

## REVIEW ARTICLE

## Computational Modeling and Experimental Facts of Mixed Self-Assembly Systems

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**Abstract:** The formation of liposomes, nanoparticle micelles, and related systems by mixtures of drugs and/or surfactants is of major relevance for the design of drug delivery systems. We can design new systems using different compounds. Traditionally these systems are created by trial and error using experimental data. However, in most cases measuring all the possible combinations represents an extensive work and almost always unaffordable. In this sense, we can use theoretical concepts and develop computational models to predict different physicochemical properties of self-aggregation processes of mixed molecular systems. In a previous work, we developed a new PT-LFER model (Linear Free Energy Relationships, LFER, combined with Perturbation Theory, PT, ideas) for binary systems. The best PT-LFER model found predicted the effects of 25000 perturbations over nine different properties of binary systems. The present work has two parts. Firstly, we carry out an analysis on the new results on the applications and experimental-theoretical studies of binary self-assembled systems. In the second part, we report for the first time, a new experimental-theoretic study of the NaDC-DTAB binary system. For this purpose, we have combined experimental procedures plus physicochemical thermodynamic framework with the PT-LFER model reported in our previous work.



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## INTRODUCTION

The interaction between different types of amphiphilic molecules in solution has long received great interest from the experimental sciences. Recently, physical chemists have devoted particular attention to these systems in a phenomenological way to get accurate information on the forces responsible for molecular interactions; this is also expected to increase the efficiency and number of uses of these systems particularly when amphiphilic drugs are involved [1]. For example, due to the impediments that nucleic acids have to cross cell membranes, the efficiency of gene therapy depends greatly on the use of vectors which could deliver genetic material into a target cell with minimum toxicity. However, the current development of both molecular medicine and biotechnology has opened up endless possibilities as can be seen in the light of the most recent studies. Thus, Nakamura *et al.* [2], reported on the controlled clustering of oppositely charged colloidal particles by the adsorption of ionic surfactants, modifying charge numbers  $Z$  of particles. They studied the heteroclustering of submicron-sized polystyrene (PS) and silica particles, both systems present negative charge, in the presence of cetylpyridinium chloride (CPC), a cationic amphiphile molecule. The surfactant concentration  $[C]$  was selected below the critical micelle concentration. When the surfactant molecules reach the interfaces, the charge of the PS and silica particles decreased, inverting to positive when  $[C]$  exceeded the

isoelectric point. The isoelectric point of hydrophobic PS particles is much lower than the hydrophilic silica particles. At  $[C]$  values ranging between isoelectric points, the particles have opposite charge, and clustering was enabled. To explain the clustering behavior, adsorption isotherms of the CPC and screened-Coulomb-type pair potential were analyzed. Expected applications of these results could be the control of colloidal stability and the construction of various particle types into heterogeneous colloidal clusters. Liang *et al.* [3], studied PAH mixtures on the Surfactant-enhanced remediation [4] context. SER has been extensively used in decontaminating PAH-polluted soil. Several authors have concentrated on the evaluation of the washing efficiency without considering the mutual interaction of pollutants. In their work, the authors studied the effect of cosolubilization between phenanthrene (Phe) and pyrene (Pyr) in the presence of nonionic surfactant Triton X-100 (TX100) on their codesorption performance from the soil. Cosolubilization experiments reveal that, when cosolubilized, solubility of Phe and Pyr in TX100 grows in 15.38% and 18.19%, respectively, values obtained from the deviation ratio of the molar solubilization in single and binary solute solubilization phases. Such potentiation arises from the volume expansion of the micelles caused by the solubilization of PAHs at the interfaces of the micelles. The cosolubilization effect was further observed in the soil washing process. The stronger solubilization ability of TX100 towards Phe and Pyr could increase the two PAHs' codesorption efficiency from soil, matching by synergistic extent of 6-15%. However, synergism in codesorption was feeble than that observed in the cosolubilization system, this fact was associated with losing surfactant from soil and PAH partition into soil organic matter and adsorbed surfactants. Even

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liquid crystals have also been studied from the point of view of mixed systems. In addition to its strong presence in information-display technology, liquid crystalline systems hold a great promise in drug solubilization and delivery. For this purpose, a mixture composed of 2,3-dihydroxypropyl oleate, medium-chain triglycerides and citrem was proposed [5]. The role of citrem is to stabilize the structure of the liquid crystal formed by the other two compounds forming a suitable platform for drug solubilization although small changes in the composition of the liquid crystal phase may change the immunological outcomes.

The use of surfactants in the remediation of contaminated areas has an important tradition. Within this field the recovery of agricultural areas devastated by the use of pesticides becomes more important with every passing day. In a very interesting document a group of researchers studied the solubilization of three different herbicides in pure and mixed different surfactant systems. Surprisingly, the use of mixed surfactant systems did not improve the solubilization of herbicides over those of the single components. Previous studies have linked increased solubilization of organic molecules in mixed micelles with the degree of attractive interactions between surfactants; however, this was not observed in the current study and the authors related this fact with the attractive interactions found in all of the binary mixtures [6]. Related with this topic, Zhao *et al.* [7], proposed another system in order to improve soil retention for phenanthrene (PHE). In this case, the vector proposed is a combination of cationic gemini (12-2-12) and nonionic surfactants (C12E10). The use of gemini surfactants is motivated by their important advantages over regular ones: lower micromolar cmc, greater surface activity, slow monomer-micelle kinetics and interesting viscoelastic properties. The sorption magnitude of the mixed system for PHE exceeded the pure surfactant. This was attributed to the formation of the most compact arrangement of surfactants in the aggregates placed onto the solid/liquid interface. The sorption capacities of surfactant mixture for PHE were greater than the summed individual results. Such enhancement was attributed to the synergetic effect of cationic gemini and nonionic surfactant on the retention of PHE.

