Surface Tensions, Critical Micelle Concentrations, and Standard Free Energies of Micellization of C_8−Lecithin at Different pHs and Electrolyte Concentrations

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The micellization of the amphiphilic zwitterionic dioctanoylphosphatidylcholine (diC_8PC) in aqueous solution has been studied as a function of pH and electrolyte concentration. Critical micelle concentration (cmc) determined by surface tension measurements at pH 1.2, 3.2, 5.8, 7.4, and 10.0 indicated an increase with pH. To analyze the influence of electrolyte on the cmc, measurements of surface tension were performed at pH 1.2 and KCl concentrations in the range 0.1 M to 2.0 M. Here, the cmc decreased with increase of KCl concentration. Areas per molecule at the interface decrease from (86.8 to 65.1) $Å^2$ $molecule^{-1}$ with increase of pH. Standard Gibbs energies of micellization were calculated from the surface tension data and are in the range of approximately (−30 to −34) kJ mol^{-1}. Data for the hydrocarbon volume of lipids, the effective area per headgroup, and the length of hydrocarbon chain have been used to estimate the shape factor of diC_8PC micelles. The value for the shape factor indicates a gradual change from nonspherical (shape factor 0.49) to bilayers (shape factor 0.59).

Introduction

The aggregation and interfacial properties of phosphatidylcholines (1,2-diacyl-*sn*-glycerol-3-phosphocholine, PCs) are of fundamental interest in biochemistry for understanding their biological function in the plasma membranes but also for different industrial applications. In aqueous solutions the synthetic PCs with two identical fatty acid chains exist primarly as monomers if the acyl chains are shorter than four carbon atoms, as a monomer−micelle equilibrium (above critical micelle concentration, cmc) if the chains consist of six to eight carbon atoms, and as swelling lamellar liquid-crystalline layers or vesicles if the chains consist of more than eight carbon atoms.[1−3] An interesting application of the aggregation properties of short-chain phospholipids in water is the study of the mechanism that is involved in the enzymatic hydrolysis of the two fatty acid ester bonds. Previous reports have shown that micellar formation dramatically enhances the enzymatic activity.[4−7] The studies by Tausk et al.[4−6] indicated the existence of a miscibility gap for the dioctanoylphosphatidylcholine + water system, which has a consequence that the lipids avoid fast enzymatic hydrolysis at room temperature. This phase separation phenomenon has been studied recently by Lo Nostro et al.[8] in terms of an adaptation of the thermodynamic model of Blankschtein, Thurston, and Benedek (BTB). NMR and Raman studies have shown that short-chain phospholipids retain many of the conformational features (chain packing and nonequivalence, headgroup orientation, etc.) of long-chain lecithins,[9,10] and their micellar form also makes these systems a useful matrix for creating model lipoproteins.

In this work we have studied the surface properties and the micellization of dioctanoylphosphatidylcholine (diC_8PC) in different conditions of pH and electrolyte concentration.

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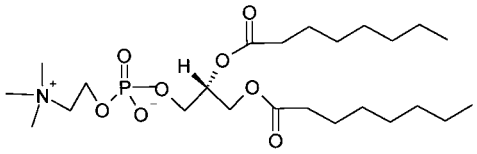
Scheme 1



As we can see in Scheme 1, diC_8PC is a zwitterion, with a negatively charged phosphate group and a positive charge on the nitrogen atom of choline. The study of molecules with a zwitterionic headgroup provides decisive advantages over the ionic or nonionic classes of surfactants. The properties of solutions of such amphiphiles depend, on one hand, on the structural parameters of the amphiphile itself, that is, on its chemical structure, and on the other hand, on the pH of the medium, which can change the charge of the headgroup.

The aim of this work is to study the following: (i) How the pH of the medium influences the micellar behavior of diC_8PC. At what pH the electrostatic interaction between the charged headgroups becomes significant. The answers to these questions will disclose ways of studying the factors that control lipid−protein interactions, which will be the objective of future publications. (ii) How the addition of electrolytes affects our systems. These effects may provide information on the interactions between polar groups in the micellar interface and thereby on the orientation of these groups. The salt can change the micellar structure and interactions between solute molecules without changing the phospholipid molecule at all.

