# Analysis of Main- and Cross-Term Diffusion Coefficients in Bile Salt Mixtures

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Ⓢ *Supporting Information*

ABSTRACT: Mutual diffusion coefficients have been measured for several average compositions of the system sodium cholate–sodium deoxycholate–water at 25 °C. The experiments have been grouped in different sets having constant concentration of one component and variable concentration of the other one. Following this approach, it has been found that the trends of the main- and cross-term diffusion coefficients can be interpreted on the basis of the diffusion and equilibrium results of similar experiments performed on the two binary systems sodium cholate–water and sodium deoxycholate–water. Implications of the presented results in the transport of lipids operated by bile salt aggregates are mentioned. The method proposed in this work, able to connect the diffusivities of an *n*-component system to those of the related *n*−1 subsystems, can be extended to obtain qualitative prediction on the diffusion coefficient trends for mixtures of other surfactants, of both industrial and biological interest.
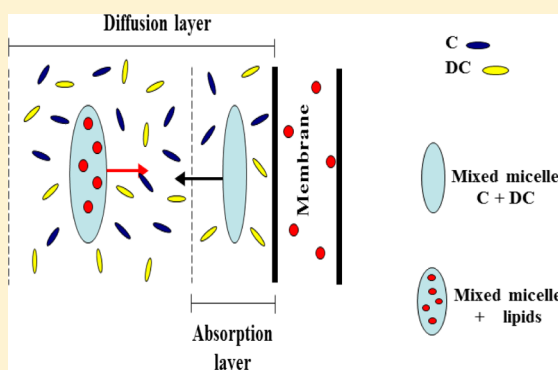
■ INTRODUCTION

It is widely known that bile salts are not conventional surfactants because of the absence of a clear-cut polarity gradient between their hydrophobic and hydrophilic parts due to their steroidal structure, see Figure 1.[1,2] Their behavior as
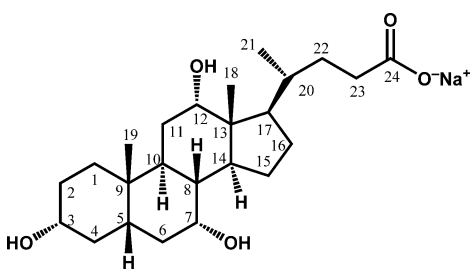

Figure 1. Structure of sodium cholate molecule.

surfactants is imputable to the presence of two or more hydroxyl groups on only one of the two surfaces of their steroidal backbone. The consequence is the higher hydrophilic character of this surface with respect to the other one where only hydrophobic groups are present. This promotes a back to back supramolecular interaction between hydrophobic surfaces through a classical entropic benefit.[3]

It is evident that the hydrophilic/hydrophobic balance of each bile salt is due to the interplay of different effects such as number and position of hydroxyl moieties and chemical characteristics of side chains. For example, the absence of one —OH on the C7 position gives rise to deoxycholic acid and its

absence on C12 to a cheno-type bile salt. The substitution of this —OH group with a chetone residue is responsible for the urso-type cholate. An amino acidic residue on C24 is responsible for glycol type, while the presence on C24 of an amino ethyl sulfonic acid gives rise to taurocholic acid. These possible substitutions on the cholic acid backbone reflect not only different HLB (hydrophilic/lipophilic balance) values but also different spatial arrangements in supramolecular aggregates.[2]

Generally speaking, the back to back interaction between the hydrophobic surfaces generates the building blocks of all bile salt aggregates. According to the most accepted hypothesis, these small blocks may interact with each other by setting directionally preferential H-bonds[1,2,4−6] to form elongated aggregates with dimensions sufficiently large to solubilize in their interior hydrophobic molecules. Indeed, the efficiency as fat emulsifier of bile is due to the capability of bile salt aggregates to accommodate in their interior molecules such as cholesterol or long aliphatic fatty acid.

Because of the importance that mixtures of cholates and cholate derivatives play in the transport, emulsification, and digestion of fats[7] and in the excretion of cholesterol and bilirubin, some of us have recently investigated the microstructural features of the ternary system sodium cholate (component 1)–sodium deoxycholate (component 2)–water

(component 0).[8,9] By adoption of a multitechnical approach, the attention has been essentially focused on the structure and on the compositions of the mixed micelles formed by the two bile salts. One set of diffusion coefficient measurements was performed at constant C_1/C_2 ratio, with increasing the total solute concentration, $C_1 + C_2$.[9] However, we found that the data, especially the cross-term coefficients, present a very complex trend, whose interpretation was not fully unsatisfactory.

In this paper four new sets of diffusion coefficients are presented with the aim to have a deeper insight into the diffusive properties of this ternary system. The measurements have been performed at constant concentration of one component and variable concentration of the other. As will be discussed later, this approach makes the trends much easier to be interpreted in terms of the behavior of the related binary systems allowing, in the absence of any predictive equation, to clarify which parameters must be used to obtain a general qualitative estimate of the diffusion coefficient trends as a function of the composition. This is a necessary prerequisite for attempting an at least qualitative prediction of the diffusive behavior of surfactant mixtures. For illustrative purposes, at the end of this work, we show that our approach allows to build a reliable basis for the modeling of the fat transport operated by the bile salt mixed micelles.

EXPERIMENTAL SECTION

Materials. Sodium cholate (NaC, stated purity >98%, molecular weight 430.57 uma) and sodium deoxyxcholate, (NaDC, stated purity >99%, molecular weight 414.58 uma) were purchased from Aldrich (St. Louis, MO) and Acros Organics (Geel, Belgium), respectively, and purified according to the procedure widely described by Kratohvil.[10] Doubly distilled and degassed water was used for preparing all solutions; its molecular weight has been chosen as 18.016 g mol^{-1}. All solutions have been prepared by weight, and their densities, necessary in passing from molality to molarity scale, have been determined by an Anton Paar DMA 500 density meter. The temperature of the cell of the density meter was set at (25.000 ± 0.002) °C, and the instrument was calibrated by water and air. The density of the water was assumed to be 0.997 047 g cm^{-3}, while the density of air was calculated for each measurement on the basis of the measured local pressure and humidity.

