min., by raising the ring upward gently and slowly till it broke away from the meniscus; two more minutes were allowed for further stabilization of the meniscus just before it ruptured.

Calculated quantities of salt solution of known strength were introduced by means of a hypodermic syringe into the bulk of the surfactant solution in a sequential cumulative manner such that the salt concentration of the system ranged from about 0.01 to 0.2 moles/l. The surface tension was taken after each addition of salt.

Measurement of Interfacial Tension at 25°—The preliminary treatment was the same as that described above. After immersing the ring in the surfactant solution an equal quantity of liquid paraffin was introduced gently to cover the aqueous phase ensuring that no air bubbles and aqueous droplets were present in the liquid paraffin phase. The interfacial tension was measured 10 min. after bringing the two phases in contact, by raising the ring upward into the liquid paraffin phase until the meniscus broke away from the ring. The salt solutions were introduced in the same manner as that stated above. The CMC was obtained from the intersection of the tangents drawn to the points of the straightest portions of the curves of surface/interfacial tensions against surfactant concentration plots.

RESULTS AND DISCUSSION

It was found that the discontinuity in the plots of surface/-interfacial tension against surfactant concentration for air-surfactant solution and liquid paraffin-surfactant solution systems, respectively, occurred at about the same concentration of surfactant. This indicated that liquid paraffin was not likely to alter the process of micellization of surfactant monomers. Generally the addition of salt resulted in a reduction of surface tension, interfacial tension and CMC of sodium lauryl sulfate, cetrimide, cetylpyridinium chloride, and cetomacrogol 1000. The magnitude of the reduction depended on the salt concentration and valency as well as the type of surfactant involved. The presence of liquid paraffin as the upper phase did not seem to affect the magnitude of the effect exerted by the salt additives in the subphase of sodium lauryl sulfate solution. The effect was observed to be similar in both the air-surfactant solution and liquid paraffin-surfactant solution systems. However, with varying concentrations of salt, the corresponding lowering of the CMC of cetrimide was greater in the air-surfactant solution than in the liquid paraffin-surfactant solution system. This could be due to the pressure effect of the hydrocarbon upper phase and that cetrimide could be sensitive to the presence of this phase.

Figure 1 shows the effect of various chlorides on the interfacial tension between liquid paraffin and sodium lauryl sulfate solution. A similar behavior was also observed with a series of sulfates in the same system. All the salts depressed the interfacial tension, the depression increased with salt concentration. The order of effectiveness of the salts on the lowering of the CMC of sodium lauryl sulfate was as follows for chlorides: $Mg^{++} > NH_4^+ > Na^+ > Li^+$ and for sulfates: $Zn^{++} \geqslant Mg^{++} > NH_4^+ > Na^+ > Li^+$. These results showed that the divalent cations produced a greater lowering of the CMC than the univalent cations. It is well known that specific ion effects are related to the size of the hydrated ion, namely, a decrease in the lyotropic number corresponds to a decrease in hydrated ionic radius. Using similar cations, but different anions, it was found that the order of effectiveness of shifting the CMC was the same for sodium and ammonium salts, namely, $Br^- > Cl^- > SO_4^{--}$ and that the order was in line with a decrease of the lyotropic number of the anion. However, with magnesium salts, it was noted that the sulfate ion was more effective than the chloride ion. On the whole, cationic gegenions were more effective than similar anions on the reduction of the CMC of sodium lauryl sulfate.

Figure 2 shows the effect of sodium salts on the interfacial tension between liquid paraffin and cetrimide solution. In the same manner as for sodium lauryl sulfate, the interfacial tension was reduced as the salt concentration was increased. Several ammonium salts also produced similar results. The order of the anionic gegenions on the depression of the CMC of cetrimide was as follows for sodium salts: $Br^- > C_6H_5O_7^{---} > Cl^- > SO_4^{--}$ and for ammonium salts: $Br^- > Cl^- > SO_4^{--}$ It was noted that the order fitted in the pattern of the Hofmeister series. Although not so markedly effective, the various cations showed a slight effect on the CMC as seen in the bromide series, the order of effectiveness being as follows: $Na^+ > NH_4^+ > K^+$. In the case of chlorides and sulfates, the lowering of the CMC by different cations was the same, namely, $Mg^{++} = Li^+ = NH_4^+ = Na^+ = K^+$. Generally the anionic gegenions were more effective in the case of cationic surfactant.

