Cholesterol solubility in mixed micellar solutions of ionic and non-ionic surfactants

Swapna Pal and S. P. Moulik

Department of Chemistry, Faculty of Science, Jadavpur University, Calcutta 700032, India

Abstract The solubility of cholesterol in mixed aqueous ionic and non-ionic micellar systems in the presence and absence of salts and ionic dyes has been studied. The mixed micellar system of cetyl trimethyl ammonium bromide and Triton X-100 solubilized more cholesterol than a system consisting of sodium dodecyl sulfate and Triton X-100. The influence of salts and dyes on these systems was moderate but different for each system. Sodium chloride, calcium chloride, and sodium citrate had mild effects on both the systems, whereas the effects of potassium hydrogen phthalate, sodium salicylate, and sodium acetyl salicylate were dramatic. The free energy, enthalpy, and entropy of solution were determined. On the basis of the energetics, the nature of the mixed systems is discussed. Solubility of cholesterol was found to bear a direct correlation with the total lipid content.—**Pal, S., and S. P. Moulik.** Cholesterol solubility in mixed micellar solutions of ionic and non-ionic surfactants. *J. Lipid Res.* 1983. **24:** 1281–1290.

Supplementary key words mixed surfactant system • thermodynamics

Solubility of cholesterol in bile is important from the point of view of its transport and specific absorption. In bile, cholesterol is solubilized in mixed micelles of bile salts and lecithin; an abnormality in bile composition is manifested by precipitation of cholesterol in the gall bladder, thus forming gallstones (1, 2). Recently, emphasis has been put on the medical management of in vivo cholesterol gallstone dissolution rather than surgical removal (3). The possibility of the use of surfactants as accelerators of gallstone dissolution has been reported (4, 5). There have also been studies on the solubility of cholesterol in surfactants in order to understand their physicochemical roles in relation to membrane lipids (6–8). However, these studies are limited, and there is no report of a study of cholesterol solubility in mixed micellar systems composed of non-ionic and ionic surfactants. Such studies with representative surfactants would be expected to offer a better understanding of the general chemical and biochemical behavior of cholesterol.

In this report we present results of studies of cholesterol solubility in mixed micelles composed of Triton X-100 (TX) and either sodium dodecyl sulfate (SDS) or cetyl trimethyl ammonium bromide (CTAB) under various conditions. Triton X-100 was selected because it is a non-ionic surfactant widely used in biochemical studies, particularly in cell lysis and membrane solubilization, phospholipid metabolism, and for various other purposes (9–11). Both cationic and anionic surfactants were used to examine the effects of charge on the solubilization process. The effects of several salts and dyes were also studied to understand the roles of minor components.

EXPERIMENTAL PROCEDURE

Materials

Cholesterol (Croda Chemicals, England) was recrystallized twice from hot distilled ethanol and dried over sulfuric acid in a desiccator. The melting point was 148°C; lit. value (12) 148.5°C.

For the makes, grades, and purities of the surfactants, TX-100, SDS, and CTAB, we refer to our earlier publication (13). Critical micelle concentrations were determined by measuring the surface tension of aqueous solutions at different concentrations by the drop volume method. Surface tension-concentration curves showed no minima (SDS showed only a very shallow minimum) suggesting a high degree of purity of the surfactants. Elemental analyses revealed SDS and CTAB to be 99.0% and 99.6% pure, respectively.

The salts NaCl, Na-citrate, K-H-phthalate, and $CaCl_2$ were of G.R. grade, Sarabhai Chemicals, India or E. Merck, Germany. Na-salicylate and Na-acetylsalicylate were prepared by recrystallizing the corresponding A.R. BDH grade acids and neutralizing weighed quantities of them with standardized NaOH solution.

Abbreviations: C, cholesterol; TX, Triton X-100 (*p*-tert-octylphenoxypolyoxyethylene ether); SDS, sodium dodecyl sulfate; CATB, cetyl trimethyl ammonium bromide; AO, acridine orange; DSB, disulphine blue; K-H-phthalate, potassium hydrogen phthalate; CMC, critical micelle concentration.

The dyes, acridine orange (AO) of E. Merck, Germany and disulphine blue (DSB) of ICI, London were used as received.

Doubly distilled water was used throughout the experiments.

Methods

Unless otherwise stated, all measurements were taken at 310°K in a temperature-controlled water bath of ±0.02° accuracy.

Cholesterol solubility was measured optically. Colloidal dispersion of cholesterol was possible at certain concentrations of the ionic surfactants (approximately ten times the CMC of the surfactants) by constant high speed agitation of cholesterol powder for at least 2–3 days. A slurry obtained in this manner was stable for hours at room temperature and did not sediment; if occasionally stirred, it could be kept as such for months. To measure the solubility, different amounts of the cholesterol slurry and a fixed volume of a standard TX-100 solution were added to glass-stoppered test tubes. Final volumes were adjusted by adding doubly distilled water resulting in a series of mixtures with a fixed TX-100 concentration and progressively increasing concentrations of cholesterol. Adjustments of ionic strength and the concentration of the additives (salts and dyes) were made whenever necessary through appropriate addition of these ingredients. The sample tubes were then immersed in the constant temperature bath with occasional shaking and left for sufficient time (24 hr) to attain equilibrium. The absorbances of the solutions were then measured in a Hilger pattern Biochem Absorptiometer of Associated Instruments, India, and in a Perkin-Elmer Hitachi Model 200 UV-visible spectrophotometer at 430 nm. Above a critical cholesterol concentration, the solutions were turbid. A plot of the absorbance versus the volume of the slurry added yielded two intersecting straight lines; the intersection was considered as the solubility point. The initial straight line ran parallel to the X-axis with a very low absorbance measured against a water blank; with surfactant mixtures as the respective blanks, these absorbances were virtually zero. For the same set of samples, both the absorptiometer and the spectrophotometer yielded the same intersecting points and the phenomenon was reproducible. The mean solubilities had a maximum deviation of 1.66%. The absorptiometer was used for most of the measurements. The attainment of maximum solubility in 1 day is presented from a typical result obtained using a slurry of 3 mg of cholesterol in 100 mM SDS, and a final concentration of 1.67 mM TX-100. Measurements were taken at intervals of 24 hr. At 24, 48, and 120 hr the solubility remained constant at 0.25 ± 0.002 mg/ml. Twenty-four hours was sufficient time to attain the solubility equilibrium and was used throughout the study. Cholesterol solubility measured by the described optical method was compared with the spectrophotometric method of assay (14). For both SDS plus TX-100 and CTAB plus TX-100 systems, the results agreed within ± 3%. The direct absorption monitoring method was fairly sensitive and was used for cholesterol solubility determinations.

