

# Wettability of Cholesterol by Bile Salt Solutions

B. Janczuk,<sup>†</sup> M. L. Kerkeb,<sup>‡</sup> F. González-Caballero,<sup>\*‡</sup> and E. Chibowski<sup>†</sup>

Department of Physical Chemistry, Faculty of Chemistry, Maria Curie-Skłodowska University, 20-031 Lublin, Poland, and Department of Applied Physics, Faculty of Sciences, University of Granada, 18071 Granada, Spain

Received April 20, 1992. In Final Form: January 24, 1994\*

Interfacial interactions between bile salt solutions, sodium cholate (NaC), sodium deoxycholate (NaDC), and sodium chenodeoxycholate (NaCDC) (in the concentration range  $5 \times 10^{-5}$  to  $5 \times 10^{-2}$  M), and the cholesterol surface (both bare and precontacted with bile salt solutions) are evaluated on the basis of the knowledge of the surface free energy components of both cholesterol and bile salt solutions. By use of these components, the contact angles are estimated and compared to experimental ones. It was found that large changes in the wettability of the cholesterol surface took place when the surface was in contact with solutions in which micelles were present. Calculations suggest that micelles adsorbed on the cholesterol surface are hydrated.

## Introduction

The importance of cholesterol and bile salts for the biochemical processes in the living organisms is well-known. In particular, gallbladder stone formation<sup>1-5</sup> is known to be closely related to aggregative phenomena that may exist in mixtures containing both cholesterol and bile salts under defined conditions. Therefore, knowledge about cholesterol/bile salts interactions may be very useful for better understanding these phenomena. One of the possibilities to get information on these interactions is to study the surface free energy of cholesterol and bile salts solutions, as well as the wettability phenomena involved when they are in contact. Since it is not possible to measure directly the surface free energy of a solid, its determination is carried out, at present, by many indirect methods. Among others, those based on contact angle measurements for appropriately chosen systems are the most popular.<sup>6-11</sup> One of the main problems, however, of using contact angle measurements for evaluation of the solid surface free energy is to express the interfacial solid-liquid free energy in Young's equation as a function of the surface free energy of the solid and liquid involved. For this purpose, various approaches to the interfacial free energy formulation have been tested,<sup>6-12</sup> among them, the commonly used geometric mean,<sup>6-8</sup> criticized by van Oss et al.,<sup>9-11</sup> and their own.<sup>9-11</sup>

In any case, determination of reliable values of the free energy components, both for solid and liquid, is still an open problem, especially in the case of complex systems

like cholesterol/bile salt solutions. In a previous paper<sup>12</sup> the free energy components, Lifshitz-van der Waals,  $\gamma_s^{LW}$ , electron acceptor,  $\gamma_s^+$ , and electron donor,  $\gamma_s^-$ , of cholesterol with preadsorbed bile salts, were determined. In this paper, the knowledge of the above components will be used for the prediction of the wettability of cholesterol, and related adsorption phenomena, at the cholesterol/bile salt solution interface. For this purpose, the free energy components of the bile salt solutions must also to be known.

They have been determined in this work from surface and interfacial (bile salt/organic liquid) tension measurements. Wettability of cholesterol by bile salts is discussed here in terms of contact angles of drops of solutions of those salts onto cholesterol surface in two experimental conditions, i.e., bare and precontacted with the bile salt solutions. The experimental contact angles are then compared with those determined theoretically by using Young's equation and the surface free energy components of cholesterol. The following bile salts were investigated: sodium cholate (NaC), sodium deoxycholate (NaDC), and sodium chenodeoxycholate (NaCDC).

## Experimental Section

**Materials.** Cholesterol was a product of Serva (anal. grade, >99%) and was the same as used in the previous studies.<sup>12</sup> Sodium cholate (NaC), sodium deoxycholate (NaDC), and sodium chenodeoxycholate (NaCDC) were from Sigma (anal. grade, >99%). The following liquids were used: dodecane (Prolabo, ch.p), benzene (Merck, p.a. 99.7%) diiodomethane (Merck, for synthesis), formamide (Carlo Erba, >99%), and glycerol (Quimon, ch.p. min. 98%). Water was doubly distilled and deionized (Milli-Q Reagent Water System, Millipore).

**Methods.** Surface and interfacial tension were determined from the shape of hanging drops at the temperature  $20.0 \pm 0.1$  °C. Standard deviation of the series of measurements was within  $\pm 0.5$  mJ/m<sup>2</sup>. Surface tension was measured for aqueous solutions of the bile salts (NaC, NaDC, NaCDC) in the presence of  $10^{-2}$  M NaCl to keep ionic strength constant. The concentration range of the solutions was between  $5 \times 10^{-5}$  and  $5 \times 10^{-2}$  M. Interfacial tensions were measured between the solutions of bile salts and dodecane, diiodomethane, and benzene, respectively, for all the concentrations of the bile salts.

Contact angles were measured on pellets of cholesterol obtained by pressing the powder up to  $2 \times 10^3$  kg/cm<sup>2</sup>.<sup>12</sup> For the measurements, only the pellets having a smooth and glossy surface were selected. In the first series, contact angles of the bile salt solutions were measured on a bare cholesterol surface, just a few seconds after settling the drop onto the surface. In the second

\* To whom correspondence should be addressed.

<sup>†</sup> Maria Curie-Skłodowska University.

<sup>‡</sup> University of Granada.

• Abstract published in *Advance ACS Abstracts*, March 1, 1994.