The increase of resistant bacteria is a major public health concern. So, developing effective new antibacterials is an important social request. Cationic surfactants have been used as antimicrobials long time ago. Marafino *et al.* [8] present a very remarkable job for several reasons. Firstly, the authors design, synthesize (triscationic, triple-headed, double-tailed amphiphiles) and characterize the compounds. Then, they study the physicochemical properties of both pure compounds and the mixtures (critical micellar concentration, thermodynamic parameters and minimum inhibitory concentration against six bacterial strains). Finally, they include combination studies, checkerboard technique, to determine if mixtures present synergism to kill *E. coli* or *S. aureus*, a first attempt to obtain structure–activity relationships. Both the designed compound and the method offer promise as future antibacterial agents in a wide variety of applications. Oliver *et al.* [9], also suggest that properties such as shape, dimension, and electric charge can be systematically and predictably tuned. Combinations of commonly used surfactants were studied by SAXS and ITC and the conclusions seem to be quite clear: the geometrical and physicochemical properties of the mixed aggregates can be expressed as a linear combination of the respective properties of the pure compounds.

The view is the most important sense. In different cultures around the world, vocabulary related to this sense represents 60% of the vocabulary referred to the senses. It is clear that we are very visual creatures, not only because the visual processing requires a large percentage of the brain but also the images capture our attention much more easily. Science has not ignored this phenomenon and in the recent years we have witnessed an incredible growth in technical and related applications. A glance at any scientific publication will corroborate this fact. Electron microscopy holds the lead

role and mixed systems have not been left out. Thus, Fox *et al.* [10], studied the structure silica nanoparticles in coated with a mixture of poly(acrylic acid)/polystyrene in two different solvents by using electron microscopy techniques: Cryo-EM and Cryo-ET. The obtained images were analyzed using the UCSF Chimera and IMOD software which allow visualization, image processing, modeling and analysis of molecular structures. The results showed that in the organic solvent, the mixed layer was highly solvated and the thickness was around 27 nm (similar to the extended length of the polymer). The layer exhibit two separate microdomains: the PS-rich one with a truncated cone shape and the PAA-rich one grafted on the silica. When water is the solvent, the thickness of the layer reduces to 18 nm, Ps microdomains became disordered tethered micelles within the swollen PAA layer. On the whole, this paper highlights the advantages of the digital images and the software packages.

Phase transitions profoundly affect the properties of the systems. Consequently, it is essential to determine their state. Although initially these studies were rare due to the difficulty in the experimental characterization, development and better access to more robust techniques have recently enabled their characterization. As an example Plazzotta *et al.* [11], studied the effect of temperature on mixed micelles as a function of temperature. The study is performed in two steps; the first one is focused on the phase diagram of the surfactant in the micellar core. After that, the influence of such changes in the mixed system is described. This study could be performed by a combination of small angle X-ray scattering (SAXS), microcalorimetry (DSC) and nuclear magnetic resonance (NMR). In conclusion, it should be noted that the phase transition is not annulled by adding another surfactant, simply, in some cases, a displacement of the lower critical temperature values was observed. Following with analogous examples; without a combination of very robust experimental techniques it would not have been possible to make such a complex study like that one carried by Klee *et al.* [12]. They determined the phase behavior and range of compositions of mixtures of ionic surfactants and ionic liquids at different temperatures. The techniques employed included: differential scanning calorimetry, polarized microscopy, surface tension, small angle neutron scattering and small angle X-ray scattering. The binary phase diagrams were constructed from DSC and polarised microscopy measurements showing textures for homogeneous phase, lamellar structures cubic or hexagonal phases as concentration is increased. From SANS measurements and using different theoretical models, authors were able to get the aggregation number revealing that the aggregation numbers are higher in C2mimFeCl<sub>4</sub> compared with those where C4mimFeCl<sub>4</sub> was used as solvent. Finally, authors were able to distinguish between the effects of the solvophobic and solvophilic parts of the molecules, showing that faults in the ability to self-assemble arise because of the surfactant's lipophobic tail.

## COMPUTATIONAL STUDIES OF THE PHYSICO-CHEMICAL PROPERTIES OF BINARY SYSTEMS

The mixtures of a huge amount of surfactant types, nonionic/nonionic anionic/nonionic, cationic/nonionic, anionic/biosurfactant, anionic/anionic, cationic/cationic, and cationic/ anionic have been studied and the theories of Clint [13], Motomura [14], Rosen [15] Rubingh [1] and Blankshtein [16] have been used to analyze and compare the experimental results in order to understand the synergism and antagonism of the binary combinations. In addition to this, a theoretical approach of regular solution and molecular thermodynamic theory [17, 18] (considering the contributions of the hydrophilic head and hydrophobic tail structures of the amphiphiles) has been applied on ternary mixtures of amphiphiles to predict the cmc and other micellar parameters on the basis of solution composition [19].