Mutual Diffusion Measurements. The four diffusion coefficients that are representative of the motion of each component in a ternary system have been obtained by Gouy interferometry at (25.00 ± 0.01) °C. The average compositions at which diffusion measurements have been performed are reported in Table 1 and in Figure 2; these compositions have been grouped in different sets with the concentration of one component kept constant and the concentration of the other one made variable. The sets are numbered from 1 to 5; Set 3 corresponds to the data already published.[8] In the figure the curved line corresponds to the cmc of the mixed micelles as determined by surface tension measurements.[9] In relation to the Gouy interferometric apparatus and its use to obtain the four ternary diffusion coefficients, we refer to the previous abundant literature.[11−17]

Just at one composition, very dilute in one component, the Gouy interferometic approach was found to be not reliable for data collection. This was due to the extremely limited number of interference fringes whose evolution with time has to be

Table 1. Measurements Sets for the Ternary System Sodium Cholate (1)−Sodium Deoxycholate (2)−Water (0)^a

set 1, $C_1^o = 0.009$ M	set 2, $C_1^o = 0.030$ M	set 3, $C_2^o = 0.004$ M	set 4, $C_2^o = 0.020$ M	set 5, $C_2^o = 0.027$ M
C_2	C_2	C_1	C_1	C_1
0.004	0.004	0.004	0.009	0.009
0.009	0.009	0.009	0.020	0.020
0.015	0.020	0.015	0.030	0.030
0.020	0.027	0.020		
0.027	0.030	0.030		

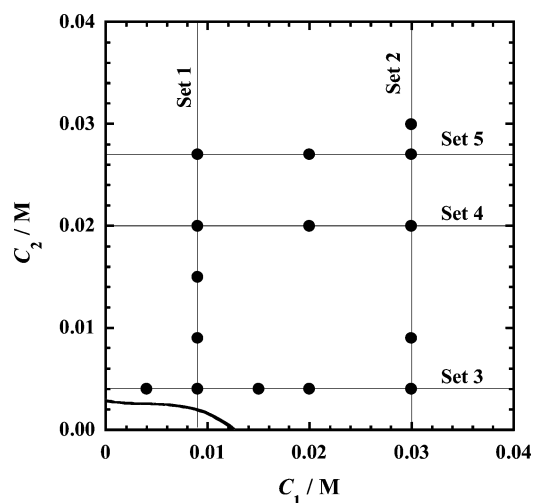^{a}All composition are expressed in M (mol/dm^3).

Figure 2. Average concentrations explored by Gouy diffusion measurements for the ternary system: sodium cholate (1)−sodium deoxycholate (2)−water at 25 °C. The curve corresponds to the cmc of the system as determined by surface tension measurements.

followed in order to determine the diffusion coefficients. For this reason, just at this single composition ($C_1 = 0.009$ mol L^{-1}, $C_2 = 0.002$ mol L^{-1}), diffusion coefficients were determined by the Taylor dispersion technique, which allows the analysis of very dilute mixtures. Also for this technique details of apparatus and raw data treatment are reported in previous papers.[18] A reasoned comparison between advantages and disadvantages of these alternative techniques has been illustrated elsewhere.[19] However, the uncertainty in the results obtained by the Taylor approach is usually higher than that in Gouy interferometry results. This was found true also in our measurements, as can be seen by the uncertainties quoted in Table ESI1. For this reason in all the graphs, the datum obtained by the Taylor technique is reported using a different marker.

In the following, all the quantities related to sodium cholate, NaC, will be indicated with subscript 1, and those related to sodium deoxycholate, NaDC, with subscript 2.

THEORETICAL BACKGROUND

In general, diffusion phenomena are present in any process that occurs in nature, and, as a consequence, the knowledge of the mutual diffusion coefficients is fundamental in studying the evolution in time of any real system. For example, referring to the action of bile salts in digestion of fats and fat-soluble vitamins in the small intestine, mutual diffusion has a relevant role in transport and adsorption of lipids.

The mutual diffusion in an n-component system $(1, 2, ..., n-1, 0$, where component 0 is the solvent), is described by the Fick equation, that for mono-directional transport can be written as

$$J_i = -\sum_{1}^{n-1} D_{ij} \cdot \nabla C_j \tag{1}$$

where J_i and ∇C_i are the flow and the concentration gradient of component i, respectively, and D_{ij} are the $(n-1)^2$ diffusion coefficients. The determination of these diffusion coefficients requires at least n independent diffusion experiments, but, to improve the statistics, a larger number of experiments is usually performed.

In modeling real diffusion processes, we have to face two main problems: the first is connected with the large number of independent components in the system, and the second with the impossibility to consider, in integrating Fick's equations, constant diffusion coefficients.[20−22] In fact, the real concentration gradients are very often so large that the dependence of the diffusion coefficients on the concentration must be taken into account. This requires a very punctual description of the diffusion properties of the considered system, i.e., a very large number of investigated compositions. This is relatively simple to do with a two-component system (solute 1, solvent 0) but is much more difficult in multicomponent systems $(1, 2, ..., 0)$.

For this reason several attempts are reported in the literature to find and test expressions able to correlate the diffusion coefficients of an n-component system $(1, 2, ..., n-1, 0)$ to those of the corresponding $(n-1)$ or $(n-2)$ related component systems.[23−27] If we refer to a ternary system containing two solutes $(1, 2, 0)$, the effort is to express the four ternary diffusion coefficients in terms of the two binary diffusion coefficients relative to the systems $(1, 0)$ and $(2, 0)$. Furthermore, for systems in which association or dissociation equilibriums are present in solution, the attempt is to express the mutual diffusion coefficients in terms of the equilibrium parameters and of the diffusivity of the species actually present in solution.[28−32]

For a binary system $(1, 0)$, eq 1 reduces to

$$J_1 = -D_1 \cdot \nabla C_1 \tag{2}$$

and a single diffusion coefficient is sufficient to describe the whole phenomenon.

In the presence of self-association of the solute, the diffusion coefficient can supply information on the equilibrium parameters. Let consider an aqueous solution of sodium cholate, Na^+C^-, as an example of the general behavior of the binary solution of ionic surfactant.