For cetylpyridinium chloride which was included to determine the effectiveness of a common gegenion effect, it was found that cations had an insignificant effect on the CMC as seen in the chlorides where $Na^+ = Li^+$ and in that of the sulfates where $Na^+ = Mg^{++}$. These results indicated that the response towards the gegenions was different from that of cetrimide, there was no indication of a common ion effect. The anionic gegenions shifted the CMC to lower concentration, the order of the effect being as follows for sodium salts: $C_6H_5O_7^{---} > SO_4^{--} > C_4H_4O_6^{--} > Br^- > Cl^-$. This showed that the citrate ion was most effective with the chloride ion as the least effective, which was different from the order for the liquid paraffin-cetrimide solution system although both are cationic surfactants. It could be assumed that the chemical nature of the surfactant has significant influence.

Cetomacrogol 1000 was found to be practically unaffected by the addition of salt. The magnitude of interfacial tension reduction with respect to salt concentration was very small when compared with corresponding systems containing ionic surfactants. However, the order of effectiveness of the anionic gegenions on the lowering of the CMC by sodium salts could be arranged as follows: $PO_4^{---} > Br^- > Cl^- > SO_4^{--}$. Cations had little effect on the CMC as seen in the chlorides which produced no appreciable change in the CMC since $Mg^{++} = NH_4^+ = Na^+$.

The factors which operate in ionic surfactant solutions tending to retard surface molecules migration are likely to be (*a*) the electrokinetic potential in the electrical double layer surrounding an ionic species that is developed when the compound dissociates; (*b*) the hydrophobic bonding between the nonpolar hydrocarbon portions of the surfactant and "iceberg" water. These factors may also govern the degree of micellization of the surfactant at high concentration. With a nonionic surfactant, in addition, account must be taken of the hydration forces due to the presence of hydroxyl groups and ether linkages which can hydrogen bond with water molecules. The observed reduction in interfacial tension and the lowering of the CMC by salts may be due to a reduction of the factors retarding migration by the salts. In addition, interfacial packing of the adsorbed monomers, particularly of the ionic type, can be increased by the presence of salts as a result of the reduction in electrostatic repulsive forces between the charged heads in the aqueous phase adjacent to the interface.

The marked effects of counterions on both the anionic and cationic surfactants support the above explanation regarding the relative influence of the various factors. The lesser effect of ions having similar charge on the surface activity of the ionic surfactants may be attributed mainly to the secondary role they play on the reduction of the cooperative structure of "iceberg" water around the hydrocarbon surface chain. The quite definite effect of anions on the surface activity of cetomacrogol 1000 seemed to support the theory of the formation of positive hydroxonium ions and their effect on the ether oxygen of polyoxyethylenic surfactant molecules (5, 8, 23) which is weakly cationic.

Cationic surfactants on the whole exhibited a greater surface activity than the anionic or nonionic surfactants in the presence of