To the best of our knowledge the first attempt to describe quantitatively the self-assembly of mixed system was performed by

Blankschtein and co-workers [20] [21]. They used a molecular-thermodynamic framework to describe micellization, phase separation and thermodynamics. From an evaluation of the Gibbs free energy modeled as the sum of different contributions (the transfer of the surfactant hydrophobic tails from an aqueous to an oil-like environment, the formation of an interface, additional entropic constraints associated with anchoring one surfactant at the interface, the formation of the micelle interfacial region, steric interactions between the surfactant heads and counterions, electrostatic interactions at the micelle interface and entropy of mixing) they were able to predict qualitative trends of micellar and phase behavior as a function of surfactant molecular structure and the solution conditions. As a logic continuation of their work they have developed different user-friendly computer programs named PREDICT, MIX2, MIXn, SURF, and DYNAMIC.

Another example of combination of computational and experimental methods was proposed by Hassan et al [22]. The focused on cationic systems (mixtures of oppositely charged surfactants). The experimental side made use of common techniques such as density and ultrasound velocities, electrical conductivities, transmission electron microscopy and dynamic light scattering. On the other hand, the computer simulation method used was molecular dynamics (MD). The force field used was GROMACS96 and The Extended Simple Point Charge model was employed for water molecules. Three dimensional periodic boundary conditions with cubic boxes were used for all the trajectories. The pressure was isotropically controlled by using a Berendsen barostat. Long range electrostatic interactions were calculated using the Particle Mesh Ewald method. Random initial velocities were assigned from a Maxwell-Boltzmann distribution at 298 K. The equations of motion were integrated using the leapfrog method with a time step of 2 fs. Bond lengths and angles in water were constrained using the SETTLE algorithm, while the LINCS algorithm was used to constrain bond lengths within the surfactant molecules. During the MD simulations, coordinates, velocities, and energies were stored every 10 ps for further analysis. Thus, they could observe that a variety of structures of different size coexist in solution with vesicles of ~160 nm diameter. Interestingly, the nanostructures were observed to self-assemble in a time scale easily accessible by atomistic classical molecular dynamics simulations, allowing to provide a comprehensive structural and dynamic characterization of the surfactant molecules at atomic level in the different aggregates.

Jórárt *et al.* [4], combined experimental studies and Molecular Dynamics (MD) to study the system formed by sodium dodecyl sulphate and sodium cholate, two molecules with different architectures. The experimental part was covered with spectrofluorimetric, surface tension and electrical measurements. For the computational part, the Martini coarse-grained method was chosen. The use of coarse grained models simulation techniques has proven to be a valuable tool to probe the time and length scales of systems beyond what is feasible with traditional atomistic models. The equilibrium is reached after 0.60  $\mu$ s. The size distribution shows a trimodal distribution. The greatest aggregate (around 41 molecules) is the dominant. It consists of 67% of SDS. The dynamics of the cluster formation was described as follows: initially SDS molecules create a stable cluster. Then sodium cholate molecules interact with the SDS cluster increasing the size of the aggregate and decreasing the number of SDS molecules.

The mixed polymer systems have also received attention. Prashanna *et al.* [23], studied co-micellization behavior for binary mixtures of Poloxamers in dilute aqueous solution. The available techniques to predict properties of mixed systems of surfactants range from Monte Carlo, self-consistent field theory, dynamic density functional theory and so on. However, a problem with these techniques is that they all describe polymers confined to lattice conformations, and are not very well suited to describe branched

polymers. DPD resembles Molecular Dynamics (MD) in that the particles move according to Newton's laws, but in DPD the inter-particle interactions allow larger time steps. Here, the interparticle force exerted between two particles is a combination of conservative, dissipative, and random forces. With suitable interaction parameters, the computational method allowed them to determine the structure of the aggregates: type, size, shape, and composition. It was found that different types of aggregates coexist at the same time: pure aggregates and mixed ellipsoidal micelles. For all cases under study, if the two polymers had similar concentrations, the proportion of mixed micelles could be increased. Also, it was shown that shorter chains prefer to straddle the core and corona in the region of ellipsoidal interface that is closer to the center of mixed micelle.

In addition, Extra-thermodynamic approaches close related to Linear Free Energy Relationships (LFER) have been successfully used in Chemoinformatics [24, 25]. The designation as LFER equations comes from the use of parameters depending on the Gibbs free energy [26] of the  $i^{\text{th}}$  process.[26] The changes on the values of this potential during a process obey a logarithmic statistical thermodynamic relationship with equilibrium constants  $K_i$ . [27]

Gonzalez-Díaz *et al.*, [28] formulated a general-purpose Perturbation Theory (PT) method for multiple-boundary Chemoinformatics problems. In our opinion, Perturbation Theory (PT) ideas could be enforced to address this issue on the context of Chemoinformatics applied to study complex molecular systems. To a large extent, sense perturbational methods start with a known exact solution of a problem and continue adding "small" terms to the mathematical description in order to approach a solution to a related problem without knowing the exact solution [29-31]. PT-LFER models offer important advantages for studies involving self-aggregation and physicochemical properties of binary systems and other mixtures of surfactants and/or drugs in solution which are of both theoretical and practical importance. However, this task becomes more difficult once we want to predict simultaneously the effect over multiple output properties of binary systems of perturbations in multiple input experimental conditions ( $b_j$ ). In addition, in a mixture of a drug and a surfactant we can clearly distinguish the role of each component. However, in surfactant – surfactant binary systems both molecules play undistinguishable roles. It could be possible to study each system experimentally but the number of possible combinations of output properties, measured in different sub-sets of conditions, for many possible mixtures will determine a very huge number of experimental outcomes to be calculated. As a consequence, we need computational chemistry or chemoinformatics models that may help us to predict different properties of these classes of mixtures in multiple conditions. In a previous work we reported a PT-LFER able to predict multiple output properties of the system obtained after changes on the chemical structure of the two components of the system and their respective contra-ions, if any, as well as perturbations on other experimental boundary conditions.