The micellization reaction can be written as

$$nC^- + zNa^+ \rightleftarrows M(C)^{-(n-z)} \tag{3}$$
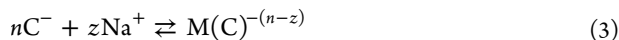
with an equilibrium constant equal to

$$K_S \cong \frac{C_{M(C)}}{C_C^n \cdot C_{Na}^z} \tag{4}$$

where $M(C)^{-(n-z)}$ is the micellar aggregate formed by n cholate ions, on which z sodium ions have condensed. From here on, C^-, Na^+, and $M(C)^{-(n-z)}$ are reported as subscripts without the charges. We remember that, in the case of electrolyte solutions, the experimental diffusion coefficient D_{NaC} accounts for the diffusion of both cation and anion, correlated by the local

electroneutrality condition. Moreover, in the system under consideration, the cholate anion diffusivity results from both the motion of the small and "fast" monomer C^- and the motion of the cholate molecules present in the bulky and "slow" micelle $M(C)^{-(n-z)}$. Therefore, it depends on the distribution of cholate between the monomeric and micellar form. In other words, it depends on the equilibrium parameters n, z, and K_S, and on the diffusivity of all species present in solution, D_C, D_{Na}, and $D_{M(C)}$:

$$D_{NaC} = f(n, z, K_C, D_C, D_{Na}, D_{M(C)}; C_{NaC}) \tag{5}$$

If the equilibrium and diffusivity parameters can be considered constant on the surfactant concentration, eq 5 takes the following form:

$$\begin{aligned} D_{NaC} = \{ & [c_C c_{Na} D_C D_{Na} + (n-z)^2 c_C c_{M(C)} D_C D_{M(C)} \\ & + n^2 c_{Na} c_{M(C)} D_{Na} D_{M(C)}] / [c_C D_C + c_{Na} D_{Na} \\ & + z^2 c_{M(C)} D_{M(C)}] \} \\ & \times \frac{c_C + c_{Na} + z^2 c_{M(C)}}{c_C c_{Na} + (n-z)^2 c_C c_{M(C)} + n^2 c_{Na} c_{M(C)}} \end{aligned} \tag{6}$$

The constancy of the equilibrium parameters implies that the micelle average size is independent of the stoichiometric sodium cholate concentration, while the constancy of diffusivities implies a very limited viscosity effect. These conditions are reasonably fulfilled in a range of surfactant concentration with an upper limit that does not exceed 4−5 times the cmc value.

Experimental diffusion data of the two binary systems $Na^+C^-(1)−W(0)$ and $Na^+CD^-(2)−W(0)$ have been recently measured and analyzed by some of us.[9] By fitting eq 6 to the experimental data, it was possible to obtain the values of the fitting parameters. In order to decrease the number of the parameters to be optimized, the D_C (or D_{DC}) and D_{Na} values have been fixed to the values obtained, applying the binary Nernst−Hartley limit equation,[14,33] from the limit diffusion coefficient of the cholates at infinite dilution. The diffusive and equilibrium parameters derived from this procedure are reported in Table 2.

For a ternary system $(1, 2, 0)$, the Fick equations are

$$J_1 = -D_{11} \cdot \nabla C_1 - D_{12} \cdot \nabla C_2 \tag{7}$$

$$J_2 = -D_{21} \cdot \nabla C_1 - D_{22} \cdot \nabla C_2 \tag{8}$$

Table 2. Aggregation and Diffusion Parameters of Sodium Cholate (NaC) and Sodium Deoxycholate (NaDC) Pure Micelles at 25 °C[a]

	sodium cholate (NaC)	sodium deoxycholate (NaDC)
*D_{Na}	1.334×10^{-5} cm²/s	1.334×10^{-5} cm²/s
*D_C or *D_{DC}	0.425×10^{-5} cm²/s	0.483×10^{-5} cm²/s
*$D_{M(C)}$ or *$D_{M(DC)}$	$(0.15 \pm 0.01) \cdot 10^{-5}$ cm²/s	$(0.13 \pm 0.01) \cdot 10^{-5}$ cm²/s
ln K	20 ± 1	28 ± 3
aggregation number	6 ± 1	6 ± 1
micellar charge	-5 ± 1	-5 ± 1
cmc	(13 ± 1) mM	(3 ± 1) mM

[a]*D_i represent the ionic diffusion coefficients. K is the equilibrium constant for micellization process. cmc values have been determined by surface tension measurements.[9]

where D_{11} and D_{22} are the main diffusion coefficients and D_{12} and D_{21} are the cross ones. In the presence of a chemical equilibrium between solutes, the four experimental diffusion coefficients can also supply information on the equilibrium parameters.

Let consider the equilibrium for the formation of mixed micelles in the ternary system $Na^+C^-(1)-Na^+DC^-(2)-W(0)$

$$nC^- + mDC^- + zNa^+ \rightleftarrows M(C, DC)^{-(n+m-z)} \qquad (9)$$
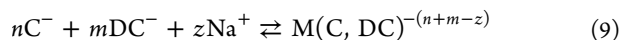
where C^- is the unimeric cholate, DC^- is the unimeric deoxycholate, n and m are the numbers of C^- and DC^- constituting the mixed micelle $M(C, DC)^{-(n+m-z)}$, and z is the number of condensed counterions Na^+. The corresponding equilibrium constant is

$$K_{C,DC} \cong \frac{C_{M(C,DC)}}{C_C^n \cdot C_{DC}^m \cdot C_{Na}^z} \qquad (10)$$

From here on, whenever C^-, DC^-, Na^+, and $M(C, DC)^{-(n+m-z)}$ will be used as superscripts or subscripts, the charge will be omitted.

As in the case of the corresponding binary systems, the ternary main-term diffusion coefficient D_{11} (or D_{22}) is an averaged parameter representative of the motion of the "fast" monomer C^- (or DC^-) and, at the same time, of the motion of the cholate (or deoxycholate) molecules embedded in the "slow" mixed micelle $M(C, DC)^{-(n+m-z)}$. They are therefore dependent on how any surfactant is distributed between the monomeric and micellar form, i.e., on the equilibrium parameters n, m, z, and $K_{C,DC}$, and on the diffusivity of all the species in solution, namely, D_C, D_{DC}, D_{Na}, and $D_{M(C)}$.