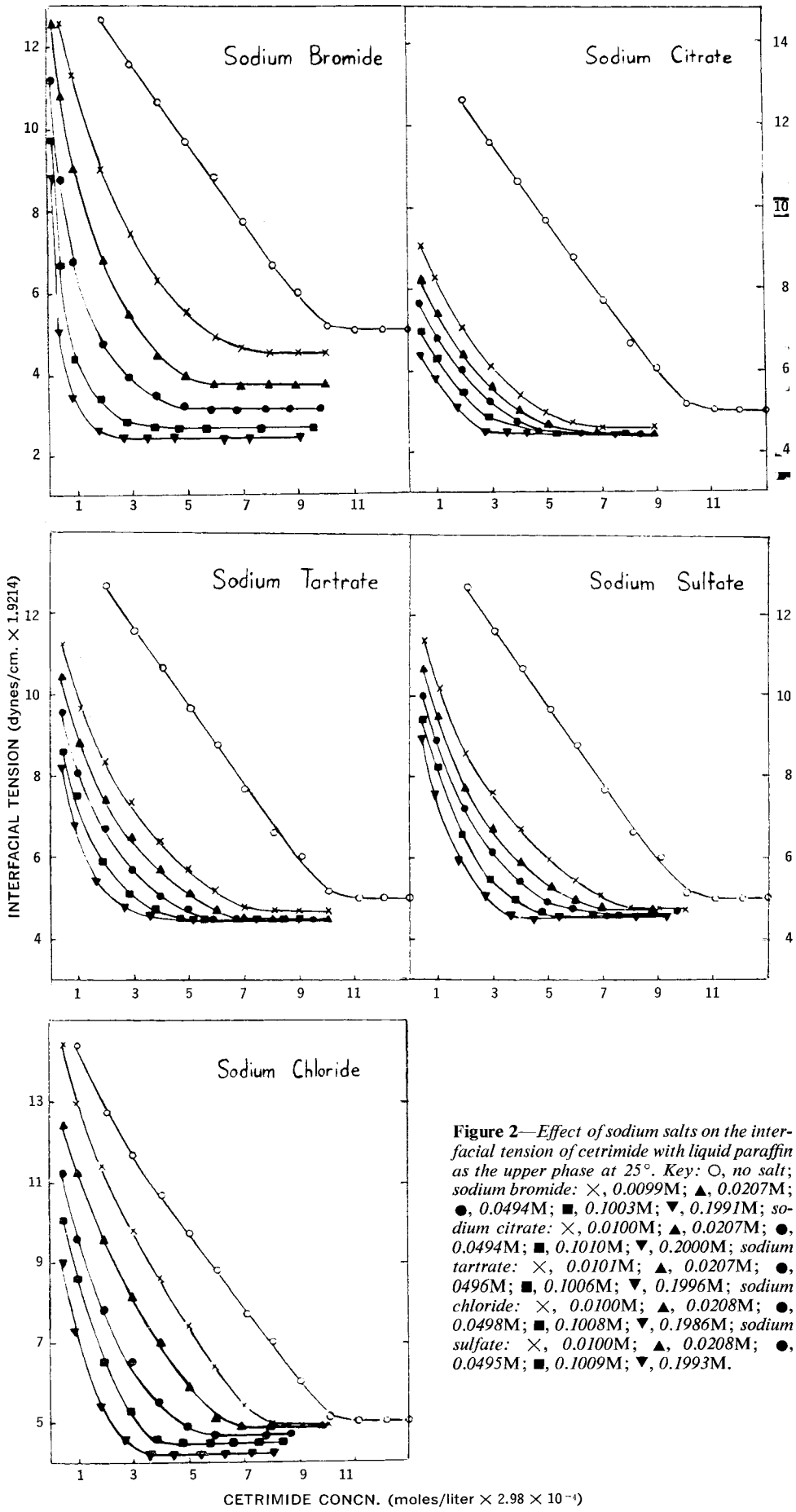

Figure 2—*Effect of sodium salts on the interfacial tension of cetrimide with liquid paraffin as the upper phase at 25°. Key:* ○, *no salt; sodium bromide:* ×, *0.0099*M; ▲, *0.0207*M; ●, *0.0494*M; ■, *0.1003*M; ▼, *0.1991*M; *sodium citrate:* ×, *0.0100*M; ▲, *0.0207*M; ●, *0.0494*M; ■, *0.1010*M; ▼, *0.2000*M; *sodium tartrate:* ×, *0.0101*M; ▲, *0.0207*M; ●, *0496*M; ■, *0.1006*M; ▼, *0.1996*M; *sodium chloride:* ×, *0.0100*M; ▲, *0.0208*M; ●, *0.0498*M; ■, *0.1008*M; ▼, *0.1986*M; *sodium sulfate:* ×, *0.0100*M; ▲, *0.0208*M; ●, *0.0495*M; ■, *0.1009*M; ▼, *0.1993*M.