(1) Musfon, D.; Higuchi, W. *J. Pharm. Sci.* 1970, 59, 601.

(2) Rastogi, R. P.; Sing, K.; Shabd, R.; Upadhyay, B. M. *J. Colloid Interface Sci.* 1981, 80, 402.

(3) Mazer, N. A.; Carey, W. C. *Biochemistry* 1983, 22, 426.

(4) O'Connor, C. J.; Wallace, R. G. *Adv. Colloid Interface Sci.* 1985, 22, 1.

(5) Jacyna, M. R. *Gut* 1990, 31, 568.

(6) Fowkes, F. M. *Ind. Eng. Chem.* 1964, 56, 40.

(7) Owens, D. K.; Wend, R. C. *J. Appl. Polym. Sci.* 1969, 13, 1741.

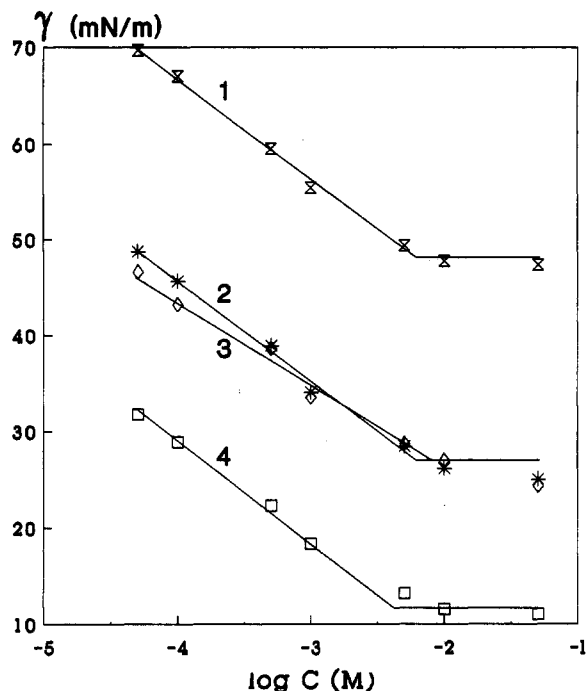
(8) Janczuk, B.; Białopiotrowicz, T. *J. Colloid Interface Sci.* 1981, 127, 189.

(9) Van Oss, C. J.; Good, R. J.; Chaudhury, M. K. *Langmuir* 1988, 4, 189.

(10) van Oss, C. J.; Ju, L.; Chaudhury, M. K.; Good, R. J. *J. Colloid Interface Sci.* 1989, 128, 313.

(11) Good, R. J.; van Oss, C. J. In *Modern Aspects of Wettability*; Schrader, M. E., Ed.; Plenum: New York, 1992.

(12) Kerkeb, M. L.; González-Caballero, F.; Janczuk, B.; Białopiotrowicz, T. *Colloids Surf.* 1991, 62, 263.



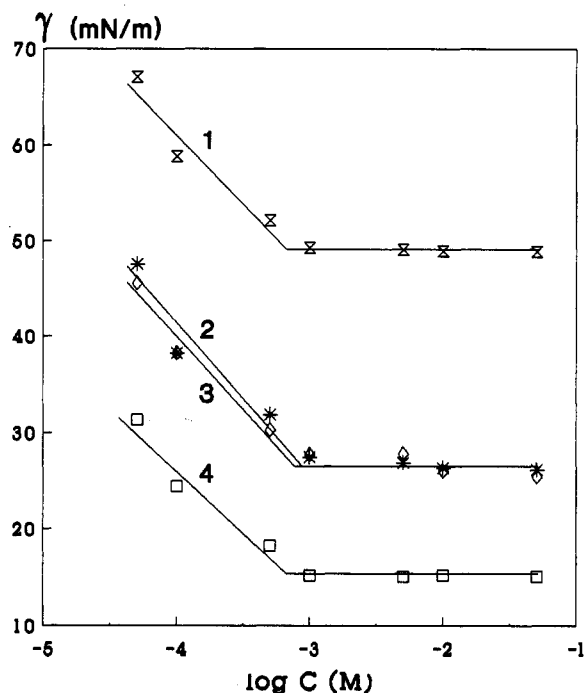
**Figure 1.** Surface tension (mN/m) of the NaC solution and interface tension of NaC solution/dodecane, diiodomethane, and benzene systems: 1, NaC solution; 2, NaC solution/dodecane; 3, NaC solution/diiodomethane; 4, NaC solution/benzene.

series, the pellets were precontacted with the particular bile salt solutions for 1.5 h.<sup>12</sup> As shown by previous kinetic measurements, this time was enough to attain adsorption equilibrium of the bile salts onto the cholesterol surface. Then, contact angles of diiodomethane, water, formamide, glycerol, and the bile salt solutions were measured. A Model 100-00 Ramé-Hart, Inc., apparatus equipped with a thermostated chamber was used for contact angle measurements. Reproducibility of the measurements was within 2°.