The cross-term diffusion coefficients are indicative of the correlation between the flows of the two surfactants; these flows are related, at least, by the fact that the two surfactants are compelled to move together in the mixed micelle. Consequently, also the cross-terms are a function of n, m, z, and $K_{C,DC}$ and of the diffusivity of all the species present therein. It is then possible to write the following general relations:

$$D_{ij} = f_{ij}(m, n, z, K_{C,DC}, D_C, D_{DC}, D_{Na}, D_{M(C,DC)}$$
$$; C_{NaC}, C_{NaDC}) \qquad (11)$$

In the case of a ternary system, the dependence of the thermodynamic parameters on the system composition is much more complex than in a binary one, mainly because the micelle composition (namely, n, m, and z) as well as $K_{C,DC}$ vary both with the total solutes stoichiometric concentration $C_{tot} = C_1 + C_2$ and with the ratio $R = C_1/C_2$. In a recent paper we reported a multitechnique analysis of the ternary system $Na^+C^-(1)-Na^+DC^-(2)-W(0)$ at constant ratio $R = 1.00$ and at increasing C_{tot}.[9] It resulted that an increase of the total concentration in a range close to the cmc of the system implies a small variation of $(n + m)$, which corresponds to a small variation of the diffusivity of the mixed micelle but to a very large variation of the ratio n/m that represents the mixed micelle composition. This last variation implies a variation of the distribution of the two surfactants between the monomeric and micellized forms, which necessarily reflects a large variation of the diffusion coefficients.

From these considerations, it is then evident that in the previous equation it is impossible to consider the equilibrium parameters and the micelle diffusivity as independent of the system composition. This "de facto" prevents obtaining equations that describe the diffusive behavior of the ternary system under consideration. This conclusion can be generalized to any aqueous mixture of two surfactants. As a consequence, the prediction, even qualitative, of the dependence of the D_{ij} on the system composition is very difficult.

In this paper we approach this issue from a different viewpoint. Our goal is to rationalize the experimental trends of both main- and cross-term diffusion coefficients for the ternary system $Na^+C^-(1)-Na^+DC^-(2)-W(0)$ on the basis of the diffusive behavior of the two related binary system. With this aim, we performed several sets of experiments at constant concentration of one surfactant, $C_i = C_i^o$, and increasing concentration of the other, C_j. This procedure simplifies the ternary/binary systems correlation. As a first advantage, as it will be discussed in the next section, this approach allows to easily predict the behavior of the main diffusion coefficients, D_{ii} and D_{jj}, in the limiting conditions $C_j \rightarrow 0$ and $C_j \gg C_i^o$. This furnishes a sort of "guidelines" for data interpretation. To a certain extent, the same holds for the cross-term diffusion coefficients. This approach is absolutely general and could be applied, in our opinion, to interpret and qualitatively predict the diffusive behavior of any ternary system containing two surfactants that form mixed micelles.

■ RESULTS AND DISCUSSION

The binary parameters that will be used in interpreting the dependence of the ternary diffusion coefficients on the composition are collected in Table 2.[9] A quick comment on some of these parameters is convenient for the following discussion on the D_{ij}. First, $\ln K$ and cmc indicate that NaDC shows a larger tendency to form micelles in comparison to NaC; on this basis it is easy to predict that NaDC will also have a larger tendency, with respect to NaC, to form mixed aggregates.[34,35] Second, the diffusion coefficients for M(C) and M(DC) micelles are very similar; this allows guessing that the mixed micelles will have a diffusivity of the same order of magnitude independently of their composition.

It is also useful to report here the assumption that will be used in interpreting the trends of the main and cross diffusion coefficients on the solute concentrations. Regarding the main terms that take into account the overall motion of any component, we will consider that the experimental D_{ii} are determined by both the diffusion coefficient of the unimeric surfactant molecules and the diffusion coefficient of the mixed micelles where each surfactant is present in a certain fraction. Regarding the cross diffusion coefficients that take into account the correlation of the motion of the two surfactants, we will try to correlate them to the composition of the mixed micelle where the two surfactants are forced to move together.

Main Diffusion Coefficients. Taking advantage of the fact that all the sets of measurements are at $C_i = C_i^o$ and $C_j = $ variable, before starting to discuss the D_{ii} trends, we remember some necessary general limit characteristics of the main diffusion coefficients, as derived from nonequilibrium thermodynamics.[36] For the limit $C_j \rightarrow 0$ it can be shown that

$$\lim_{C_j \rightarrow 0} D_{ii}(C_i^o, C_j) = D_i(C_i^o) \qquad (12)$$

and by definition

$$\lim_{C_j \rightarrow 0} D_{jj}(C_i^o, C_j) = D_{jj}^\infty(C_i^o) \qquad (13)$$
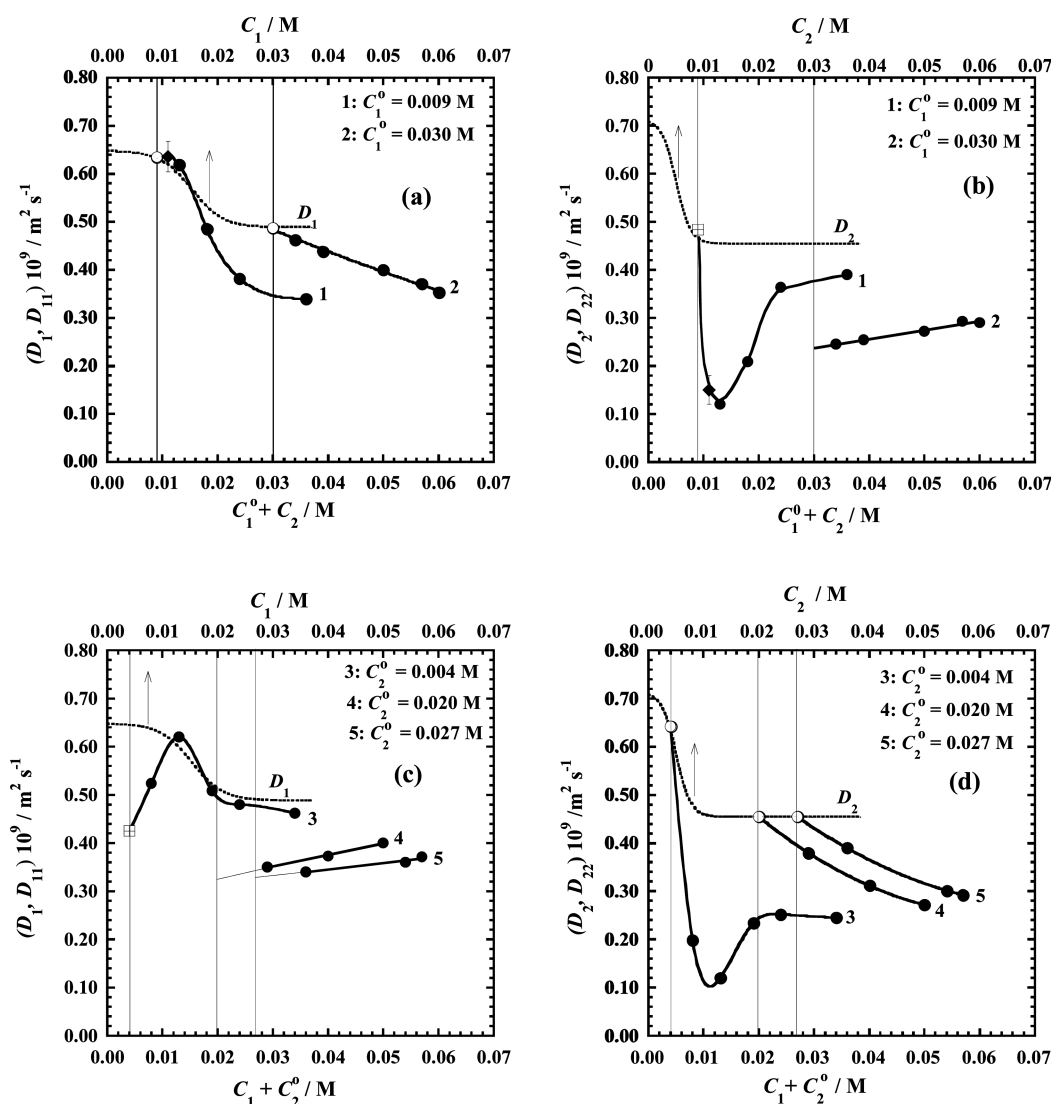
Figure 3. D_{11} (panel a and c) and D_{22} (panel b and d) main diffusion coefficients for the different sets at 25 °C: ●, Gouy experimental data; ◆, Taylor experimental data; ○, binary diffusion coefficient D_i at $C_i = C_i^o$; ⊞, limit values calculated by the Nernst−Hartley equations. Solid lines connecting the data are just guides to the eyes. The D_1 and D_2 trends of the binaries are also shown (dashed lines). Vertical lines represent the constant C_i^o values reported in the legends and correspond to the infinite dilution conditions for the jth component in the $(i, 0)$ mixture.