Cholesterol solubility was also studied in presence of different additives. The upper limit of $CaCl_2$ concentration used in the TX-SDS system was kept well below the point of precipitation of the calcium salt of the surfactant. In the case of dyes, controls contained equal amounts of the dyes as in the mixtures and the measurements were taken at the wavelengths at which the dyes absorbed at a minimum (520 nm for AO and 470 nm for DSB). In the SDS-TX system, the total lipid concentrations were in the ranges 27.4–32.3 mM and 27.4–30.3 mM for the salts and the dyes, respectively. The respective ranges for the salts and the dyes were 6.2–9.7 mM and 5.9–6.4 mM in the CTAB–TX system.

The cholesterol solubilities in both systems were reversible. This was tested by successively raising and lowering the environmental temperature of individual samples. Measurements in the temperature range 298–318°K were useful in evaluating the energetics of the process.

RESULTS

Table 1 presents the cholesterol solubilities in individual surfactants at concentrations below and above their respective CMC's. The solubility in mixed surfactant systems has been described under two classifications: System 1 (cholesterol + SDS + TX + water) and System 2 (cholesterol + CTAB + TX + water). The results were obtained at different ionic and non-ionic surfactant compositions (**Fig. 1**). The non-ionic surfactant, TX, increased the solubility in both systems, but System 2 could solubilize more cholesterol than System 1. Representative results are presented in **Table 2** and **Table 3** in terms of mole percent compositions of the three lipids (SDS or CTAB, TX-100, and cholesterol) assuming invariant solvent (water) in large excess. The total lipid concentrations are also given. The compositions indicate the boundaries between the clear and turbid solutions in aqueous medium, i.e., the boundary between the colloidal and noncolloidal states of the solutions.

Effects of additives

The effects of the additives are shown in **Fig. 2** and **Fig. 3A** and **B.** It was observed that the equilibrium cholesterol solubility increased with ionic strength up to 0.1 M NaCl in System 1 (Fig. 2, curve 5), whereas it

TABLE 1. Cholesterol solubility in pure surfactant solutions at 310°K

Surfactant[a]	Solubility	
	μg/ml	μmol/l
CTAB (100 μM) (<CMC)	3.12	8.08
CTAB (10 mM) (>CMC)	4.00	10.36
SDS (1 mM) (<CMC)	3.50	9.07
SDS (10 mM) (>CMC)	4.08	10.57
TX-100 (24 μM) (<CMC)	5.20	13.47
TX-100 (20 mM) (>CMC)	16.00	41.45

[a] $(CMC)_{CTAB}$ = 0.76 mM; $(CMC)_{SDS}$ = 8 mM; $(CMC)_{TX\text{-}100}$ = 0.30 mM.

decreased with concentration up to 0.05 M NaCl in System 2 (Fig. 3A, curve 3). Beyond these limits, the solubilities remained invariant. Thus, NaCl had opposite effects on the solubilities in the two systems. The addition of $CaCl_2$ (at concentrations much lower than the critical concentration at which SDS precipitates as the calcium salt) decreased the solubility of cholesterol in

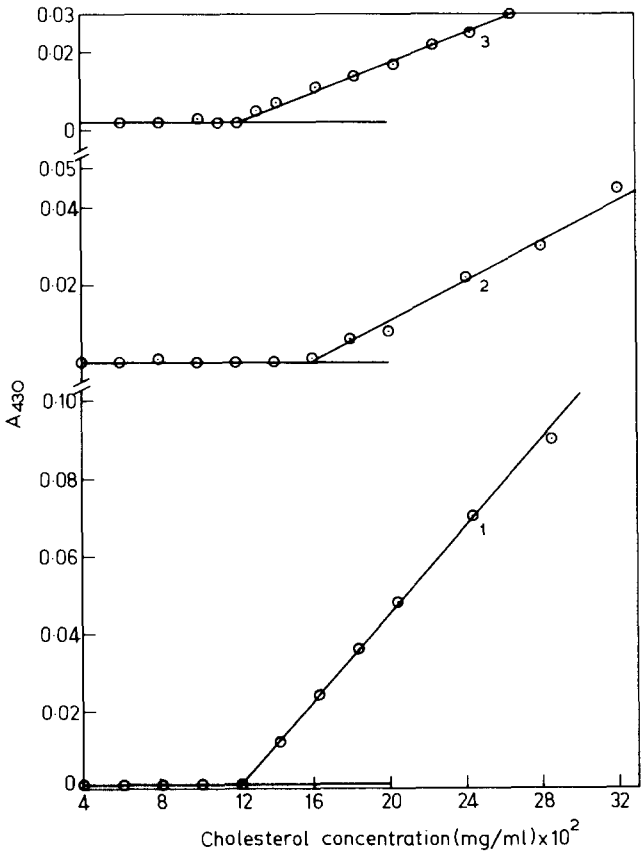

Fig. 1. Plots of absorbance versus cholesterol concentration at 37°C for Systems 1 and 2. Curve 1, slurry: 2.036 mg cholesterol per ml of 10 mM CTAB. Concentration of TX, 4 mM. Curve 2, slurry: 2.0 mg cholesterol per ml of 100 mM SDS. Concentration of TX, 20 mM. Curve 3, slurry: 1.02 mg cholesterol per ml of 80 mM SDS. Concentration of TX, 30 mM.

TABLE 2. Compositions of the soluble mixed lipid system (TX + SDS + C) with and without NaCl at 310°K

Total Lipid × 10^2 (M)	% TX	% SDS	% C
NaCl = 0			
0.58	86.01	13.76	0.23
1.05	79.11	20.25	0.67
2.26	73.81	25.39	0.84
2.74	72.89	26.24	0.87
2.84	70.38	28.15	1.46
3.99	75.15	24.05	0.79
5.68	73.33	25.81	0.85
11.20	71.44	27.15	1.41
23.33	68.57	29.48	1.94
24.91	66.91	31.05	2.04
NaCl = 0.1 M			
0.66	75.46	24.15	0.40
1.28	65.20	33.90	0.89
2.42	68.89	30.31	0.80
2.56	65.06	33.83	1.11
2.98	66.85	32.09	1.06
3.05	65.53	32.77	1.70