### Results and Calculations

**Surface and Interfacial Tensions.** Values of surface tension of the bile salt solutions and interfacial tension of the solution/dodecane, diiodomethane, and benzene systems are shown in Figures 1–3 for NaC, NaDC, and NaCDC, respectively. It can be seen that both surface and interfacial tensions decrease linearly with the logarithm of the concentration of the bile salt solution, up to a given concentration, whereas for higher concentrations these quantities remain constant. This obviously means that micellization of the bile salt molecules takes place in the solutions at that concentration which corresponds to cmc.<sup>12,13</sup> Values of the cmc for NaC, NaDC, and NaCDC solutions obtained from Figures 1–3 on the basis of the surface tension of bile salt solution (1) and interfacial tensions in the dodecane/bile salt solution (2), diiodomethane/bile salt solution (3), and benzene/bile salt solution (4) systems are given in Table 1. From this table it appears that there is good agreement between cmc values determined from surface and interfacial tensions in the above mentioned systems. These values are close to those reported earlier in the literature.<sup>4,13–15</sup>

By use of the values of surface and interface tensions (Figures 1–3) surface tension components of the bile salt solutions can be calculated from eqs 16–8 and 2,<sup>9–11</sup> respectively



**Figure 2.** Surface tension (mN/m) of the NaDC solution and interface tension of NaDC solution/dodecane, diiodomethane, and benzene systems: 1, NaDC solution; 2, NaDC solution/dodecane; 3, NaDC solution/diiodomethane; 4, NaDC solution/benzene.

$$\gamma_1 + \gamma_2 - \gamma_{12} = 2(\gamma_1^d \gamma_2^d)^{1/2} + 2(\gamma_1^n \gamma_2^n)^{1/2} \quad (1)$$

$$\gamma_1 + \gamma_2 - \gamma_{12} = 2(\gamma_1^{LW} \gamma_2^{LW})^{1/2} + 2(\gamma_1^+ \gamma_2^+)^{1/2} + 2(\gamma_1^- \gamma_2^-)^{1/2} \quad (2)$$

where subscript 1 means the bile salt solution and subscript 2 means the organic liquid (dodecane, diiodomethane, or benzene). The superscripts mean as follows: d, dispersion; n, nondispersion; LW, Lifshitz-van der Waals; plus (+), electron acceptor component; minus (−) electron-donor component.

In the case of dodecane/bile salt solution systems ( $\gamma_2 = \gamma_2^d$ ), eq 1 can be rewritten as

$$\gamma_1^d = \frac{(\gamma_1 + \gamma_2 - \gamma_{12})^2}{4\gamma_2} \quad (3)$$

The dispersion components of the surface tension of the bile salt solutions were calculated only from eq 3 on the basis of dodecane/bile salt solution interfacial tension measurements and then  $\gamma_1^n = \gamma_1 - \gamma_1^d$ .

Because  $\gamma_2^+ = 0$  for benzene, and in the case of diiodomethane,  $\gamma_2^- = 0$ , the simultaneous solution of two equations like (2) allows calculation of the three components of the surface tension of the bile salt solutions, if one takes into account that<sup>9–11</sup>

$$\gamma^{AB} = 2(\gamma^+ \gamma^-)^{1/2} = \gamma - \gamma^{LW} \quad (4)$$

where  $\gamma^{AB}$  is the acid-base contribution to the surface free energy of the phase. The  $\gamma_1^{LW}$ ,  $\gamma_1^+$ , and  $\gamma_1^-$  components of  $\gamma_1$  were then determined from eq 2 from only interfacial tension measurements in the diiodomethane/bile salt solution and benzene/bile salt solution systems.

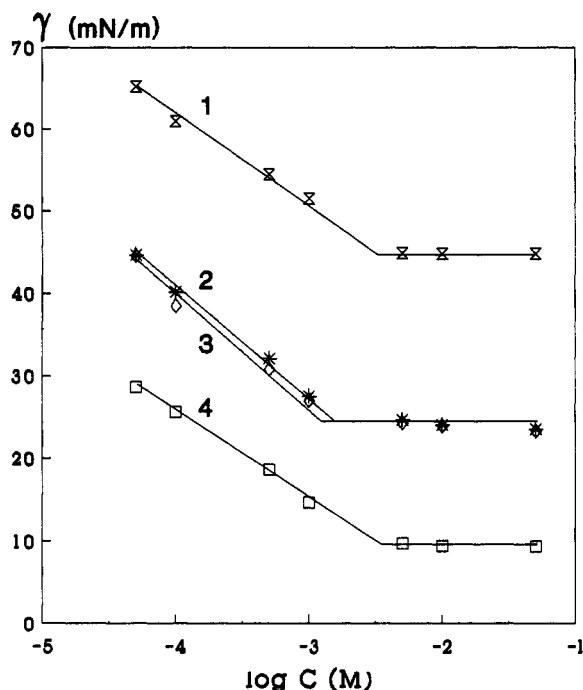
The literature<sup>16,17</sup> values of surface tension components of the liquid used for the calculations are listed in Table

(13) Hofmann, A. F.; Mysels, K. J. *Colloids Surf.* 1988, 30, 145.

(14) Igini, H.; Carey, M. C. *J. Lipid Res.* 1980, 21, 72.

(15) Hofmann, A. F. *J. Infect. Dis.* 1977, 135, 126.

(16) Van Oss, C. J.; Good, R. J. *J. Macromol. Sci. Chem.* 1989, A26, 1183.



**Figure 3.** Surface tension (mN/m) of the NaCDC solution and interface tension of NaCDC solution/dodecane, diiodomethane, and benzene systems: 1, NaCDC solution; 2, NaCDC solution/dodecane; 3, NaCDC solution/diiodomethane; 4, NaCDC solution/benzene.