Material and Methods

DiC_8PC (CAS Reg. No. 41017-85-0) was purchased from Avanti Polar Lipids Inc. (Birmingham, AL) in a powder form with purity greater than 99%, stored at 253.15 K, and used without any further purification. Five buffers were

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Table 1. Surface Tension, γ, as a Function of Concentration, c, of Dioctanoylphosphatidylcholine in Aqueous Solution at a Temperature of 298.15 K and Different pHs

pH 1.2		pH 3.2		pH 5.8		pH 7.4		pH 10	
c	γ	c	γ	c	γ	c	γ	c	γ
mmol dm^{-3}	mN m^{-1}	mmol dm^{-3}	mN m^{-1}	mmol dm^{-3}	mN m^{-1}	mmol dm^{-3}	mN m^{-1}	mmol dm^{-3}	mN m^{-1}
1.654	24.59	1.793	25.31	1.762	25.42	1.810	25.26	1.671	25.44
1.460	24.57	1.585	25.42	1.547	25.24	1.590	25.42	1.475	25.37
1.270	24.32	1.374	25.33	1.339	25.42	1.390	25.47	1.280	25.36
1.079	24.47	1.163	25.52	1.131	25.20	1.170	25.23	1.086	25.37
0.891	24.22	0.952	25.40	0.928	25.25	0.960	25.28	0.891	25.50
0.698	24.35	0.740	25.44	0.721	25.45	0.750	25.33	0.693	25.39
0.498	24.35	0.529	25.38	0.512	25.28	0.530	25.42	0.494	25.53
0.292	24.35	0.315	25.43	0.307	25.37	0.320	25.72	0.296	25.69
0.245	24.35	0.263	25.42	0.256	25.11	0.260	26.52	0.247	26.37
0.197	24.42	0.211	25.76	0.205	25.53	0.213	27.49	0.197	27.56
0.149	24.93	0.158	27.62	0.154	26.86	0.159	28.78	0.148	28.21
0.099	27.61	0.105	29.17	0.102	29.83	0.106	31.92	0.098	32.30
0.079	28.67	0.084	30.62	0.082	31.05	0.085	33.55	0.079	33.62
0.059	29.70	0.063	32.13	0.061	32.42	0.064	35.00	0.059	35.64
0.040	31.32	0.042	34.68	0.041	36.15	0.042	37.32	0.039	38.08

used: KCl/HCl, pH 1.2; glycine/HCl, pH 3.2; hydrogen phosphate/dihydrogen phosphate, pH 5.8 and 7.4; and glycine/NaOH, pH 10.0. The concentration of the buffers was chosen so that all would have the same ionic strength. Water was from a Milli Q reverse-osmosis purification system. Potassium chloride, obtained from Fluka, was of analytical grade.

Surface tensions were measured by the Wilhelmy plate method using a Krüss K12 surface tension apparatus, equipped with a processor to acquire the data automatically. Precautions of time were taken to ensure that equilibrium was reached. The reproductibility of the surface tension was ±0.01 mN m^{-1}. The equipment was connected to a circulating water bath to keep the temperature constant at (298.15 ± 0.01) K. Phospholipid solutions were prepared by diluting with buffer, determining the concentration by mass, with a precision of ±0.00001 g. The usual precautions were taken to ensure cleanliness.

Results and Discussion

(A) Influence of the pH. Table 1 shows the experimental surface tension data for diC_8PC at different pHs. The critical micelle concentrations are found by the intersection of the best straight lines through the two branches of γ−ln c curves (see Figure 1) and their values are given in Table 2. These values compare with those reported in the literature. Tausk et al.[4] give cmc values for diC_8PC in the range (0.23−0.31) mmol dm^{-3}, obtained by surface tension measurements using the drop volume method.[11] Their experiments were carried out at room temperature, in 10^{-2} M phosphate buffer, pH 6.9. Bian and Roberts[12] report a value of 0.27 mmol L^{-1} for the cmc at 298.15 K in water. An important fact that must be highlighted is the absence of a minimum in our curves (see Figure 1), indicating the high purity of the product utilized. The presence of curvature has been attributed to a surface-active impurity.[13]

Electrophoretic mobility measurements[14] show an isoelectric point for synthetic lecithins of about 6. From this we can accept the gradual change of charge sign in the molecule from positive to zwitterionic in the (1.2−10.0) pH interval. The increase of the cmc with increase in pH (see Table 2) needs a detailed analysis. First, it is very important to point out that this variation is very small, with two well-defined parts below and above the isoelectric point (see Figure 2). These results agree with the observation that at low pH fewer monomers have accommodation in the aqueous solution/air interface and must pass to the bulk. In accordance with the pH increase, the number of monomers at the interface increases. This fact could be explained by the orientation of the polar group in the interface. At low pH the net positive charge in the headgroup produces an electrostatic repulsion with the subsequent increase in the area of the molecule at the interface as it will be demonstrated with area values. Nevertheless, at pH near the isoelectric point the orientation of the zwitterion is parallel to the surface, giving rise to electrostatic attractions with a big number of monomers at the interface.