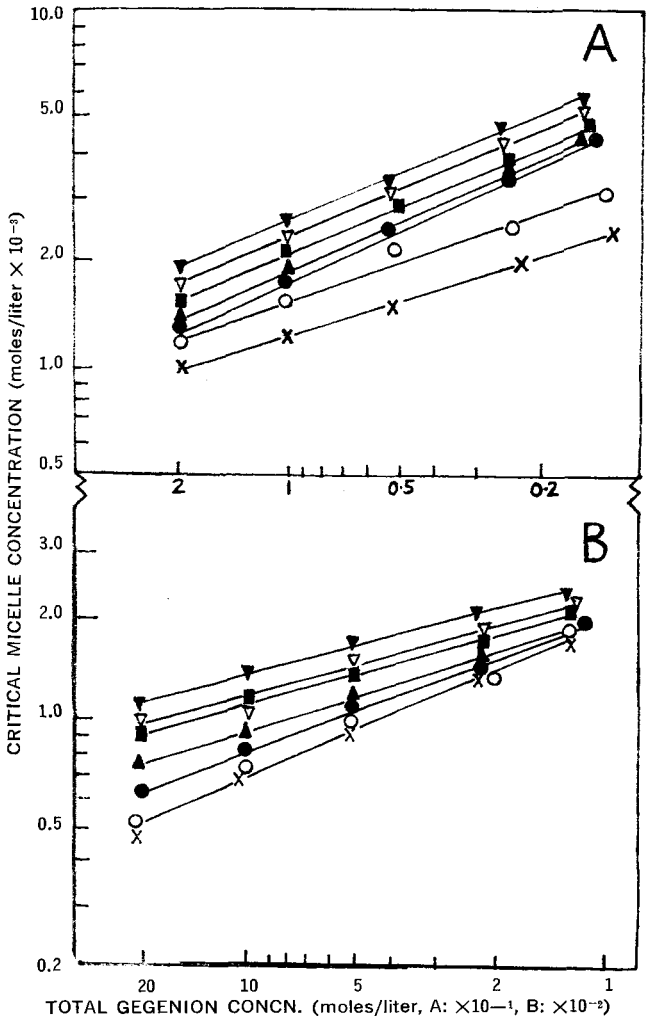

Figure 3—*Variation of the logarithm of CMC of sodium lauryl sulfate* (A) *and cetrimide* (B) *with liquid paraffin as the upper phase at 25° as a function of the logarithm of the gegenion concentration. Key:* A: ×, *sulfates of magnesium and zinc;* ○, *magnesium chloride;* ●, *ammonium bromide;* ▲, *ammonium chloride, sodium bromide;* ■, *ammonium sulfate, sodium chloride;* ▽, *sodium sulfate, lithium chloride;* ▼, *lithium sulfate, sodium phosphate;* B: ×, *potassium bromide;* ○, *ammonium bromide;* ●, *sodium bromide;* ▲, *sodium citrate;* ■, *sodium tartrate;* ▽, *chlorides of sodium, potassium, ammonium, lithium;* ▼, *sulfates of magnesium, sodium, ammonium, lithium.*

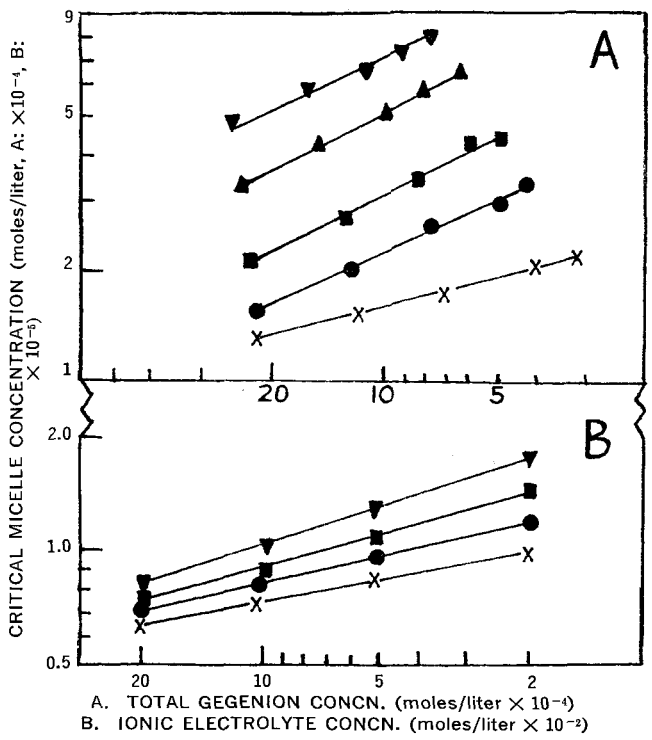

Figure 4—*Variation of the logarithm of CMC of cetylpyridinium chloride* (A) *and cetomacrogol 1000* (B) *with liquid paraffin as the upper phase at 25° as a function of the logarithm of the concentration of gegenion and ionic electrolytes, respectively. Key:* A: ▼, *sodium chloride, lithium chloride;* ▲, *sodium bromide;* ■, *sodium tartrate, magnesium sulfate;* ●, *sodium sulfate;* ×, *sodium citrate;* B: ×, *sodium phosphate;* ●, *sodium bromide;* ▼, *sodium sulfate;* □, *sodium chloride, magnesium chloride, ammonium chloride.*

salts. This is likely to be due to a preponderance of negatively charged ions in the electrical barrier when a salt is introduced, the surface migration of the cationic surfactant monomer may be facilitated. The greater activity of cetylpyridinium chloride as shown in the finding that the salt concentration 100 times less than that required to produce a similar effect with cetrimide, indicates the greater sensitivity of the delocalized charge in the polar pyridinium ring to the counterion than to the "point charge" of the alkyltrimethylammonium cation in the ionized cetrimide. Cetomacrogol 1000 showed a slight lowering of the interfacial tension in the presence of salt additives. This suggests that the relatively high surface activity of cetomacrogol 1000 can be due to the multiple polyoxyethylenic groups, which because of their high degree of hydration (24) and configuration entropies (25) may have rendered the salt effect insignificant.