**Table 1.** Values of cmc Obtained from Figures 1–3 on the Basis of Surface Tension of Bile Salt Solution, 1 (Curve 1), and Interfacial Tensions in the Dodecane/Bile Salt Solution, 2 (Curve 2), Diiodomethane/Bile Salt Solution, 3 (Curve 3), and Benzene/Bile Salt Solution, 4 (Curve 4), Systems and cmc Taken from Literature<sup>4,12–15</sup>

bile salt	cmc			
	1	2	3	4
NaC	$7.2 \times 10^{-3}$	$7.6 \times 10^{-3}$	$9.5 \times 10^{-3}$	$7.2 \times 10^{-3}$
NaDC	$7.1 \times 10^{-4}$	$9.4 \times 10^{-4}$	$7.6 \times 10^{-4}$	$7.9 \times 10^{-4}$
NaCDC	$4 \times 10^{-3}$	$2 \times 10^{-3}$	$1.8 \times 10^{-3}$	$4 \times 10^{-3}$

**Table 2.** Literature Values of the Liquids Surface Tension and Cholesterol (Reference 17) Surface Free Energy Components

substance	$\gamma^{LW}$	$\gamma^+$	$\gamma^-$	$\gamma$
n-dodecane	25.08			25.08
diiodomethane	50.80	0.72	0.0	50.8
benzene	28.88	0.00	3.04	28.88
water	21.80	25.50	25.50	72.80
glycerol	34.00	3.92	57.4	64.00
formamide	39.00	2.28	39.60	58.00
cholesterol	34.62	0.96	1.16	36.73
cholesterol	34.92 <sup>a</sup>		2.06 <sup>b</sup>	

<sup>a</sup> Dispersion component. <sup>b</sup> Nondispersion component.

2. The values of the surface tension components of the bile salt solutions calculated from eqs 2 and 3, respectively, are listed in Table 3. This table shows that for all the solutions studied  $\gamma_1^d$  is close to  $\gamma_1^{LW}$ , and their values do not depend on the concentration or type of bile salt. In fact, they are almost equal to the dispersion component of the surface tension of water (21.8 mN/m).<sup>6</sup> Because of  $\gamma_1^d = \gamma_1^{LW}$ ,  $\gamma_1^a$  must be equal to  $\gamma_1^{AB}$ . The nondispersion or acid–base component of the surface tension of bile salt solutions decreases with concentration until the cmc of the bile salt is reached. It is worth noting that for most

**Table 3.** Values of the Components of Surface Tension of Bile Salt Solution Calculated from Equations 2 and 3, Respectively (in mN/m)

$-\log c$	$\gamma_1^d$	$\gamma_1^a$	$\gamma_1^{LW}$	$\gamma_1^+$	$\gamma_1^-$
NaC					
4.3	21.14	48.52	21.19	24.79	23.69
4.0	21.49	45.49	21.85	23.23	21.92
3.3	20.81	38.67	20.82	24.01	15.56
3.0	21.50	33.86	21.67	20.71	13.70
2.3	21.09	28.19	21.44	19.11	10.14
2.0	21.66	26.02	21.73	18.44	9.13
1.3	22.36	24.92	23.07	15.25	9.61
NaDC					
4.3	19.85	47.17	20.05	22.36	24.67
4.0	20.81	39.02	20.06	18.92	19.86
3.3	20.50	31.62	21.28	14.34	16.58
3.0	21.89	27.63	21.46	14.14	13.62
2.3	22.23	26.78	21.26	13.92	13.83
2.0	22.69	26.24	22.19	11.93	14.98
1.3	22.80	26.07	22.52	11.37	15.27
NaCDC					
4.3	20.72	44.41	20.00	24.87	20.47
4.0	21.00	39.92	21.04	18.43	21.57
3.3	22.44	31.98	22.51	15.50	16.42
3.0	23.96	27.50	23.57	15.09	12.89
2.3	20.46	24.39	21.78	15.88	8.38
2.0	20.98	23.82	22.02	15.84	8.19
1.3	21.46	23.32	22.38	14.97	8.38

cases the electron-acceptor and electron-donor parts of the acid–base component are almost equal.

**Contact Angles.** The values of the contact angles of bile salt solutions measured on bare cholesterol surfaces and on the surface equilibrated with the bile salt solutions are depicted in Figures 4–6 and Figure 7, respectively. From these figures it is clearly seen that contact angles drop down at a concentration close to the cmc (Figures 1–3). In the case of precontacted cholesterol, the decrease in contact angle values is much bigger (Figure 7) than in the case of bare surfaces (Figures 1–3).

Wettability of bare cholesterol by bile salts can be predicted by estimating the contact angles of their solutions onto cholesterol surface. For this purpose, either of the following eqs<sup>6–11</sup> can be used

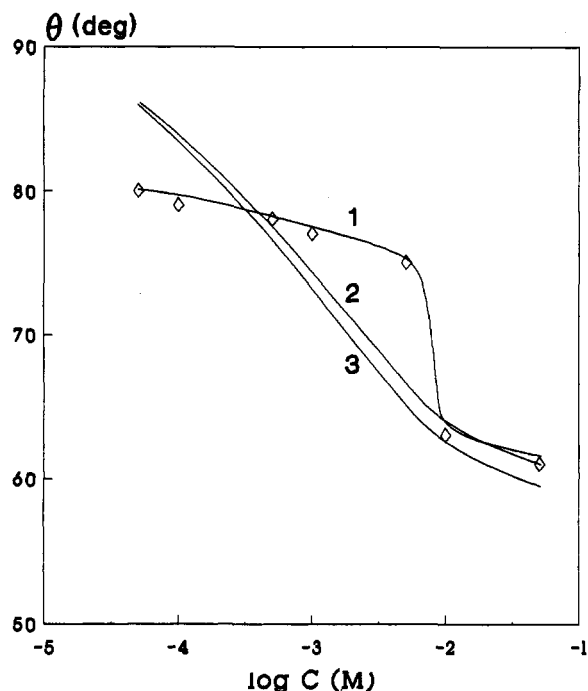
$$\gamma_1 \cos \theta_1 = -\gamma_1 + 2(\gamma_s^d \gamma_1^d)^{1/2} + 2(\gamma_s^a \gamma_1^a)^{1/2} \quad (5)$$

$$\gamma_1 \cos \theta_1 = -\gamma_1 + 2(\gamma_s^{LW} \gamma_1^{LW})^{1/2} + 2(\gamma_s^+ \gamma_1^+)^{1/2} + 2(\gamma_s^- \gamma_1^-)^{1/2} \quad (6)$$