$${}^0f(\epsilon_{ij})_{\text{new}} = -0.275152 \cdot f(\epsilon_{ij})_{\text{ref}} - 0.158186 \cdot \Gamma_{\text{pj}}(\text{dip})^0 + 0.037112 \cdot \Gamma_{\text{pj}}(\text{solv})^0 \quad (1)$$

$$+ 0.017595 \cdot \Gamma_{\text{pj}}(\text{part})^0 - 0.150110 \cdot \Gamma_{\text{pj}}(\text{solv})^0 + 0.095564 \cdot \Gamma_{\text{pj}}(\text{solv})^0 + 0.181375$$

$$N = 25000 \quad R = 0.93 \quad F = 25840.0 \quad p < 0.001 \quad SEE = 0.01595$$

Where, the output function  ${}^0f(\epsilon_{ij})_{\text{new}}$  is a multi-output function that quantify the numerical values ( $\epsilon$ ) of different p-th physicochemical properties of the i-th binary system that have been experimentally determined under a certain set of j-th boundary conditions ( $c_j$ ). The statistical parameters used were the Regression coefficient of training series (R), the Fisher ratio (Sn), the probability of error or p-level (p), and the Regression coefficient of leave-one-out validation ( $q^2$ ). This new model predicted (with R = 0.93) the effects over 12 different experimental properties of thousands of changes or perturbations (N = 25000) in initial conditions ( $b_j$ ).

## EXPERIMENTAL-THEORETICAL STUDY OF THE NaDC-DTAB SYSTEM

Nowadays, the main gene transfer routes are focused on the use of viral vectors which exhibit better gene transfer efficiency but are immunogenic and potentially mutagenic. To circumvent these limitations, substantial efforts have been made in developing nonviral synthetic vectors like cationic liposomes or mixed micelles where some of their components play a certain role in living organisms [32-34]. Bile acids and their salts are one of the most evident examples of surfactants with biological activity[35]. Bile salts are the final products of the hepatic biotransformation of cholesterol and perform main roles in the gastrointestinal absorption of lipo-soluble compounds and in their deliver through the aqueous domains of humans. These amphiphilic molecules can be found in bile as mixed aggregates that serve to deliver additional cholesterol from the liver into the intestine [36]. Although their best known role is their process in the digestive function and absorption of fat, they also are present in other important physiological processes [37]. Sodium deoxycholate (NaDC) is a bile salt, soluble in water, very frequently used in applications ranging from cell lysis, vesicles preparation and insulation of transmembrane proteins. Owing to their biological compatibility and fascinating aggregation behavior, research on bile salts and their interactions with lipids, cyclodextrins or other additives have been explored in recent years [19].

From the points discussed above, it is clear that subtle changes in compounds, ratio and environment conditions can lead to striking changes in the parameters and morphology of the aggregates. In order to shed light over the mechanism of formation and the ability to tune these intriguing structures at will, we present herein a experimental/computational study of the association behavior and corresponding properties of the mixed amphiphilic system sodium deoxycholate (NaDC)-dodecyltrimethylammonium bromide (DTAB). In addition to its scientific charm, we believe that once the underpinning factors and mechanisms are fully understood, the phenomenon can be harnessed for potential applications at an early stage in fabrication of highly efficient drug vectors, templates for biosensor devices, and so forth.

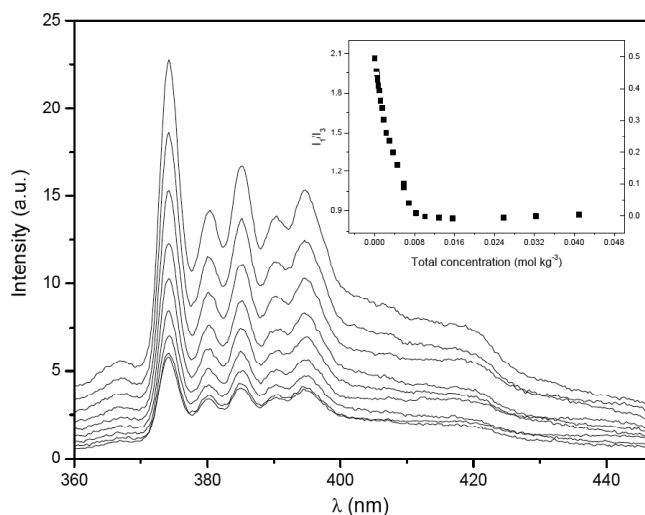
The system studied here is a binary mixture of dihydroxy bile salt sodium deoxycholate (NaDC) and dodecyltrimethylammonium bromide (DTAB). NaDC of purity  $\geq 97\%$  and DTAB of purity  $\geq 98\%$  were purchased from Sigma Aldrich and used as received. Pyrene (Sigma-Aldrich, 99%) was used as fluorescence probe.