Equation 12 means that at infinite dilution of component j, the main diffusion coefficient of component i, D_{ii}, tends to the value of the corresponding binary, while eq 13 states that in the same conditions the main diffusion coefficient of component j tends to its tracer diffusion coefficient, D_{jj}^∞.

We can also predict the D_{jj} trend for $C_j \gg C_i^o$. In this condition the ternary system $(i, j, 0)$ approaches the binary one $(j, 0)$ with solute i in trace. As a consequence, the behavior of component j will approach its own behavior in the binary system. In other words for large value of the ratio C_j/C_i^o, $D_{jj}(C_i^o, C_j)$ tends to the value $D_j(C_j)$. In contrast, in the same experimental conditions, $D_{ii}(C_i^o, C_j)$ depends on the characteristic of the system, and it will be discussed below.

The experimental D_{ii} values relative to sets 1−5 are reported in Figure 3. In particular, in panels a and b D_{11} and D_{22} are reported for sets 1−2 as a function of $C_1^o + C_2$, while in panels c and d D_{11} and D_{22} are reported for sets 3−5 as a function of $C_1 + C_2^o$. In the same figure the binary trends of D_1 vs C_1, panels a and c, and of D_2 vs C_2, panels b and d, are also reported (dotted lines obtained as described elsewhere[9]). Indeed, the use of total

concentrations $C_i^o + C_j$ on abscissae allows a direct comparison between the data for ternary and corresponding binary systems: in fact, in Figure 3a and 3d, starting from the vertical solid line (corresponding to a binary system with concentration C_i^o), we compare what happens if one adds alternatively further component i (i.e., one increases C_i, upper scale, binary system) or component j (i.e., one increases $C_i^o + C_j$, lower scale, ternary system). Finally, lines passing throughout the experimental D_{ii} points are just a guide to the eye; therefore, the positions of the maxima or the minima are only indicative.

We start analyzing D_{ii} for set 1 and set 2. In panel a, the empty circles are the binary diffusion coefficients, D_1, at $C_1 = C_1^o$. By inspection of this panel, it appears that the condition expressed in eq 12 is fulfilled by the experimental data; in fact D_{11} data are very well connected to the curve of D_1 vs C_1. At finite C_2, the D_{11} trends are very different for the two sets of experiments. In fact for $C_1^o = 0.009$ mol L^{-1}, the D_{11} shows a steep decrease in a small concentration range with an S-shaped trend, while for $C_1^o = 0.030$ mol L^{-1} it decreases slowly with a linear trend. This different behavior is strictly connected with
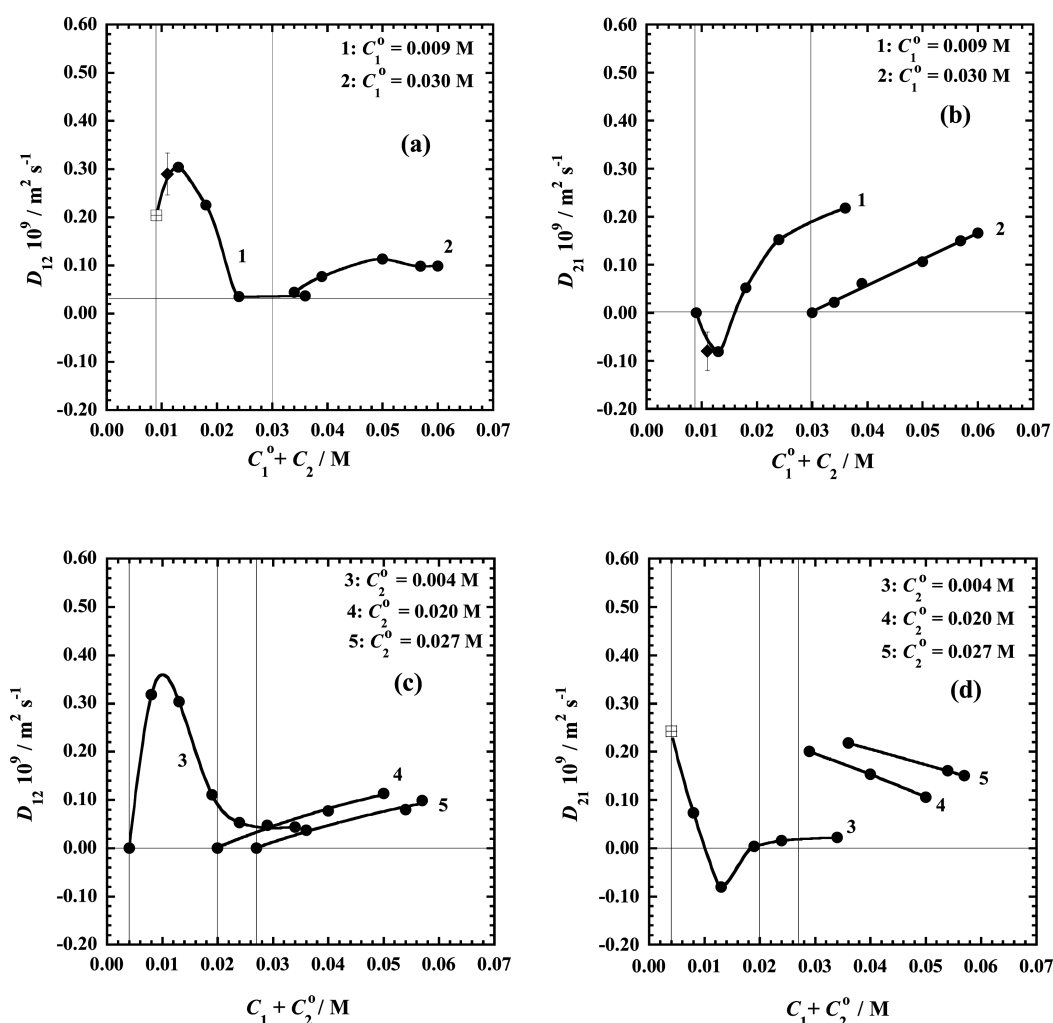