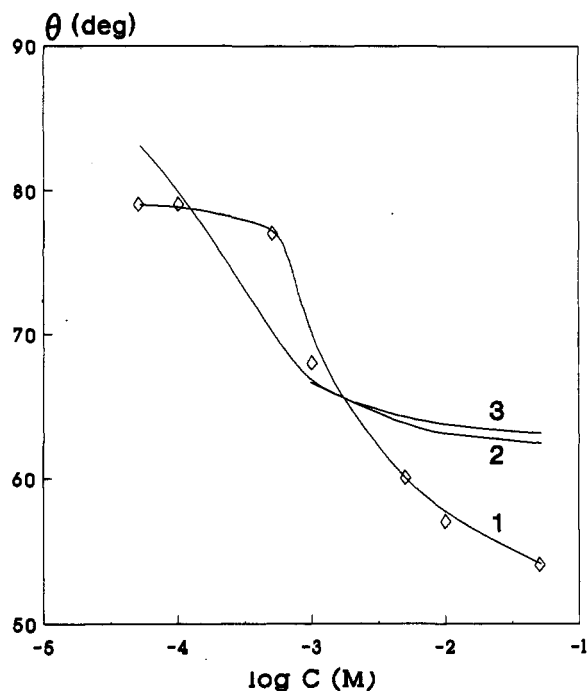
where subscripts s and l refer to solid and liquid, respectively. By use of previously determined surface free energy components of cholesterol<sup>12</sup> (Table 2) and surface tension components of the bile salt solutions (from Table 3), the contact angles in the system cholesterol–solution drop–air were calculated from eqs 5 and 6, respectively. The values obtained are presented in Figures 4–6.

The contact angles determined on the basis of the geometric mean method (eq 5) are represented by curves labeled 2 and those based on van Oss et al. are represented by curves labeled 3 in these figures. As observed in Figures 4–6, calculated contact angles are higher than those measured at low concentrations, and in the range of middle concentrations, up to cmc, the situation is reversed. At concentrations higher than cmc, except for NaDC, the calculated contact angles are close to measured ones.

It is worth noting that at concentrations lower than cmc, the contact angles calculated by the geometric mean method<sup>6–8</sup> are equal to those deduced from van Oss et al.'s<sup>9–11</sup> theory. At concentrations higher than cmc, however, some differences are observed.



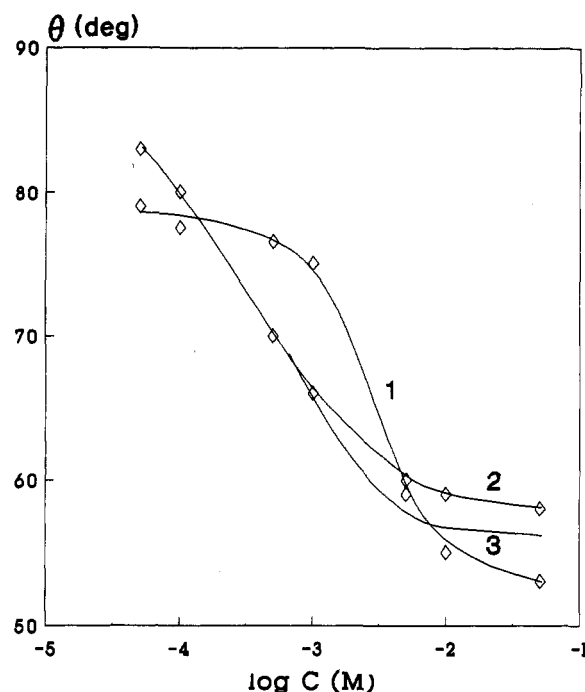
**Figure 4.** Contact angles,  $\theta$  (deg), of the NaC solution on the "bare" cholesterol surface, as a function of the NaC concentration: 1, measured; 2, calculated from eq 5; 3, calculated from eq 6.



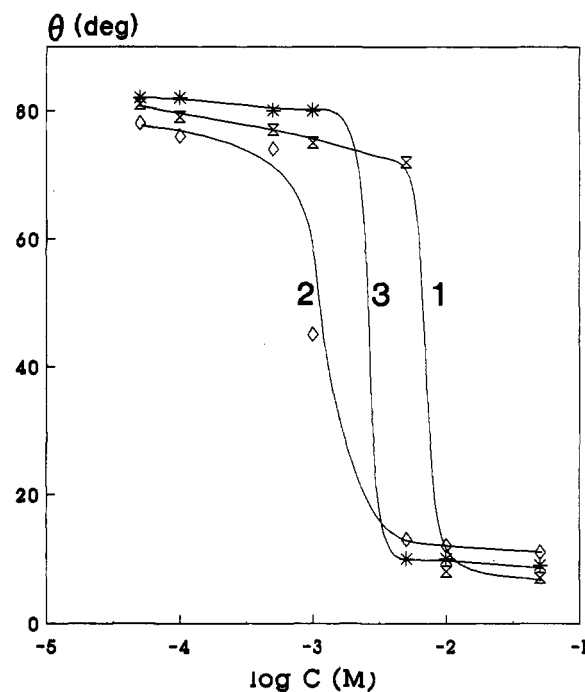
**Figure 5.** Contact angles,  $\theta$  (deg), of the NaDC solution on the "bare" cholesterol surface, as a function of the NaDC concentration: 1, measured; 2, calculated from eq 5; 3, calculated from eq 6.