Stock NaDC and DTAB solutions ( $0.1 \text{ mol dm}^{-3}$ ) were prepared and diluted as required for each experiment. The appropriate amounts of NaDC and DTAB stock solutions were mixed to obtain the different NaDC-DTAB mixture solutions. All surfactants and fluorescence probe solution were prepared using double-distilled water. Fluorescence spectra of pyrene were obtained with a Cary Eclipse spectrophotometer equipped with a temperature control device and a multicell sample holder (Varian Instruments Inc.). All samples were prepared with saturated solutions of pyrene ( $3 \times 10^{-7} \text{ mol dm}^{-3}$ ). Measurements were performed at 298 K and the fluorescence intensities ratios ( $I_1/I_3$ ) of the first ( $I_1$ , 373 nm) and third ( $I_3$ , 384 nm) peaks from the short wavelength in the spectra of pyrene were obtained with excitation at  $\lambda = 335 \text{ nm}$ . The excitation and emission slit widths were set to be 5 and 1.5 nm, respectively. Regarding the computational analysis we have our PT-LFER method to predict multiple output properties of the system.

Fluorescence spectroscopy measurements are highly sensitive to the intermolecular interactions having place into the solution, and so to the aggregation process (see Fig. 1). Plots of  $I_1/I_3$  against total concentration of the mixture in aqueous solutions show two different regions, separated by sharp slope changes (inset, Fig. 1). Since this property usually change linearly when only one sort of molecule or cluster is present in the solution, each of these regions must correspond to a different aggregation state. The low concentration area corresponds to a monomeric state of the compounds, meanwhile higher ones are identified with the aggregate state of the molecules. The critical concentrations values were obtained from breaks in plots of  $I_1/I_3$  against total concentration of the mixture in aqueous solutions. Since there was no clear inflection point in these plots, the results were analysed to detect a precise value of cmc, using the Phillips definition of the critical micelle concentration, in which the cmc is defined as the concentration corresponding to the maximum change in gradient in plots of some colligative property against total concentration. An algorithm was applied in the numerical analysis of the data that the determination of precise values of the critical concentrations of drugs and surfactants of low aggregation number. The method consists of a Gaussian approximation of the second derivative of the conductivity concentration data, followed by two consecutive numerical integrations by the Runge-Kutta method and the Levenberg-Marquardt least-squares fitting algorithm. The inset in Fig. 1 show the maximum of the second derivative for the  $\alpha_{\text{NaDC}} = 0.1$  system. The results obtained for all systems under study can be consulted in Table 1.

**Table 1. Critical micelle concentration, mixed micelle composition, activity coefficients  $f_i$ , interaction parameter and Gibbs free energy as a function of NaDC molar fraction**

| $\alpha_{\text{NaDC}}$ | cmc   |      | $f_1$ | $f_2$ | $\beta$ (k <sub>B</sub> T) | $\Delta G_m^0$ (kJ mol <sup>-1</sup> ) |
|------------------------|-------|------|-------|-------|----------------------------|--|
| 0                      | 15.01 | --   | --    | --    | --                         |  |
| 0.1                    | 10.40 | 0.32 | 0.92  | 0.68  | -0.84                      | -11.31                                 |
| 0.2                    | 9.31  | 0.43 | 0.87  | 0.78  | -0.74                      | -11.68                                 |
| 0.3                    | 6.82  | 0.51 | 0.65  | 0.67  | -1.66                      | -12.35                                 |
| 0.4                    | 5.66  | 0.57 | 0.53  | 0.69  | -1.97                      | -12.82                                 |
| 0.6                    | 5.36  | 0.68 | 0.44  | 0.84  | -1.74                      | -12.95                                 |
| 0.7                    | 5.33  | 0.74 | 0.41  | 0.89  | -1.63                      | -12.97                                 |
| 0.8                    | 5.82  | 0.84 | 0.48  | 0.97  | -1.02                      | -12.75                                 |
| 0.9                    | 5.62  | 0.97 | 1.25  | 1.00  | 0.23                       | -12.83                                 |
| 1                      | 5.8   | --   | --    | --    | --                         |  |



**Fig. (1).** Emission fluorescence spectra of pyrene in aqueous mixed micellar solutions of NaDC/DTAB ( $\alpha_{\text{NaDC}} = 0.1$ ) as a function of total surfactant concentration. The inset shows the Fluorescence intensities ratios of pyrene emission spectra ( $I_1/I_3$ ) as a function of total surfactant concentration for the  $\alpha_{\text{NaDC}} = 0.1$  system. The dashed line represents the second derivative of the fluorescence-concentration curve.

The composition of the mixed micelles as well as the changes in the distribution of the components of each system between monomeric and micellar phases have been evaluated by the analysis of the variation of the cmc with composition using different approaches. The ideal approximation of the cmc of mixed micelles as a function of composition is done by Clint's model:

$$\frac{1}{\text{cmc}_{\text{ideal}}} = \sum_i \frac{\alpha_i}{\text{cmc}_i} \quad (2)$$

where this expression constitutes a relationship between the critical micellar concentration of the mixed system ( $\text{cmc}_{\text{ideal}}$ ) and of the  $i$  pure components ( $\text{cmc}_i$ ), and the molar fraction ( $\alpha_i$ ). The mole fraction of the components in the micelle in a binary ideal mixed state can be obtained by using the equation

$$X_1 = \frac{\alpha_1 \text{cmc}_2}{\alpha_1 \text{cmc}_2 + \alpha_2 \text{cmc}_1} \quad (3)$$