System 1 (Fig. 2, curve 1) but had no influence on System 2 (Fig. 3A, curve 6). Calcium was specific towards System 1. At a much higher concentration, it only lowered the cholesterol solubility in System 2 to some extent. Thus, NaCl and $CaCl_2$ have dissimilar effects on the solubility in the two systems. In System 2, Na-citrate (up to a concentration of 2 mM) decreased the solubility, which did not change at higher concentrations (Fig. 3A, curve 1). The other organic salts, phthalate, salicylate, and acetyl salicylate had striking effects on the cholesterol solubility in System 2 (Fig. 3B). Solubility increased

TABLE 3. Compositions of the soluble mixed lipid system (TX + CTAB + C) with and without NaCl at 310°K

Total Lipid × 10^2 (M)	% TX	% CTAB	% C
NaCl = 0			
0.24	70.76	28.31	0.93
0.25	68.14	31.34	0.52
0.36	82.23	12.88	4.89
0.43	77.94	19.49	2.57
0.44	75.77	22.73	1.50
0.49	81.36	12.20	6.44
0.62	80.47	15.45	4.07
1.96	81.59	13.26	5.15
3.07	81.47	14.66	3.87
NaCl = 0.1 M			
0.22	76.63	22.99	0.38
0.22	76.34	22.90	0.76
0.36	84.46	11.26	4.28
0.41	81.54	16.31	2.15
0.42	78.96	19.74	1.30
0.47	85.34	9.60	5.06
0.57	87.55	7.00	5.44
0.59	84.97	11.89	3.14
1.88	85.21	10.65	4.14

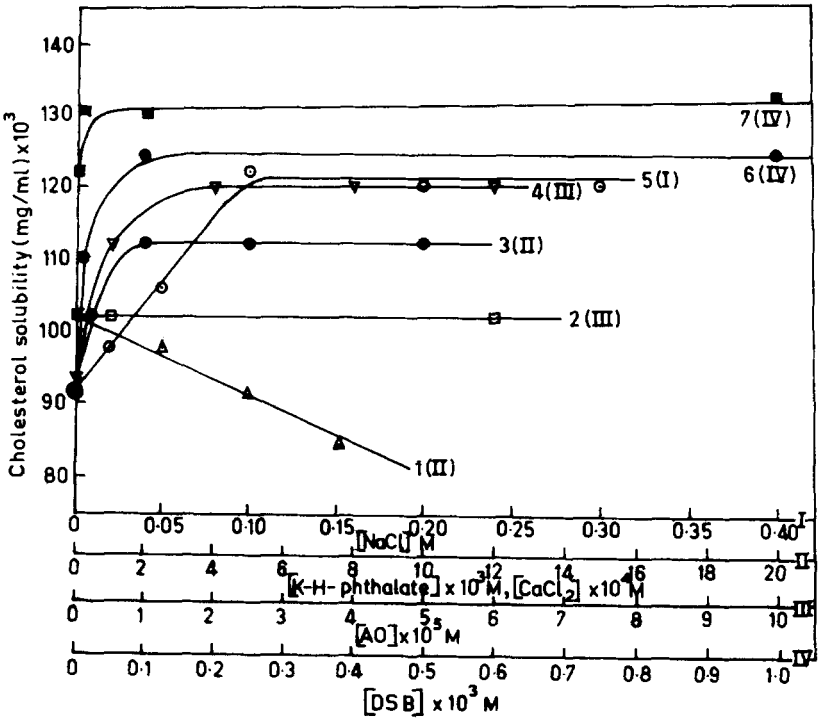

Fig. 2. Additive effects on cholesterol solubility in SDS + TX system at 37°C (System 1). Curve 1, $CaCl_2$; curve 2, AO at 0.1 M NaCl; curve 3, K-H-phthalate; curve 4, AO (no NaCl); curve 5, NaCl; curve 6, DSB (no NaCl); curve 7, DSB at 0.1 M NaCl.

appreciably and progressively with the addition of these salts. The effects were much less in System 1 (Fig. 3B). The phenyl ring present in these salts played a special role.

Low concentrations of dyes were used in the study. They were found to affect the cholesterol solubility in the mixed micellar systems. Systematic effects were not observed. AO increased the solubility in System 1, whereas in the presence of 0.1 M NaCl it was ineffective (Fig. 2, curves 2 and 4). In this system, DSB increased the solubility which increased further in the presence of 0.1 M NaCl (Fig. 2, curves 6 and 7). The effect of DSB was greater than that of AO. In System 2, AO increased the solubility with no NaCl: the solubility decreased in the presence of 0.1 M NaCl (Fig. 3A, curves 2 and 7). Under similar situations, the effects of DSB were just the reverse (Fig. 3A, curves 4 and 5).

Effect of temperature

The solubility showed a small temperature dependency. The extent depended on the total lipid concentration. Considering solubilization as the transfer of cholesterol from the solid state to the micellar environment (15), the standard free energy of solubilization ($\Delta G^{\circ}_{S\rightarrow M}$) was calculated from the relation

$$\Delta G^{\circ}_{S\rightarrow M} = -RT \ln X_c \qquad \text{Eq. 1)}$$

where R and T have their usual significance and X_c is the mole fraction of cholesterol ($X_c = n_c / \sum_i n_i$, where n_i refers to the number of moles of the *i-th* component and n_c is the number of moles of cholesterol in the system). $\Delta G^{\circ}_{S\rightarrow M}$ thus refers to the unitary free energy of the micellar solubilization process. Knowing the cholesterol solubility in water (7), the standard free energy of transfer of cholesterol from water to the micellar environment was computed; such transfers were thermodynamically favorable. The enthalpy of the process was estimated from the relation

$$\ln \{(X_c)_1/(X_c)_2\} = -\frac{\Delta H^{\circ}_{S\rightarrow M}}{R}\left[\frac{1}{T_1} - \frac{1}{T_2}\right] \qquad \text{Eq. 2)}$$

assuming $\Delta H^{\circ}_{S\rightarrow M}$ to be temperature independent in the range 303°–318°K. From the free energy and enthalpy of transfers, the entropy of transfer, $\Delta S^{\circ}_{S\rightarrow M}$ was calculated from the Gibb's equation. These thermodynamic parameters are shown in **Table 4** and **Table 5**. It is seen from the tables that the solubilization process is exothermic in System 1 with a large negative entropy, whereas it is endothermic in System 2 with a small negative entropy: the process in System 2 is thus more favorable.