Figure 4. D_{12} (panels a and c) and D_{21} (panels b and d) cross diffusion coefficients for the different sets at 25 °C: ●, Gouy experimental data; ◆, Taylor experimental data; ⊞, limit values calculated by the Nernst−Hartley equations. Solid lines connecting the data are just guides to the eyes. Vertical lines represent the constant C_i^o values reported in the legends and correspond to the infinite dilution conditions for jth component in the $(i, 0)$ mixture.

the C_1^o values. For set 1 it results that $C_1^o < (cmc)_1$ so that component 2 is added to a solution where only unimers of component 1 are present; with increasing C_2 involvement of component 1 in mixed micelle formation causes a steep decrease of D_{11}. For set 2 it results that $C_1^o > (cmc)_1$, and component 2 is added to a solution where pure micelles of component 1, in equilibrium with their free unimers, are already present. As a consequence, the addition of component 2 only causes an increase of the number of micelles in solution and a change of their composition. The continuous decrease of D_{11} with increasing C_2 can be explained as follows. In general, with increasing C_j at constant C_i, component i is increasingly involved in the formation of mixed micelles, with the consequence that i unimers participate in a negligible way to the transport of component i. This implies that D_{ii} will tend to the diffusion coefficient of aggregates whose composition is approaching that of pure j. In other words, the following relation must hold:

$$D_{ii}(C_i^o, C_j) = D_{M(j)} \quad C_j \gg C_i \tag{14}$$

This condition explains the decreasing experimental trend of D_{11} in Figure 3, panel a.

Panel b of the same figure shows D_{22} as a function of $C_1^o + C_2$. In the case of set 1 ($C_1^o < (cmc)_1$), the limit value, $D_{jj}^\infty(C_i^o)$, eq 13, can be obtained as limit of the Nernst−Hartley expression valid for the mixture of two electrolytes with a common ion:

$$\lim_{C_2 \to 0} D_{22}(C_1^o, C_2) = D_{22}^\infty(C_1^o) = D_{DC} \tag{15}$$

In eq 15, D_{DC} represents the diffusivity of the free deoxycholate ion. The last equality is valid because, for $C_2 \to 0$, Na$^+$ concentration ($C_{Na} = C_1^o + C_2$) is very large in comparison with that of deoxycholate C_2, and this prevents the formation of any Na$^+$ gradient able to drag the heavy deoxycholate ion. As a consequence, the motion of component 2 is exclusively determined by the diffusivity of the deoxycholate ion, D_{DC}. The predicted limiting value is reported in panel b as a cross in square. It is important to note that this value does not belong to the curve D_2 vs C_2 as indeed it could appear.

In the case of set 2 ($C_1^o > (cmc)_1$), even though no theoretical motivation can be invoked, it appears reasonable to linearly extrapolate, for $C_2 \to 0$, the experimental D_{22} data to a finite value. A finite value of the limit simply means that, in the presence of mixed micelles, each component even at extremely

low concentration is compelled to distribute itself between the bulk as monomer and the micelles. The same happens for the distribution between two different phases for which a defined partition coefficient can be estimated. The finite value of this partition coefficient is directly reflected in the finite value of the limit D_{22}.

At finite C_2 concentration the D_{22} data relative to set 1 show a very steep decrease followed by a deep minimum. The observed drop-off is indicative of the formation of mixed micelles while the value of the minimum ($D_{22} \cong 0.1 \cdot 10^{-9}$ m^2 s^{-1}), very similar to that of micellar aggregates (see Table 2), indicates that a very high fraction of the more hydrophobic deoxycholate molecules is initially involved in the micelles formation. The further deoxycholate addition imposes a redistribution of component 2 between the unimeric and micellar form and a consequent increase of the experimental diffusion coefficient.

The linearity of D_{22} in set 2, $C_1^o > (\text{cmc})_1$, can be explained as done for the analogous D_{11} trend in panel a. Finally, for both sets D_{22} shows an ascending trend. In the case of set 1, the trend seems compatible with the prediction that for large C_2/C_1^o the $D_{22}(C_1^o, C_2)$ has to approach the value $D_2(C_2)$, as discussed at the beginning of this subsection. For Set 2, D_{22} also increases, as expected. However, the tendency to approach the binary diffusion coefficient cannot be verified in the considered concentration range because of the higher value of C_1^o.

The trends of the D_{ii} data at constant deoxycholate composition C_2^o and varying C_1, reported in panels c and d of Figure 3, allow an internal test of our interpretative approach. In the case of sets 4 and 5, D_{11} shows the same behavior of D_{22} for set 2. Conversely, the trend of D_{22} in panel d corresponds to that of D_{11} in panel a. Thus, the same interpretation holds.