Rubingh's model [1] based on the regular solution approach for the treatment of nonideal mixing, considers the nonideality by introducing the activity coefficients,  $f$  in eq(3), as follows

$$\frac{1}{\text{cmc}^*} = \sum_i \frac{\alpha_i}{f_i \text{cmc}_i} \quad (4)$$

For a binary solution, for example, according to this model we have:

$$f_i = \exp[\beta(1 - X_i)^2] \quad (5)$$

Here,  $X_i$  is the molar fraction of the  $i^{\text{th}}$  surfactant in the micelle and the  $\beta$  parameter (in  $kT$  units, where  $k$  is the Boltzmann constant and  $T$  the absolute temperature) can be interpreted in terms of an energetic parameter that represents the excess Gibbs free energy of mixing. However, this theory does not take into account the non-ideality behavior of real mixed systems. For this reason, the composition of the monomeric and micellar phases of these systems was evaluated by the treatment of Motomura *et al.* [14] based on excess thermodynamic quantities, where micellization is considered similar to a macroscopic bulk phase, and energetic parameters associated with the process are expressed by excess thermodynamic quan-

ties. The critical concentration, and the mole fraction of components in the system,  $\bar{X}_i$ , are given by

$$\text{cmc}^* = (v_1 \alpha_1 + v_2 \alpha_2) \text{cmc} \quad (6)$$

$$\bar{X}_i = v_i \alpha_i / (v_1 \alpha_1 + v_2 \alpha_2) \quad (i = 1, 2) \quad (7)$$

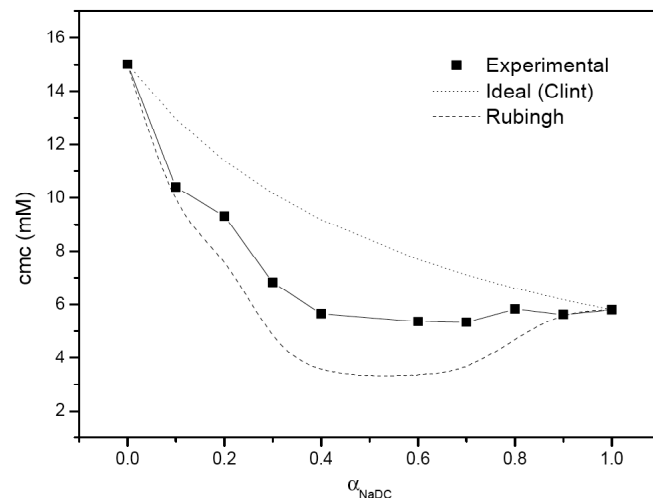
where  $\alpha_1$  and  $\alpha_2$  are the mole fractions of component 1 and 2 and  $v_i = v_{1,a} + v_{1,c}$  and  $v_2 = v_{2,b} + v_{2,d}$ ;  $v_{1,a}$  and  $v_{1,c}$  are the number of cations and anions produced on dissociation of component 1 and  $v_{2,b}$  and  $v_{2,d}$  are the number produced on dissociation of component 2. The compounds under investigation are 1:1 electrolytes with identical counterions charge, then:  $v_{1,a} = v_{1,c} = v_{2,b} = v_{2,d} = 1$ , so  $\text{cmc}^* = 2\text{cmc}$ . The composition of the mixed micelle is determined using the relationship

$$X_2^m = \bar{X}_2 - \left( \frac{\bar{X}_1 \bar{X}_2}{\text{cmc}^*} \right) \left( \frac{\partial \text{cmc}^*}{\partial \bar{X}_2} \right)_{T,p} \left[ 1 - \frac{\delta_d^c v_{1,c} v_{2,d}}{v_{1,c} v_2 \bar{X}_1 + v_{2,d} v_1 \bar{X}_2} \right] \quad (8)$$

The Kronecker delta,  $\delta_d^c$ , for systems investigated here, in which counterions are identical, is 1 and Eq. (8) reduces to:

$$X_2^m = \bar{X}_2 - \left( \frac{\bar{X}_1 \bar{X}_2}{\text{cmc}^*} \right) \left( \frac{\partial \text{cmc}^*}{\partial \bar{X}_2} \right)_{T,p} \quad (9)$$

In Fig. 2 we have plotted the experimental values of cmc for the mixed system with the ideal approximation, as a function of  $\alpha_{\text{NaDC}}$ . Even just from visual inspect it is clear that Rubingh's model exhibit best agreement with experimental points. However, the correlation is not constant for all molar fraction range: the highest correlations correspond to the lowest and highest values of  $\alpha_{\text{NaDC}}$ , meanwhile poor correlation values were found for intermediate values of  $\alpha_{\text{NaDC}}$ . We were not able to obtain cmc values for  $\alpha_{\text{NaDC}} = 0.5$ , the reason was that at this concentration, a liquid-liquid phase separation is observed when the mixing ratio is close to equimolar. Such behavior is quite common in catanionic systems (cationic-anionic mixtures) [19].



**Fig. (2).** Plots of critical micelle concentration (cmc) dependence on the molar fraction of NaDC obtained from different sources: experimental values (black squares), ideal (dotted line) and Rubingh (dashed line).