Effect of total lipid

At constant temperature, the equilibrium cholesterol solubility is directly proportional to the total lipid con-

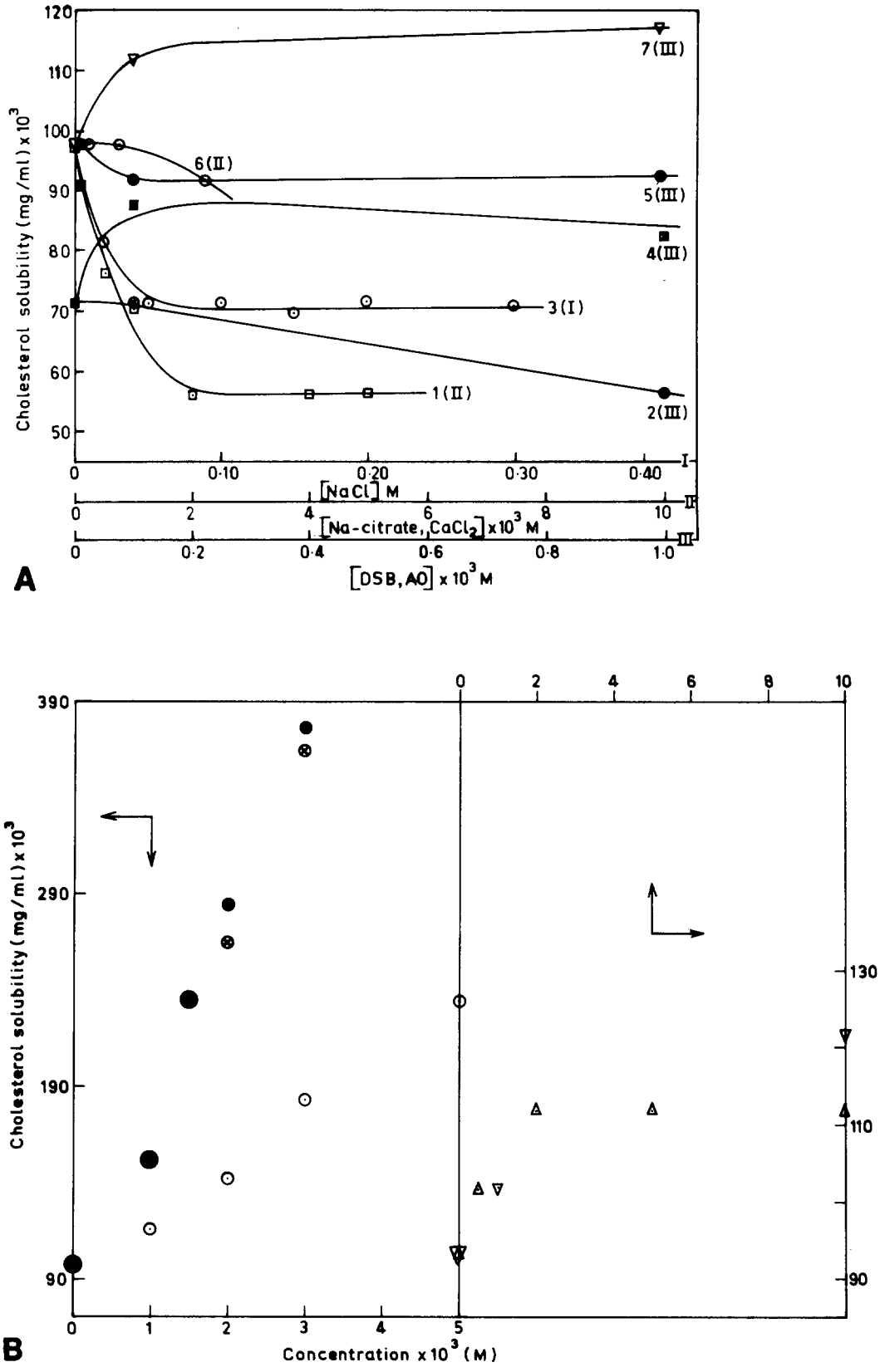

Fig. 3. A, Additive effects on cholesterol solubility in CTAB + TX system at 37°C. Curve 1, Na-citrate; curve 2, AO at 0.1 M NaCl; curve 3, NaCl; curve 4, DSB at 0.1 M NaCl; curve 5, DSB (no NaCl); curve 6, $CaCl_2$: curve 7, AO (no NaCl). B, Additive effects on cholesterol solubility in systems 1 and 2. System 1: △, K-H-phthalate; ▽, Na-salicylate. System 2: ⊙, K-H-phthalate; ●, Na-salicylate; ⊗, Na-acetyl salicylate.

TABLE 4. Energetics[a,b,c] of cholesterol solubility in SDS + TX + water system at 310°K

Total Lipid × 10^2 (M)	SDS / TX	$\Delta G^\circ_{S\rightarrow M}$	$\Delta H^\circ_{S\rightarrow M}$	$\Delta S^\circ_{S\rightarrow M}$	$\Delta G^\circ_{W\rightarrow M}$
0.58	0.16	39.32	−77.41	−376.55	−2.50
0.66	0.32	(37.52)	(−45.28)	(−267.12)	(−4.29)
2.26	0.344	32.43	−33.44	−212.46	−9.39
2.56	0.52	(31.32)	(−6.99)	(−123.58)	(−10.49)
2.74	0.36	31.83	−16.22	−155.01	−9.99
2.84	0.40	30.39	−35.65	−213.02	−11.43
2.98	0.48	(31.09)	(−23.46)	(−175.97)	(−10.72)
11.20	0.38	26.83	−5.76	−105.11	−14.99
11.75	0.45	(26.39)	(−16.32)	(−137.76)	(−15.43)

[a] Due to lack of cholesterol solubility data at 37°C, the value reported (7) at 298°K was used to calculate $\Delta G^\circ_{W\rightarrow M}$.
[b] ΔG° and ΔH° are in kJ mol^{-1} and ΔS° in $J \cdot K^{-1} mol^{-1}$.
[c] Values in parentheses are with reference to 0.1 M NaCl in the medium.

centration. The results are depicted in **Fig. 4**. It is seen that the initial slope is higher suggesting greater effectiveness at lower concentrations. The importance of the total lipid concentration on the cholesterol solubility in synthetic and natural bile samples has been stressed by Carey (16).