**Surface Free Energy.** Using the values of contact angles for the triads of liquids diiodomethane, water, and formamide or diiodomethane, water, and glycerol, the surface free energy components of cholesterol precoversed with bile salts were calculated from eq 6. These values are presented in Table 4. It may be stated that relatively consistent values of the components were obtained from both sets of the three liquids.

From Table 4, it appears that  $\gamma_s^{LW}$  decreases when the concentration of the solution of bile salt used for adsorption



**Figure 6.** Contact angles,  $\theta$  (deg), of the NaCDC solution on the "bare" cholesterol surface, as a function of the NaCDC concentration: 1, measured; 2, calculated from eq 5; 3, calculated from eq 6.



**Figure 7.** Contact angles,  $\theta$  (deg), of the bile salts (NaC, NaDC, and NaCDC) on the cholesterol surface precontacted with the solution, as a function of the bile salt concentration.

increases. There are only slight differences between particular bile salts at the same concentration. In the case of  $\gamma_s^+$  a small increase is observed as a function of bile salt concentration, whereas the increase in  $\gamma_s^-$  is much more considerable. For all the cases studied,  $\gamma_s^-$  is higher than  $\gamma_s^+$ , but large differences between these parameters are observed only at concentrations higher than the cmc. In Table 5 the total surface free energy of cholesterol covered with bile salt is presented together with the values previously determined.<sup>12</sup> Also the values of  $\gamma_s$  calculated on the basis of Young's equation, using contact angles of

**Table 4.** Calculated from Equation 6 Average Values of the Free Energy Components of Cholesterol with Adsorbed Bile Salts from Solutions at Different Concentrations in mJ/m<sup>2</sup>

$-\log C$	$\gamma_s^{LW}$	$\gamma_s^+$	$\gamma_s^-$
Cholesterol Precontacted with NaC Solutions			
4.3	29.69	1.25	4.76
4.0	28.89	1.52	5.67
3.3	28.79	1.66	6.05
3.0	28.15	1.92	6.44
2.3	27.85	2.01	7.73
2.0	20.27	4.54	63.66
1.3	20.24	4.61	64.17
Cholesterol Precontacted with NaDC Solutions			
4.3	26.50	1.93	6.55
4.0	26.26	2.14	7.58
3.3	26.17	2.29	8.02
3.0	22.22	3.67	33.22
2.3	19.36	4.41	63.25
2.0	18.82	4.66	64.26
1.3	18.77	4.63	64.96
Cholesterol Precontacted with NaCDC Solutions			
4.3	27.04	1.65	4.51
4.0	27.04	1.65	4.51
3.3	26.94	1.79	4.84
3.0	26.55	2.04	6.33
2.3	25.68	2.56	7.78
2.0	19.21	4.51	65.51
1.3	18.76	4.84	65.11
1.3	18.82	5.04	64.25

bile salt solutions on the bare and precovered cholesterol surfaces, are given in Table 5 (see below for explanation of this table). Young's equation for the system "bare" cholesterol-bile salt solution drop-air and precovered cholesterol-bile salt solution drop-air can be expressed, respectively, in the forms

$$\gamma_s(b) - \gamma_{sl}(b) = \gamma_l \cos \theta_l(1) \quad (7)$$

$$\gamma_s - \gamma_{sl} = \gamma_l \cos \theta_l(2) \quad (8)$$

where  $\gamma_s(b)$  and  $\gamma_{sl}(b)$  are surface free energy of "bare" cholesterol and interfacial free energy of "bare" cholesterol/bile salt solution, respectively.  $\gamma_s$  and  $\gamma_{sl}$  stand for the surface free energy of cholesterol covered by bile salt molecules, and the interfacial free energy of cholesterol (covered by bile salt molecules)/bile salt solution system, respectively.

Because cholesterol is a hydrophobic solid for which the surface free energy results mainly from Lifshitz-van der Waals intermolecular interactions,<sup>12</sup> it can be expected that the excess of bile salt molecules is similar for the drops settled on precovered and bare cholesterol so that we can assume that  $\gamma_{sl}(b)$  is close to  $\gamma_{sl}$ . Thus, from eqs 7 and 8 it results:

$$\gamma_s = \gamma_s(b) + \gamma_l[\cos \theta_l(2) - \cos \theta_l(1)] \quad (9)$$