To gain quantitative understanding of the mixing process is worth applying the Regular Solution Theory to obtain  $\beta$ , the dimensionless intramicellar interaction parameter, which is related with

the molecular interactions in the mixed micelles and can be interpreted in terms of an energetic parameter that represents the excess Gibbs free energy of mixing [38, 39]:  $\beta = \beta_{12} = N_A(W_{11} + W_{22} - 2W_{12})$ . Where  $W_{11}$  and  $W_{22}$  are the energies of interaction between molecules in the pure micelle and  $W_{12}$  is the interaction between two species in the mixed micelle. In the case of binary nonideal mixtures the regular approximation for the activity coefficients in the mixed micelles are given by:

$$f_1 = \exp \beta (X_2^m)^2 = \exp \beta (1 - X_1^m)^2 \quad (10)$$

$$f_2 = \exp \beta (X_1^m)^2 \quad (11)$$

The value of  $\beta$  (in  $k_B T$  units) can be calculated from the equations [21]:

$$(X_1^m)^2 \ln \left( \frac{\alpha_1 cmc_m}{X_1^m cmc_1} \right) = (1 - X_1^m)^2 \ln \left[ \frac{(1 - \alpha_1) cmc_m}{(1 - X_1^m) cmc_2} \right] \quad (12)$$

$$\exp \beta (1 - X_1^m)^2 = \frac{\alpha_1 cmc_m}{X_1^m cmc_1} \quad (13)$$

In an ideal mixed micelle  $\beta$  is equal to zero whereas repulsive interactions yield a positive values indicating a possible antagonism. Positive  $\beta$  values have been found in mixtures of fluorocarbon-hydrocarbon surfactants. Negative  $\beta$  values indicate attractive synergic interactions which imply that the  $cmc_m$  is lower than the averaged value of the  $cmc$  of each surfactant. Although theoretically  $\beta$  is independent on temperature and composition of the micelles, some papers show temperature dependence and also a micelle composition dependence [40, 41]. Table 1 listed the mixed micelle composition and the interaction parameter, respectively as a function of bile salt concentration by applying the RST.  $\beta$  indicates the nature and strength of the interaction between both amphiphilic molecules, this means, it is a measure of the degree of nonideality in mixed micelles, i.e., negative value of  $\beta$  is associated with a stronger attractive interaction between the two different molecules [17]. On the other hand, positive values yield repulsive interactions. Table 1 shows the interaction parameter  $\beta$  over the complete range of NaDC molar fraction and the tendency of this parameter increase with molar fraction of the bile salts (except for  $\alpha_{NaDC} = 0.9$ ). Mixed micelles for  $\alpha_{NaDC} = 0.1$  to  $\alpha_{NaDC} = 0.8$  (range of synergism between building units of mixed micelles), have lower  $cmc$  values than the values of ideal critical micelle concentration. This means that the formation of real mixed micelles is thermodynamically more favorable than formation of ideal mixed micelles where no attractive interactions exist (ideal micelle formation is spontaneous due to positive entropy of mixing). Attractive interactions in real mixed micelles exist due electrostatic interaction of polar heads and hydrogen bonds between alkyl chain of DTAB and OH groups in the steroid skeleton of bile acid anions. For larger mole fraction values of bile salts in the binary surfactant mixture (above  $\alpha=0.8$ ) the formation of real mixed micelle is less favored than the formation of ideal mixed micelle. Interaction parameter is now positive showing antagonism between building units (repulsive interactions). Usually, according to the RST, electrostatic repulsive interactions between ionic polar heads are responsible for  $\beta > 1$ , this is true because of the high proportion of bile salt monomers in the mixed micelles [42].

The micellar composition,  $X_{NaDC}$ , predicted by the above described models is plotted versus total molar fraction Fig. 3. It can be observed that the best model to parametrize this system is the Rubingh's model. In the case of Motomura's model, deviations are much more accentuated.

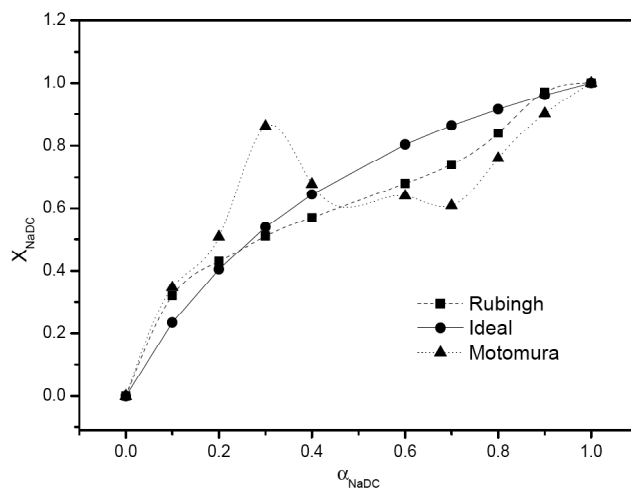


Fig. (3). Plots of mixed micelle composition,  $X_{NaDC}$  obtained from different sources: Rubingh (black squares), Ideal (black circles) and Motomura (black triangles) versus total molar fraction.

We also used the CPT-LFER model published by our work to carry out a simulation of the effect of perturbations on the initial composition over the stability of the NaDC-DTAB micelle nanoparticle system. The perturbations studied here appear after changes on the composition of the system ( $\alpha_{DDBA}$  vs.  $\alpha_{NaDC}$ ). In so doing, we introduced the values of the new free energy covariance functions  $\Gamma_{jp}$  (see Equation 1). The  $\Gamma_{jp}$  values were calculated with the new potentials of the molecules as well as the average values of the functions. In the Table 2 we depict the values of the average functions for different properties.

We begin the simulation generating 1000 perturbations in the system at random. It means that we selected at random 1000 pairs of new states vs. their states of reference. We selected only states with perturbations in the composition of the initial system and final micelle nanoparticles. As these compositions are tied,  $\alpha_{NaDC} = 1 - \alpha_{DDBA}$ , we focused only on  $\alpha_{DDBA}$  values. Other state functions were kept constant  $T = 298$  K,  $I = 1$ , etc. The 1000 perturbations generated randomly included changes in initial values of  $\alpha_{DDBA}^{ref}$  to obtain new values  $\alpha_{DDBA}^{new}$  in the full range 0-1. In this sense, this is other example on the use of this method to predict multiple properties of binary systems of bile salts towards a computational design of more stable micelle nanoparticles useful in pharmaceutical design.