DISCUSSION

In aqueous medium cholesterol monomers exist up to a concentration limit of 10^{-8} M. Stacked aggregates occur up to 10^{-6} M and thereafter the aggregates coalesce into a separate phase (17). The solubilities of anhydrous cholesterol in this study in surfactant solutions below their CMC's were in the range of 8–14 μM (Table 1). We presumed that aggregates of cholesterol were stabilized by surfactant monomers in solution. The results presented in Tables 2 and 3 represent cholesterol solubilities in mixed micellar environments. The surfactant media contained TX-100 at concentrations nearly tenfold the respective CMC's of CTAB and SDS. The ionic surfactants were nearly at their CMC values. Under these conditions, a major part of the ionic and non-ionic surfactants remained in the mixed state and the concentrations of the mixed micelles were also well above their respective CMC's. Although the mixed micellar CMC's for ideal and non-ideal binary surfactant mixtures may be theoretically evaluated (18, 19), this was not the goal of the present investigation. The mixed micellar CMC's at 1:1 mole ratio (0.5 mole fraction) for SDS-TX-100 and CTAB-TX-100 systems determined in our laboratory were 2.6 mM and 0.4 mM, respectively. The present working media contained SDS and CTAB in the mole fraction range of 0.1–0.2. The mixed micellar CMC values must be then lower than those obtained at 0.5 mole fraction (19). The solution media therefore contained predominantly mixed micelles of more or less constant proportion and of appreciable concentration.

The enhanced cholesterol solubility with the addition of TX-100 in the ionic micellar solutions is in line with the general solubility enhancement observed in mixed surfactant systems. System 2 solubilized more cholesterol than System 1. An ion-dipolar interaction between the cationic group on CTAB and the −OH group on cholesterol is possible: existence of interaction of cholesterol with cationic surfactant has been suggested (8). This is supported by the actions of NaCl. The mixed micelles of colloidal dimension in System 1 and System 2 had opposite effective surface charges. Addition of NaCl reduced the influence of these charges through the collapse of their electrical double layers, and the repulsion effect of anionic SDS on the cholesterol molecule was thus minimized by Na^+ ions, whereas the attractive influence of the cationic CTAB on cholesterol was also minimized by the influence of Cl^- ions. This caused a solubility increase in System 1 and decrease in System 2. Such a salt effect was not observed in the case of $CaCl_2$. In System 2, this salt had no influence, whereas it decreased the solubility of cholesterol in System 1. The concentration of $CaCl_2$ used was much lower. In all probability, greater affinity of SDS for Ca^{2+} (they may form an ion pair) is a specific effect that altered the micellar characteristic in System 1 causing lower solubility. The Cl^- ion concentration was too low to cause any decrease in the solubility (as with NaCl) through a surface charge decrease phenomenon in System 2. At higher concentrations, $CaCl_2$ was effective. The general salt effect to increase the cholesterol solubility through the increased micellar concentrations and aggregation could not be the explanation as both increased and decreased solubility resulted from salt effects.

Dramatic effects of the phenyl ring containing phthal-

TABLE 5. Energetics[a,b,c] of cholesterol solubility in CTAB + TX + water system at 310°K

Total Lipid × 10^2 (M)	CTAB / TX	$\Delta G^\circ_{S\rightarrow M}$	$\Delta H^\circ_{S\rightarrow M}$	$\Delta S^\circ_{S\rightarrow M}$	$\Delta G^\circ_{W\rightarrow M}$
0.24	0.460	39.43	27.23	−39.35	−2.39
0.36	0.133	(33.01)	(22.86)	(−32.72)	(−8.81)
0.36	0.157	32.60	19.75	−41.45	−9.22
0.41	0.200	(34.41)	(22.91)	(−37.11)	(−7.40)
0.42	0.250	(35.63)	(34.54)	(−3.50)	(−6.18)
0.43	0.250	33.85	18.71	−48.84	−7.97
0.44	0.300	35.17	29.47	−18.38	−6.66
0.47	0.113	(31.85)	(20.57)	(−36.38)	(−9.96)
0.49	0.150	31.12	15.81	−49.40	−10.70
0.57	0.080	(31.16)	(22.89)	(−26.68)	(−10.65)
0.59	0.140	(32.50)	(25.79)	(−21.65)	(−9.31)
0.62	0.130	29.91	14.68	−49.16	−11.91
0.62	0.192	31.70	13.94	−57.26	−10.13

[a,b,c] See legend to Table 4.

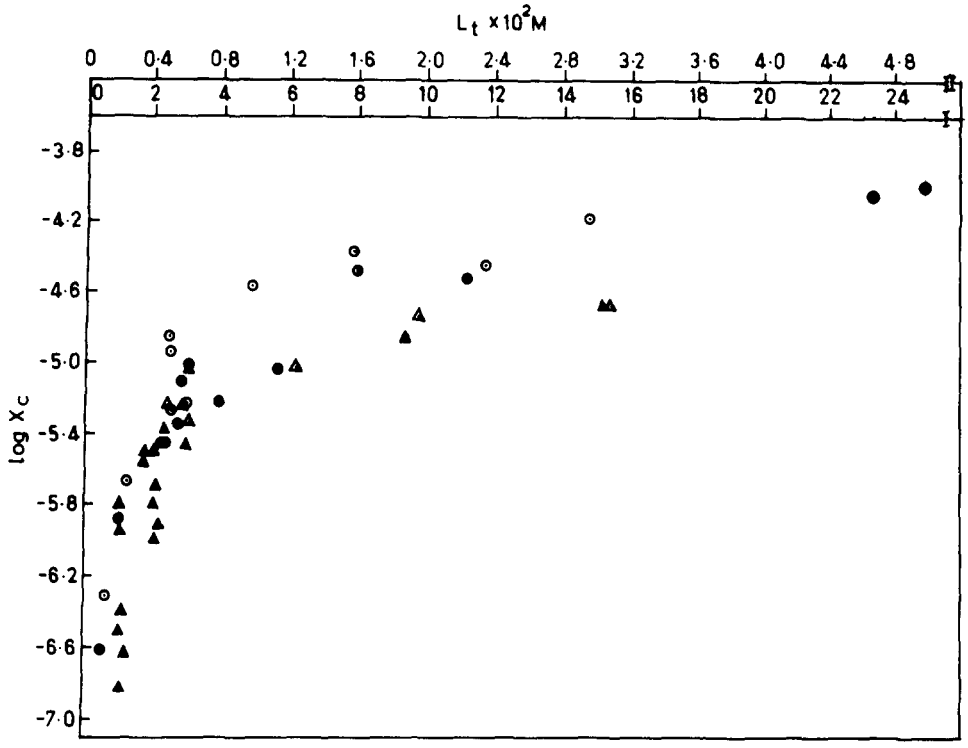

Fig. 4. Plot of log X_c versus the total lipid concentration, L_t at 37°C. System 1 (Scale I): ●, no NaCl; ⊙, 0.1 M NaCl. System 2 (Scale II): △, no NaCl; ▲, 0.1 M NaCl.