The data relative to set 3 ($C_2^o \simeq (\text{cmc})_2$) deserve some further considerations. For both D_{11} and D_{22} the behavior for $C_1 \gg C_2^o$ is similar to that observed for set 1. Also, the limiting values predicted for $C_1 \to 0$ connect very well with the trends of the experimental data. However, substantial differences exist in the trends at low C_1. In this case the data can be analyzed assuming that almost no pure micelles are present in the binary deoxycholate−water at $C_2^o = 0.004$ mol L^{-1} and that micelles are formed upon the addition of component 1. For D_{22} the initial minimum can be explained in terms of mixed micelle formation, analogously to that observed in panel b; also in this case the minimum value, $D_{22} \cong 0.1 \cdot 10^{-9}$ m^2 s^{-1}, is similar to the diffusion coefficient expected for the micellar aggregate, indicating that the fraction of component 2 present in the micellar form is very high in comparison with the fraction of unimers in solution.

Regarding D_{11}, its limit value can be obtained by the application of the Nernst−Hartley equation:

$$\lim_{C_1 \to 0} D_{11}(C_1, C_2^o) = D_{11}^\infty(C_2^o) = D_C \tag{16}$$

The limit D_{11}^∞ value coincides with the diffusivity of cholate anion because of the absence of dragging effect of the Na$^+$ ions in very dilute solutions of component 1. The successive steep and positive increase of D_{11} can be explained considering that, at increasing C_1, similar values are assumed by C_{Na} and C_C, so that the Na$^+$ dragging effect becomes effective. This increase is an indirect confirmation that the fraction of cholate anion participating in the micellar aggregates is relatively low. As a consequence, $D_{11}(C_1, C_2^o)$ approaches the value $D_1(C_1)$. For a further increase of C_1, the micellized fraction of cholate anion increases and $D_{11}(C_1, C_2^o)$ starts to decrease.

Cross Diffusion Coefficients. The experimental D_{ij} values relative to sets 1−5, representative of a correlation between the motion of two solutes, are reported in Figure 4. In particular in panels a and b are reported D_{12} and D_{21} for sets 1−2 as a function of $C_1^o + C_2$, while in panels c and d are reported D_{12} and D_{21} for sets 3−5 as a function of $C_1 + C_2^o$. In these graphs, we have also reported the predicted limiting values. Concerning panels b and c one has to take into account that the following condition holds:

$$\lim_{C_j \to 0} D_{ji}(C_i^o, C_j) = 0 \tag{17}$$

The null limit in eq 17 can be explained as follows: at infinite dilution of component j its flow J_j must tend to zero, and according to eqs 7 and 8 this implies that the product $D_{ji} \cdot \nabla C_i$ must be null too. Due to the absence of any restriction on the ∇C_i values, this condition is fulfilled only if $D_{ji} \to 0$.

Concerning panels a and d one has to consider that, by definition

$$\lim_{C_j \to 0} D_{ij}(C_i^o, C_j) = D_{ij}^\infty(C_i^o) \tag{18}$$

In eq 18, D_{ij}^∞ represents the tracer diffusion coefficient of component i under the concentration gradient of component j. If $C_i^o \leq (\text{cmc})_i$, the limits in eq 18 can be computed by the limiting Nernst−Hartley equations. Thus for set 1 and set 3 we have

$$\lim_{C_2 \to 0} D_{12}(C_1^o, C_2) = \frac{D_C}{D_C + D_{Na}}(D_{Na} - D_{DC}) \tag{19}$$

$$\lim_{C_1 \to 0} D_{21}(C_1, C_2^o) = \frac{D_{DC}}{D_{DC} + D_{Na}}(D_{Na} - D_C) \tag{20}$$

respectively. For the other sets the $D_{ij}^\infty(C_i^o)$ must be obtained by extrapolation of the experimental trends.

At finite concentration, the interpretation of D_{ij} is quite complex. In fact, because of the presence of the micellization equilibrium, any initial gradient of surfactant i creates a gradient of the micelles in the same direction and a gradient of the unimers of the other surfactant j in the opposite one. This is the reason why any cross diffusion coefficient is given by the sum of at least two opposite contributions that, moreover, are difficult to quantify individually. For this reason it is not possible to simply correlate the cross diffusion coefficients to the amount and composition of the mixed micelles in which the two surfactants are constrained to move together. Nevertheless, taking advantage of the fact that our measurement sets have been carried on at constant concentration of one component, it is possible to give an interpretation of D_{ij} trends for $C_j \gg C_i^o$. As said above, the large excess of one surfactant over the other not only imposes its prevalence into the mixed micelles but also compels the less abundant surfactant preferentially to participate with the aggregates. In this condition it is possible to assume that both the gradients of the unimeric surfactants are close to zero. The consequence is that the D_{ij} values exclusively depend on the flow's correlation due to the motion of the mixed micelles. In this case, for $C_2 \gg C_1^o$, in a single micelle a large amount of surfactant 2 moves a small and decreasing amount of surfactant 1, so that small and decreasing values of D_{12} are expected. Conversely, in the same mixed micelle, a small and decreasing amount of surfactant 1 moves a

large and increasing amount of surfactant 2; this should imply large and increasing values of D_{21}. The same argument can be used to predict, for $C_1 \gg C_2^o$, an increasing trend of D_{12} and a decreasing trend of D_{21}. Despite the rough approximation used in describing the system, these predictions are clearly confirmed by the experimental trends reported in Figure 4.

We would like to conclude this subsection with some general considerations on the cross-term diffusion coefficients in multicomponent systems. Usually, in modeling processes occurring in biological and technological complex systems, the impact of these coefficients on the kinetics is neglected. This could cause errors that often lead to a meaningless interpretation of the molecular processes. In Figure 5 the D_{ij}
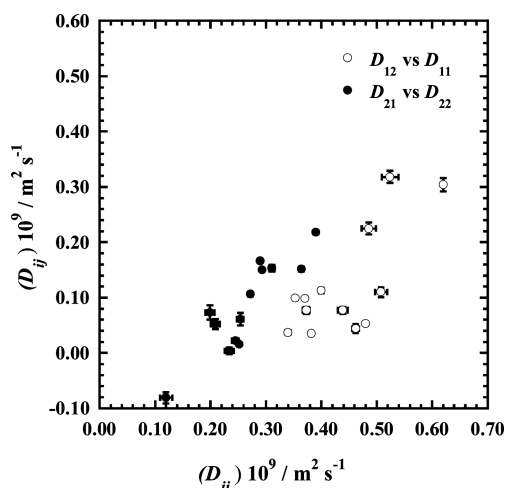

Figure 5. Cross-term diffusion coefficients D_{ij} vs the corresponding main-term diffusion coefficients, D_{ii}.

determined in this work are reported as a function of the corresponding D_{ii}; it is to point out that the cross terms can reach values of about 50% of the corresponding main ones. Thus, the cross diffusion coefficients are necessary to correctly describe the system behavior.