The variations of the CMC of surfactants in the aqueous subphase with liquid paraffin as the upper phase with respect to added salts gave a log-log relationship (Figs. 3 and 4). Similar relationships had been established with air-surfactant solution systems (1, 3, 7). Substitution of the air phase with a liquid phase would probably be without much effect. From Figure 3 A, the slopes of the lines, except that of ammonium bromide, did not vary to any appreciable degree, demonstrating that the lowering of the CMC of sodium lauryl sulfate was approximately the same for a fixed concentration of gegenion. This was also true for cetylpyridinium chloride (Fig. 4A) with the exception of sodium citrate. The slopes of the lines for plots of log CMC of both cetrimide and of cetomacrogol 1000 against log gegenion concentration varied but the lines in such plot for cetrimide apparently had the tendency to converge at low concentration of gegenion while those for cetomacrogol 1000 were more inclined toward convergence at high gegenion concentration. The change in CMC of cetrimide with various salts (Fig. 3B) was more marked at high concentration of gegenion than at low concentration and the converse was true for cetomacrogol 1000 (Fig. 4B). However, with sodium lauryl sulfate and cetylpyridinium chloride the change in CMC was without much difference at high or at low concentration of gegenion. It appeared that the pattern of such log-log plots would be dependent on the surfactant itself.

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COMMUNICATIONS

Possible Role for Trichloroacetate in Pharmaceutical Formulations

Keyphrases ☐ Trichloroacetate role—pharmaceutical formulations ☐ Thiopental sleeping time—trichloroacetate effect ☐ Protein binding, thiopental—trichloroacetate effect

Sir:

Trichloroacetic acid (TCA) has a pKa of 0.70 at 25° (1) and our investigations into its absorption from the rat's stomach using isotonic TCA buffers of pH 2.0 and pH 3.0 (2, 3) suggest it to be mainly absorbed as the undissociated acid. These experiments were performed to determine whether or not dextromethorphan (DMX) and tetracycline were absorbed in association with TCA (that is as ion pairs) following our earlier observations that DMX (4) was absorbed as a protonated species from chloride buffers. The investigations suggested that the drugs were not absorbed as ion pairs, and in the case of DMX the enhanced absorption from the TCA buffers seemed to be due to the increased surface activity of the drug. In a recent publication (5) the enhanced biological activity of a quaternary ammonium compound (isopropamide iodide) administered orally with a large excess of TCA has been reported; however, it is possible that the increased biological activity reported is not due to increased absorption but due to a biological availability phenomenon such as protein binding. TCA, a common protein precipitant, is strongly bound to serum proteins, and if, as suggested above, TCA is absorbed as the undissociated species from the upper alimentary tract, then this TCA could prevent or minimize the binding of the drug to the plasma proteins or tissues, thus freeing the drug for absorption at the biophase.

To check this possibility, we determined the LD_{50} and the sleeping time of mice after the intravenous injection of thiopental in solutions made isotonic with sodium chloride or trichloroacetic acid with the pH adjusted to 7.4. Thiopental (Pentothal sodium, Abbott Laboratories, North Chicago, Ill.) was used so that no ion-pair phenomenon could account for the results. It is very strongly protein bound (6, 7), and the biological responses are comparatively easy to evaluate. The drug in 0.2-ml. doses was injected into the tail vein of a 20-g. mouse (male, albino, Rolfsmeyer, Madison, Wis.); the duration of anaesthesia was determined by the righting reflex, the mouse being required to right itself three times in a period of 30 sec.

Table I—Sleeping Times

Thiopental Dose Level, mg./kg.	Trichloroacetate	Chloride
20	3.9 min., 3.5–4.8	2.2 min., 1.8–2.5
25	6.2 min., 4.8–9.0	3.4 min., 2.0–4.3
30	11.5 min., 6.5–22.0	4.1 min., 2.8–6.5

Using six animals at each dose level, the LD_{50} of thiopental in the TCA solution was 41.0 mg./kg. and in the sodium chloride solution was 61.0 mg./kg. The sleeping times for several dose levels using six animals at each dose level are summarized in the table. Animals injected with the isotonic solutions alone showed no untoward effects either immediately or during a period of 2 weeks (see Table I). Using the Student t test of significance ($p = 0.01$) the duration of sleeping times at each dose level was significantly different for the two solutions. In preliminary dialysis experiments using 50 mcg./ml. thiopental and 1% human albumin crystalline 100% (Mann Research Labs., New York, N. Y.), it was found that 55.0% of the thiopental was bound using physiological phosphate buffer whereas only 21.6% thio-