ate, salicylate, and acetyl salicylate salts were observed in System 2: cholesterol solubility increased almost linearly with the salt concentration. Although their individual effects were comparable, salicylate and acetyl salicylate, on a relative basis, solubilized more cholesterol than phthalate. We anticipated expanded mixed micelle formation which made room for more cholesterol. The distribution of the salts between the aqueous and the micellar phases determined the final mixed micellar composition, and in such a comparison salicylates sur-

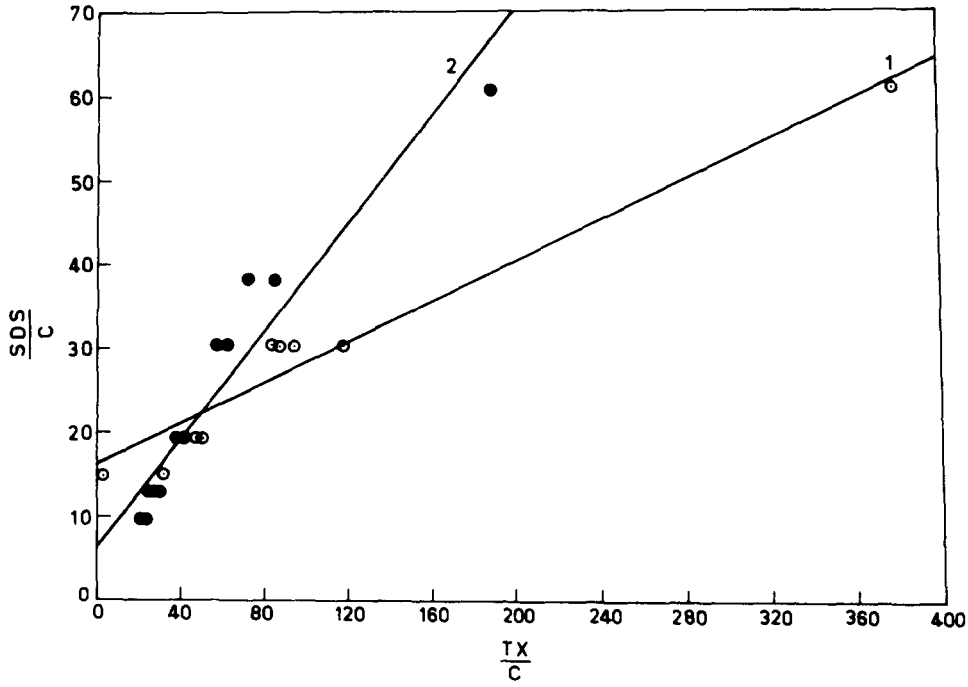

Fig. 5. Plot of SDS/C versus TX/C at 37°C. Curve 1, no NaCl; curve 2, 0.1 M NaCl (cf. equation 3).

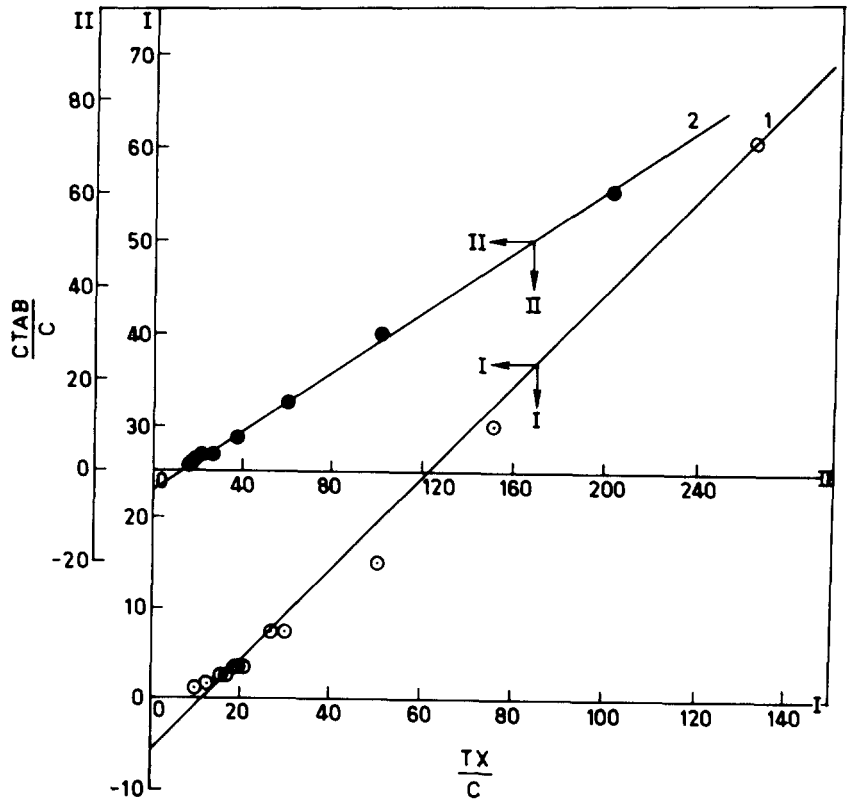

Fig. 6. Plot of CTAB/C versus TX/C at 37°C. Curve 1, no NaCl; curve 2, 0.1 M NaCl (cf. equation 4).

passed the phthalate, as less lipid solubility was observed in presence of the latter. The significant role of the phenyl ring was envisaged by the equal effects of salicylate and the acetyl salicylate, although the latter contained an extra hydrophobic (micellophilic) group. The minor effect of the aliphatic salt, Na-citrate (actually a decreasing effect was observed in line with the action of NaCl) further supported the special role of the phenyl ring. The charge repulsion between SDS and salicylate anions countered the phenyl ring-induced modified mixed micellization resulting in less cholesterol solubility in System 1. But the effects were comparatively greater than NaCl, thus the aromatic salts are better cholesterol solubilizers also in this system.

The effects of the dyes (acridine orange (cationic) and disulphine blue (anionic)) on the cholesterol solubility in Systems 1 and 2 were dependent on the presence and absence of NaCl. The effects of the dyes at low concentrations (0.01–10 mM) are in favor of positive actions of minor components towards cholesterol solubility; this may have relevance to its solubility in bile (a mixed micellar system) where the dye bilirubin is normally present as a minor component.