By means of eq 9, we have calculated  $\gamma_s$  using the  $\gamma_s(b)$  values previously reported,<sup>12</sup> determined on the basis of geometric mean<sup>6-8</sup> ("g-g"<sup>12</sup> in Table 5) and van Oss et al.<sup>9-11</sup> ("O<sub>2</sub>" in Table 5) approaches. The values of  $\gamma_s$  determined from eq 6 are denoted in Table 5 as "O<sub>1</sub>". It results from this table that  $\gamma_s(g-g)$  and  $\gamma_s(O_2)$  values are almost equal for a given system. For cholesterol precontacted with NaCDC solutions, the agreement is excellent between the values of  $\gamma_s(g-g)$ ,  $\gamma_s(O_2)$ , and  $\gamma_s(O_1)$ . However, for cholesterol precontacted with NaC and NaDC solutions, better agreement is observed between  $\gamma_s(g-g)$  and  $\gamma_2(O^{12})$  or  $\gamma_s(O_2)$  and  $\gamma_s(O^{12})$  than between  $\gamma_s(g-g)$  and  $\gamma_s(O_1)$  or  $\gamma_s(O_2)$  and  $\gamma_s(O_1)$ . The  $\gamma_s(g-g)^{12}$  values at concentrations

**Table 5.** Values of the Total Surface Free Energy for Cholesterol Surface with Adsorbed Bile Salts from Solutions at Different Concentration, and Values Taken from Literature<sup>12</sup> (g-g<sup>12</sup> and O<sup>12</sup>), in mJ/m<sup>2</sup> (Calculated from Equations 5 and 9, See Text for Meaning of Symbols)

$-\log C$	$\gamma_s$				
	g-g	g-g <sup>12</sup>	O <sub>1</sub>	O <sub>2</sub>	O <sup>12</sup>
Cholesterol Precontacted with NaC Solutions					
4.3	36.38	35.80	34.57	36.13	35.78
4.0	36.98	36.08	34.76	36.73	36.07
3.3	37.99	36.47	35.13	37.74	36.47
3.0	38.85	36.47	35.18	38.60	36.45
2.3	39.04	37.32	35.73	38.79	37.32
2.0	62.55	71.31	54.27	62.30	63.87
1.3	61.35	71.78	54.64	61.10	64.15
Cholesterol Precontacted with NaDC Solutions					
4.3	38.13	34.88	33.61	37.88	34.80
4.0	39.99	35.75	34.32	39.74	35.75
3.3	40.06	36.21	34.74	39.82	36.22
3.0	53.34	52.32	44.30	53.10	48.24
2.3	60.23	69.71	52.76	59.98	58.24
2.0	57.48	70.36	53.43	57.23	63.40
1.3	56.23	70.67	53.45	55.98	65.58
Cholesterol Precontacted with NaCDC Solutions					
4.3	33.62	35.03	32.50	33.37	38.91
4.0	32.27	35.37	32.83	32.02	36.22
3.3	33.73	36.47	33.74	33.48	36.68
3.0	32.60	37.33	34.60	32.35	37.69
2.3	58.05	71.41	53.59	57.80	74.38
2.0	55.40	71.41	54.26	55.15	73.57
1.3	54.26	71.41	54.81	54.01	73.57

of bile salt higher than cmc are considerably higher than those determined in the other different ways ( $\gamma_s(O^{12})$  values for cholesterol precontacted with NaCDC solution are an exception of this rule).

## Discussion

It is well-known that cholesterol and bile salts are similar compounds from the structural point of view. However, cholesterol is an unsaturated alcohol (it has one  $\pi$ -bond) with only one hydroxyl group, while bile salts contain two (NaDC and NaCDC) or three (NaC) hydroxyl groups, as well as an acidic carboxyl group. This makes an essential difference as to the solubility and polarity of cholesterol and bile salts. The molecular structure of these compounds suggests that they should possess higher electron-donor than electron-acceptor acid-base interactions. This conclusion was verified experimentally by measurements of contact angles of diiodomethane, bromoform, and water, from which the surface free energy components of the bile salts were calculated.<sup>17</sup> It was found there that  $\gamma_s^+$  amounted to about 10–12 mJ/m<sup>2</sup>, whereas the typical values of  $\gamma_s^-$  were 35–41 mJ/m<sup>2</sup>. However, in the case of aqueous solutions of bile salts, both acid-base components are of similar magnitude, and for the highest concentrations used (where molecular association takes place, Figures 1–3)  $\gamma_l^-$  is, in some cases, even somewhat smaller than  $\gamma_l^+$  (Table 3). This means that both at the solution/air and solution/apolar liquid interfaces the water molecules are not strongly oriented. The similarities between the cmc values obtained for a given salt, from different solution/organic liquid or solution/air systems (Table 1), and the fact that  $\gamma_l^d \approx \gamma_l^{LW}$  (Table 3) confirm this conclusion.

Bile salts in water are partly dissociated, but both dissociated and undissociated forms can interact with hydrophobic solids or liquids mainly by dispersion forces. Because cholesterol is a hydrophobic solid, the interaction between water molecules and the cholesterol surface is probably weaker than that between this surface and bile salt molecules. Furthermore, there has been also found

**Table 6.** Values of "Bare" Cholesterol/Bile Salt Solution Interface Free Energy Calculated from Equation 7

$-\log c$	NaC	NaDC	NaCDC
4.3	25.48	24.19	24.55
4.0	24.20	25.75	23.79
3.3	24.61	25.70	24.27
3.0	24.53	18.54	23.66
2.3	23.81	12.47	13.88
2.0	15.33	9.62	11.28
1.3	14.42	8.25	10.03

a negative value of the free energy of interaction between cholesterol and bile salt molecules in water.<sup>17</sup> Therefore, there could be an adsorption of bile salt molecules at the cholesterol/solution interface, which influenced the wettability of the cholesterol.