Bile Salt Diffusion Coefficients and Lipid Absorption Process. The comprehension of the diffusive properties of bile salt mixtures furnishes a robust basis for a reliable modeling of the physiological processes to which they participate. NaC and NaDC are two of the most abundant bile components, concurring to the formation of bile salt aggregates present in this complex fluid.[7] The bile salt mixed micelles play an important role in fats digestion.[37,38] The main steps of this process are the following: (i) the degradation of lipids (fats), by action of pancreatic lipase, into fatty acids and monoglycerides, (ii) the solubilization of fatty acids in the mixed micelles formed by the bile salts secreted by the liver and the gall bladder, (iii) transport of the fatty acids to the small intestine where they are absorbed by enterocytes with a mechanism of "passive" diffusion. The composition of bile salt mixed micelles depends on the amount of the various bile components. Assuming for simplicity a prevalence of NaC and NaDC, the formation of mixed micelles can be described as the following:

$$n\mathrm{C}^- + m\mathrm{DC}^- + z\mathrm{Na}^+ \rightleftarrows \mathrm{M}^{-(n+m-z)}$$
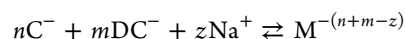
The upload of the degraded lipids in the mixed micelle can be described by the following reaction:

$$\mathrm{M}^{-(n+m-z)} + l\mathrm{Lip} \rightarrow \mathrm{M(Lip)}_l^{-(n+m-z)}$$
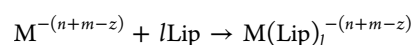
We implicitly suppose that the loading of the mixed micelle does not change its composition parameters (n, m, z). This assumption can be considered reasonable for a low uploading of micellar aggregates.[34,39−41] The uptake of the lipids by the small intestine enterocytes, which happens in a thin layer close to their membrane, can be described as the following:

$$\mathrm{M(Lip)}_l^{-(n+m-z)} + \text{membrane}$$
$$\rightarrow \mathrm{M}^{-(n+m-z)} + [\text{membrane} + l\mathrm{Lip}]$$
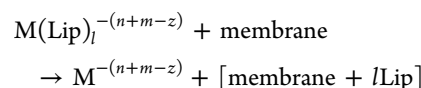
The system constituted by the intestinal liquid and the intestinal membrane is schematized in Figure 6 where the
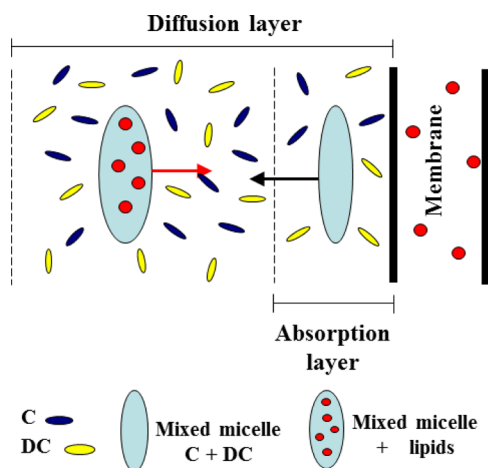

Figure 6. Schematic representation of the fatty acids transport in the intestinal lumen and their uptake operated by the small intestine membrane.

diffusion and the absorption layers are also indicated. Starting with a system with homogeneous concentration, because of the uptake of the lipid molecules by the membrane there will be a concentration gradient of the "empty" mixed micelle, $\mathrm{M}^{-(n+m-z)}$, and of all its constituents, C^-, DC^-, and Na^+, from the bulk of the system to the membrane and a concentration gradient of the "loaded" mixed micelles in the opposite direction. The flows are obviously in the opposite direction with respect to the concentration gradients. For simplicity in Figure 6 only the flows of the two aggregates $\mathrm{M(Lip)}_l^{-(n+m-z)}$ and $\mathrm{M}^{-(n+m-z)}$ are indicated while the flows of the simple molecules are omitted.

According to this model, the species C^-, DC^-, Na^+, $\mathrm{M}^{-(n+m-z)}$, and $\mathrm{M(Lip)}_l^{-(n+m-z)}$ are present and diffuse in solution; the concentrations of the first four are defined by the micellization parameters while the ratio $C_{\mathrm{M(Lip)}l}/C_\mathrm{M}$ is defined by the initial amount of lipids. The species $\mathrm{M}^{-(n+m-z)}$ and $\mathrm{M(Lip)}_l^{-(n+m-z)}$ differ for their hydrodynamic volume which is presumably larger for the uploaded mixed micelle. The difference in volume, which in turn depends on the number of lipids carried by the mixed micelles, results in a different value of the diffusivity of the two species. If l is not very large the two micelles will have similar mobilities. At the end of this analysis, one realizes that on the basis of the proposed model, the diffusion of the species involved in the fats' digestion process can be reasonably evaluated by the diffusivity of the same species that define the four diffusion coefficients of the system NaC−NaDC−water. This is equivalent to using the four diffusion coefficients experimentally determined for the system NaC−NaDC−water in evaluating the time evolution of

the concentrations of the different species involved in the model.

CONCLUSIONS

For aqueous ternary systems containing two surfactants that form mixed micelles, the interpretation of the experimental diffusion coefficients is a very difficult task. Moreover, no predictive equations for the four coefficients can be obtained. In this paper the D_{ij} for the system NaC (1)−NaDC (2)−water (0) have been determined for different measurement sets at constant concentration of one component and variable concentration of the other one. Following this approach, it has been found that the system diffusive behavior can be easily related to that of the two binary systems NaC (1)−water (0) and NaDC (2)−water (0). By using general argumentations we succeeded to obtain an excellent qualitative explanation of the experimental trends of the main-term diffusion coefficients as a function of C_{tot}. The trends of the cross-term diffusion at large total concentration, which is the most relevant condition in modeling biological and technological processes, were also correctly predicted. We are confident that this kind of analysis can be extended to similar systems, and this is, in our opinion, the preeminent value of this paper.

ASSOCIATED CONTENT

Ⓢ Supporting Information

Diffusion coefficients measured for the ternary system sodium cholate−sodium deoxycholate−water. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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