Carey (16) has recently stressed the importance of the total lipid concentration on the cholesterol solubility in artificial and normal bile systems. In Fig. 4, we have shown that cholesterol solubility varies curvilinearly with the total lipid concentration for both the systems. When moles of SDS per mole of solubilized cholesterol was plotted against moles of TX per mole of solubilized cholesterol, linearity was obtained with good correlation. The results are shown in **Fig. 5** and **Fig. 6.** For a

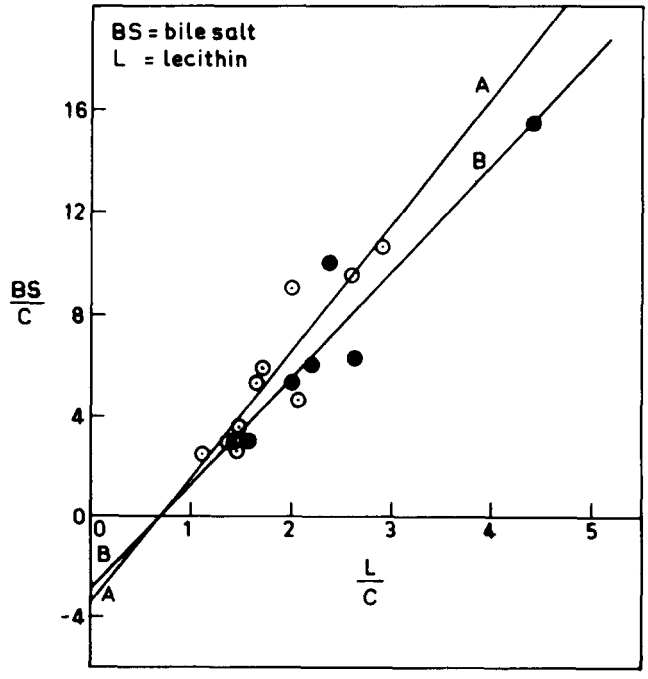

Fig. 7. Plot of $\frac{BS}{C}$ versus $\frac{L}{C}$ for hepatic biles. Curves: A, patients with gallstones; B, control subjects without gallstones. Correlation coefficients: A, 0.9172; B, 0.9313.

mere comparison with another mixed micellar system, results on hepatic bile (with and without gallstones (16)) are also included in **Fig. 7**. The observed linear relations are the following.

System 1

No NaCl:

$$\frac{SDS}{C} = 0.1231\left(\frac{TX}{C}\right) + 15.98; \quad r = 0.9697. \quad 3a)$$

0.1 M NaCl:

$$\frac{SDS}{C} = 0.3224\left(\frac{TX}{C}\right) + 5.787; \quad r = 0.9572. \quad 3b)$$

System 2

No NaCl:

$$\frac{CATB}{C} = 0.4886\left(\frac{TX}{C}\right) - 5.753; \quad r = 0.9957. \quad 4a)$$

0.1 M NaCl:

$$\frac{CATB}{C} = 0.3240\left(\frac{TX}{C}\right) - 4.258; \quad r = 0.9976. \quad 4b)$$

The two systems yielded opposite intercepts.

The free energy of transfer of cholesterol from water into the mixed micellar solutions was negative. At equal solubilities, the total lipid concentrations were greater in System 1 than in System 2. The $\Delta G^{\circ}_{S \rightarrow M}$ values are presented in Tables 4 and 5 with the total lipid concentrations. They were mainly calculated from the solubility data presented in Tables 2 and 3. Although the free energies of transfer, $\Delta G^{\circ}_{S \rightarrow M}$ for both the systems were close, $\Delta H^{\circ}_{S \rightarrow M}$ and $\Delta S^{\circ}_{S \rightarrow M}$ significantly varied, one compensating the other. In System 1, the enthalpies were negative (exothermic process), while those in System 2 were positive (endothermic process). The entropy values were much less negative in the second system than in the first. System 1 became ordered through liberation of heat and production of large negative entropy; System 2 absorbed heat and became less ordered producing low negative entropy. The counter cation Na^+ in System 1 is known to be a water structure promoter, while the counter anion Br^- in System 2 is a structure breaker (20). To bring cholesterol from an aqueous milieu to the mixed micellar core, its environmental structure has to be disrupted. This disrupted structure was more prevalent in System 1 than in System 2, and the solubility in the latter was therefore higher.

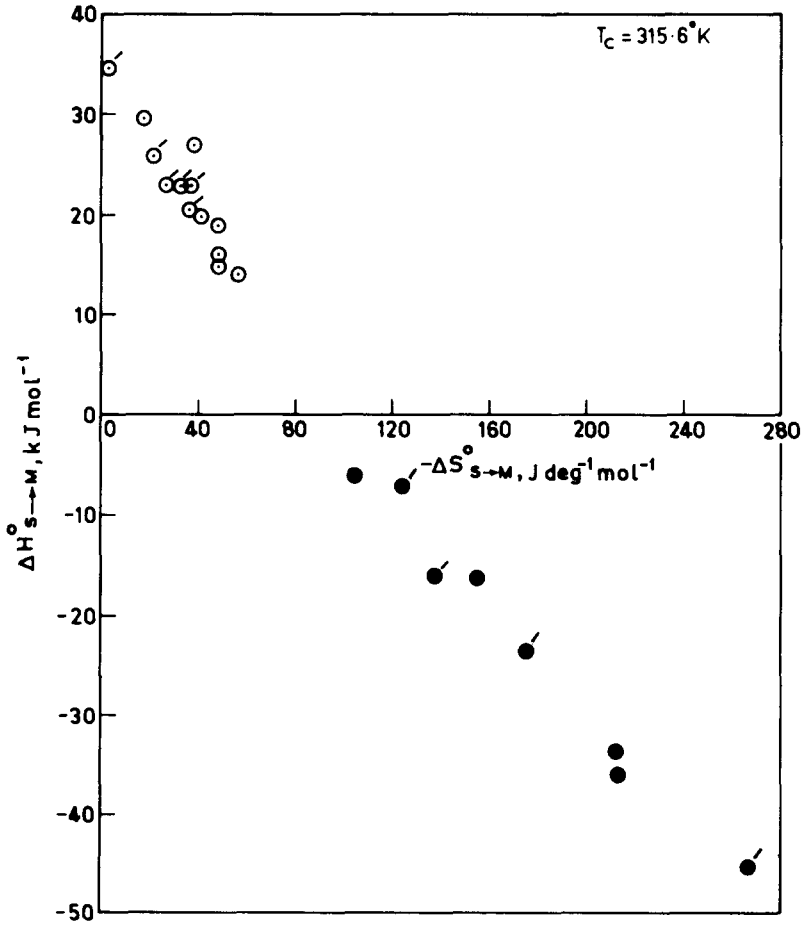

Fig. 8. Enthalpy–entropy compensation plot. System 1: ●, no NaCl; ◐, 0.1 M NaCl. System 2: ⊙, no. NaCl; ⊘, 0.1 M NaCl. Correlation coefficient, 0.9924; compensation temperature (T_c), 315.6°K.