According to eq 7, wettability of cholesterol in the "bare" cholesterol-solution drop-air system depends on the quantities  $\gamma_{sl}(b)$  and  $\gamma_l$ . Since we have determined here experimentally  $\gamma_l$  and  $\theta_l(1)$  values and earlier<sup>17</sup> and  $\gamma_s(b)$  value, it is possible to evaluate the  $\gamma_{sl}(b)$  from eq 7 as a function of bile salt concentration. The results are shown in Table 6. From this table it appears that at bile salt concentration lower than cmc, constant values of  $\gamma_{sl}(b)$  are attained, which do not depend on the kind of bile salt.

On the basis of the data presented in Table 6 and Figures 4-6, we can conclude that in the case of the bare cholesterol surface, adsorption of bile salt molecules may occur after contact of the drop with the surface. However, this practically does not affect the measured contact angles until micellization of the bile salt molecules takes place in the solutions (constant  $\gamma_{sl}(b)$  values, Table 6). Therefore, the contact angles measured at low concentrations on the bare surface can change only as a consequence of changes in surface tension of the solutions. However, at concentrations higher than cmc, the surface tension is constant, and contact angle can change only because of variations in  $\gamma_{sl}(b)$ . Comparing the values of contact angles on bare cholesterol surfaces, calculated from eqs 5 and 6, and the measured ones (Figures 4-6), we can suggest that at low concentrations the adsorption of bile salt at the cholesterol/solution interface is higher than at the solution/air interface. The values of contact angle calculated for bile salt solutions are higher than those experimentally determined. This fact suggests that  $\gamma_l^N$  or  $\gamma_l^{AB}$  ( $\gamma_l^d$  and  $\gamma_l^{LW}$  are constant for all studied solutions, Table 3) should be lower than those resulting from solution/air and solution/apolar liquid interface. Because at concentrations lower than cmc the  $\gamma_{sl}(b)$  values are constant (Table 6), it is very easy to calculate this value from, for example, eq 5. This value is equal to approximately 38.7 mN/m. In the concentration range in which the association of bile salt molecules probably start to take place, the calculated values of contact angles are lower than measured ones.

We must note that from the mathematical point of view, the calculated contact angles should be close to measured ones in the case that the adsorption of bile salt at cholesterol/solution and solution/air interfaces were the same, and water molecules were not strongly oriented at any interface. Such a situation can take place if there are intersection points on the curves obtained from measurements and calculations (Figures 4-6). With the exception

of NaC, the contact angles calculated from eqs 5 and 6 are higher than the measured ones for concentrations higher than cmc. This suggests that the adsorption of bile salt molecules at the cholesterol/solution interface should be higher than at the solution/air one. Because in this range of concentration the calculated values of  $\gamma_{sl}(b)$  depend on the kind of bile salt and concentration, and surface tension of bile salt solution is constant (Figures 1-3), it can suggest that reorientation of bile salt molecules at the cholesterol/solution interface occurs in comparison to micelles and that water molecules are stronger oriented than at the solution/air interface. This conclusion is confirmed by the values of  $\gamma_s^+$  and  $\gamma_s^-$  determined for cholesterol covered with bile salt molecules (Table 4). At concentrations higher than cmc,  $\gamma_s^-$  is much more higher than  $\gamma_s^+$ . It indicates that the surface of cholesterol covered with bile salt molecules at concentrations higher than cmc is strongly hydrated.

At this point, we must note that geometric mean<sup>6-8</sup> and van Oss et al.<sup>9-11</sup> methods give a different result for surface free energy of cholesterol. At concentrations higher than cmc, the values of  $\gamma_s(O_1)$  (determined in this paper on the basis of the van Oss et al.<sup>9-11</sup> approach, from contact angles of liquids, two of which were strongly polar and one apolar) are considerably lower than those determined earlier on the basis of the geometric mean approach<sup>12</sup> (from contact angles of two-liquid systems, polar and apolar, respectively). Thus, a question can arise about which values are more real as to the surface free energy of cholesterol covered by bile salt molecules. From a mathematical point of view, it is clear that in the case when  $\gamma_l^+ = \gamma_l^-$  and  $\gamma_s^+ = \gamma_s^-$  the geometric mean<sup>6-8</sup> and van Oss et al.<sup>9-11</sup> approaches give the same result. Therefore, the theoretical curves (labeled 2 and 3) shown in Figures 4-6 are almost identical. Also, the values of  $\gamma_s$  for cholesterol surface covered by bile salt molecules from solutions of low concentration, determined from both approaches, are similar. This suggests that the above mentioned condition is fulfilled. However, great differences exist between the electron-acceptor and electron-donor components of surface free energy for surfaces covered from solutions of high concentration.

Calculations of  $\gamma_s$  made on the basis of eq 9 suggest that at a high concentration of bile salt, the van Oss et al.<sup>9-11</sup> method gives the more clear picture of interfacial interactions. The  $\gamma_s$  values obtained from eq 9 are quite similar to those from eq 2 (Table 5). Thus, taking into account the results obtained using the van Oss et al.<sup>9-11</sup> approach, we can conclude that bile salt increases the hydrophilicity of the cholesterol surface, but drastic changes in wettability take place only after micelles are formed in the solution and then adsorbed onto the surface. So, it may be stated that in these systems, a much more important role is played by micelles than by individual molecules. This conclusion may be helpful for better understanding of the mechanism of the gallbladder stone formation.

**Acknowledgment.** E.Ch. expresses his gratitude to Spanish Ministerio de Educacion y Ciencia for financial support of his stay at the Departamento de Fisica Aplicada, Facultad de Ciencias, Universidad de Granada, Spain. We thank also support given by DGICYT, Spain, under Project No. 0461/89.