## CONCLUSION

Impelled by the pair of current imperatives constituted by experimental techniques and computational methods, in this paper we have tried to provide, from the beginning, a plural approach to the characterization of the physicochemical properties of the nanoparticles spontaneously formed within aqueous mixtures of the bile salts sodium glycodeoxycholate and the surfactant didodecyltrimethylammonium bromide (NaDC-DTAB) as a function of concentration. The experimental study has been carried on by means of a fluorescence spectroscopy experiments and the consequent thermodynamic analysis. The mixed systems were analyzed using system different models, being Rubingh's model the best one which reproduced the experimental data. Then, we check our CPT-LFER model to carry out a simulation of the effect of perturbations on the initial composition over the stability of the NaDC-DTAB micelle nanoparticle system showing a very good agreement with experimental data, with uncertainties less than 0.02 %. In addition, we have predicted results for our system under different conditions that were not experimentally studied. Finally, we show how experimen-

Table 2. Average properties of potentials for different input/output conditions.

| Property              | $\langle\mu_{ij}(\text{dip})\rangle$   | $\langle\mu_{ij}(\text{sol})\rangle$       | $\langle\mu_{ij}[35]\rangle$       | $\langle^P\varepsilon_{ij}\rangle$ | SD( $^P\varepsilon_{ij}$ ) |
|-----------------------|--|--|------------------------------------|------------------------------------|----------------------------|
| $\beta$               | 1.14319809                             | 0.01131703                                 | 4701.567                           | -0.040                             | 6.42                       |
| cmc                   | 1.16341030                             | 0.01178695                                 | 5395.792                           | 0.248                              | 1.18                       |
| $\Delta G$            | 1.16517857                             | 0.01184776                                 | 5479.602                           | -14.975                            | 7.87                       |
| $X_{m1}$              | 1.13449024                             | 0.01029247                                 | 8892.178                           | 0.520                              | 0.34                       |
| $X_{m2}$              | 1.14123007                             | 0.01077712                                 | 9978.641                           | 0.481                              | 0.34                       |
| A                     | 1.00000000                             | 0.00000916                                 | -10256.956                         | 41.807                             | 25.42                      |
| $\gamma$              | 1.00000000                             | 0.00000916                                 | -10256.956                         | 27.602                             | 4.83                       |
| $\Delta H$            | 1.00000000                             | 0.00000925                                 | -8477.038                          | 3.336                              | 7.53                       |
| $\Delta S$            | 1.00000000                             | 0.00000925                                 | -8477.038                          | 82.596                             | 26.16                      |
| N                     | 1.70000000                             | 0.00005235                                 | -13978.519                         | 70.300                             | 48.14                      |
| f1                    | 1.00000000                             | 0.00065068                                 | -9917.067                          | 0.694                              | 0.26                       |
| f2                    | 1.00000000                             | 0.00065068                                 | -9917.067                          | 0.815                              | 0.12                       |
| Counter - anion       | $\langle\mu_{ij}(\text{dip})\rangle$   | $\langle\Delta\mu_{ij}(\text{sol})\rangle$ | $\langle\Delta\mu_{ij}[35]\rangle$ | SMART code                         | Free                       |
| Bromide               | 1.460585                               | 0.000089                                   | -15223.868                         | [Br <sup>-</sup> ]                 | Yes                        |
| Carboxylate           | 1.000000                               | 0.000059                                   | 61488.694                          | C(=O)[O <sup>-</sup> ]             | No                         |
| Chloride              | 1.000000                               | 0.000011                                   | -8530.522                          | [Cl <sup>-</sup> ]                 | Yes                        |
| Sulphate              | 1.000000                               | 0.000009                                   | -8318.713                          | S(=O)(=O)[O <sup>-</sup> ]         | No                         |
| Oxide                 | 3.718750                               | 0.214099                                   | -9012.713                          | [O <sup>-</sup> ]                  | No                         |
| 2-Methoxy-ethan-olate | 1.000000                               | 0.000009                                   | -10136.560                         | COCC[O <sup>-</sup> ]              | No                         |
| Counter - cation      | $\langle\mu_{ij}(\text{dip})^+\rangle$ | $\langle\mu_{ij}(\text{sol})^+\rangle$     | $\langle\mu_{ij}[35]^+\rangle$     | SMART code                         | Free                       |
| Phosphine             | 1.000000                               | 0.000009                                   | -28602.581                         | [P <sup>+</sup> ]                  | No                         |
| Sodium                | 1.000000                               | 0.000038                                   | 32631.454                          | [Na <sup>+</sup> ]                 | Yes                        |
| Amine-ter             | 1.482998                               | 0.038085                                   | -11207.204                         | [N <sup>+</sup> ]                  | No                         |
| Amine-sec             | 1.377358                               | 0.000034                                   | -10700.483                         | [NH <sup>+</sup> ]                 | No                         |
| -                     | 0.000000                               | 0.000000                                   | -10640.488                         | no ionic                           | -                          |

tal and theoretical approaches are complementary for a full characterization of these kinds of systems and represent a very promising route to design new nanoparticles based on surfactants mixtures in a fast and economical way.

#### CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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