Higher entropy of solubilization also supported more spontaneity of the process in the second system. The $\Delta H^{\circ}_{S \rightarrow M}$ and $\Delta S^{\circ}_{S \rightarrow M}$ were found to more or less exactly compensate each other. A linear regression line of such a compensation is presented in **Fig. 8.** The compensation temperature (T_c) was 315.6°K (the experimental temperature was 310°K). Like many kinetic and equilibrium systems (21, 22), such compensation has been observed also in micellar media (23, 24). Identical compensation behavior of both the mixed micellar systems 1 and 2 spoke in favor of overall order–disorder controlled cholesterol dissolution. The explanation given above is a simplified version of a complex situation. Other factors, such as local dielectric, specific interaction, micellar volume, etc., may have their roles in the process of solubilization. CTAB having longer chain length than SDS is expected to have a larger nonpolar micellar core and on a relative basis may also solubilize more cholesterol than the SDS micelles. The present knowledge is not sufficient to understand the individual roles of all these factors. Further discussion and comment must be kept pending until further work is done.

One of us (S.P.) is thankful to the University Grants Commission for a junior fellowship.

Manuscript received 12 March 1981, in revised form 2 November 1982, and in re-revised form 14 June 1983.

REFERENCES

1. Admirand, W. H., and D. M. Small. 1968. The physicochemical basis of cholesterol gallstone formation in man. *J. Clin. Invest.* **47:** 1043–1052.
2. Carey, M. C., and D. M. Small. 1978. The physical chemistry of cholesterol solubility in bile: relationship to gallstone formation and dissolution in man. *J. Clin. Invest.* **61:** 998–1026.
3. Hofmann, A. F. 1977. Desaturation of bile and cholesterol gallstone dissolution with chenodeoxycholic acid. *Am. J. Clin. Nutr.* **30:** 993–1000.
4. Vicedo Saez, J. L., and G. E. Vilanova. 1978. Effects of sodium oleate in bile cholesterol solubilization. *An. Quim.* **74:** 1314–1317.
5. Kwan, K. H., W. I. Higuchi, A. M. Molokhia, and A. F. Hofmann. 1977. Cholesterol gallstone dissolution rate accelerators. 1. Exploratory investigations. *J. Pharm. Sci.* **66:** 1105–1108.
6. Ekwall, P., and L. Mandell. 1961. Occurrence of cholesterol in water containing liquid crystalline form IV. Solubility of cholesterol in sodium caprylate solutions at 20°. *Acta Chem. Scand.* **15:** 1404–1406.
7. Gilbert, D. B., and J. A. Reynolds. 1976. Thermodynamic equilibria of cholesterol–detergent–water. *Biochemistry.* **15:** 71–74.
8. Han, S. K., and N. H. Kim. 1977. Micellar solubilization of cholesterol, cholesteryl myristate and gallstones by synthetic surfactants. *Yakhak Hoe Chi.* **21:** 135–140.
9. Helenius, A., and K. Simons. 1975. Solubilization of membranes by detergent. *Biochim. Biophys. Acta.* **415:** 29–79.
10. Robson, R. J., and E. A. Dennis. 1978. Characterization of mixed micelles of phospholipids of various classes and a synthetic, homogeneous analogue of the nonionic detergent Triton X-100 containing nine oxyethylene groups. *Biochim. Biophys. Acta.* **508:** 513–524.
11. Inoue, K., and T. Kitagawa. 1976. Effect of lipid composition on sensitivity of lipid membranes to Triton X-100. *Biochim. Biophys. Acta.* **426:** 1–16.
12. Hand Book of Chemistry and Physics. 1975. R. C. Weast, editor. CRC Press Inc., Cleveland, OH. C-240.
13. Mandal, A. B., A. M. Biswas, and S. P. Moulik. 1980. Physicochemical studies on the characterization of Triton X-100 micelles in an aqueous environment and in the presence of additives. *J. Phys. Chem.* **84:** 856–859.
14. Sperry, W. M., and M. Webb. 1950. A revision of the Schoenheimer-Sperry method for cholesterol determination. *J. Biol. Chem.* **187:** 97–106.
15. Molyneux, P., and C. T. Rhodes. 1972. Calculation of the thermodynamic parameters controlling micellization, micellar binding, and solubilization. *Kolloid Z. Z. Polym.* **250:** 886–890.
16. Carey, M. C. 1978. Critical tables for calculating the cholesterol saturation of native bile. *J. Lipid Res.* **19:** 945–955.
17. Tanford, C. 1973. The Hydrophobic Effect: Formation of Micelles and Biological Membranes. John Wiley & Sons, New York. 106–107.
18. Clint, J. H. 1975. Micellization of mixed nonionic surface active agents. *J. Chem. Soc. Faraday Trans. 1.* **71:** 1327–1334.
19. Rubingh, D. N. 1979. Mixed micelle solutions. *In* Solution Chemistry of Surfactants. Vol. 1. K. L. Mittal, editor. Plenum Press, New York. 337–354.
20. Gurney, R. W. 1953. Ionic Processes in Solution. McGraw-Hill, New York. 159–185.
21. Lumry, R., and S. Rajender. 1970. Enthalpy–entropy compensation phenomena in water solutions of proteins and small molecules: a ubiquitous property of water. *Biopolymers.* **9:** 1125–1227.
22. Pimental, G. C., and A. L. McClellan. 1971. Hydrogen bonding. *Annu. Rev. Phys. Chem.* **22:** 347–85.
23. Moulik, S. P., S. Ray, and A. R. Das. 1977. Interaction of *p*-nitrosalicylic acid with ethylenediamine in the presence of cetyl trimethyl ammonium bromide, sodium dodecyl sulfate, Triton X-100, polyethylene glycol, and their binary mixtures. A proton donor–acceptor equilibrium in micellar solution. *J. Phys. Chem.* **81:** 1766–1769.
24. Moulik, S. P., S. Ray, and A. R. Das. 1979. Proton transfer complexing equilibrium in micellar solution. *Colloid Polym. Sci.* **257:** 182–191.