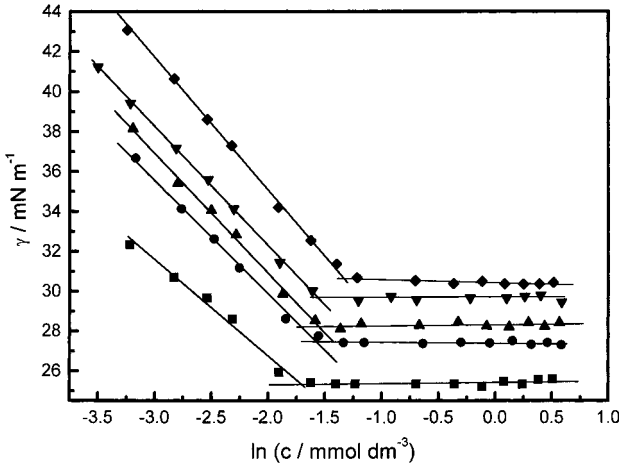

Figure 1. Surface tension values (in mN m^{-1}) as a function of the logarithm of concentration (in mmol dm^{-3}) of dioctanoylphosphatidylcholine at 298.15 K and different pHs: (■) pH 1.2; (●) pH 3.2; (▲) pH 5.8; (▼) pH 7.4; (◆) pH 10.0. For clarity the data are displaced vertically by 1, 2, 3, 4, and 5 mN m^{-1} for pH 1.2, 3.2, 5.8, 7.4, and 10.0, respectively.

Table 2. Critical Micelle Concentrations (cmc), Area per Molecule (A_{min}), and Standard Free Energies of Micellization (ΔG^0_m), for Dioctanoylphosphatidylcholine at 298.15 K and Different pHs

pH	cmc[a] / mmol dm^{-3}	A_{min} / Å^2 molecule^{-1}	ΔG^0_m [b] / kJ mol^{-1}
1.2	0.18	86.8	−31.3
3.2	0.19	72.7	−31.2
5.8	0.20	68.9	−31.1
7.4	0.21	68.7	−30.9
10.0	0.28	65.1	−30.2

[a] Deviations in cmc values are ±0.01. [b] Deviations in the ΔG^0_m are ±0.1.

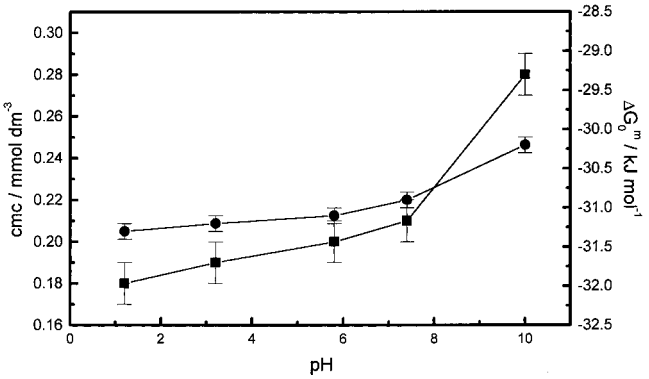

Figure 2. Critical micelle concentration (in mmol dm^{-3}) and standard Gibbs energies of micellization (in kJ mol^{-1}) of aqueous solutions of dioctanoylphosphatidylcholine at 298.15 K as a function of pH: (■) experimental values of cmc; (●) standard Gibbs energies calculated by expression (3).

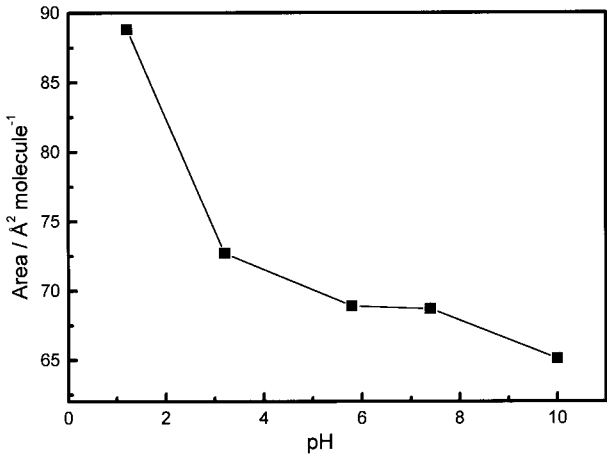

Figure 3. Minimum molecular area (in Å^2 molecule^{-1}) as a function of pH for aqueous solutions of dioctanoylphosphatidylcholine at 298.15 K.

The minimum molecular area, A_{min}, of the diC$_8$PC at the interface was calculated, assuming ideality, from the Gibbs equation[15]

$$\frac{1}{A_{\min}} = -\frac{1}{k_B T}\left(\frac{\partial \gamma}{\partial(\ln c)}\right)_T \qquad (1)$$

where k_B is the Boltzmann constant, T the temperature in Kelvin, and c the molar concentration. The slope $\partial\gamma/[\partial(\ln c)]$ was determined below the cmc, in the monomers zone. The values obtained for different pHs are given in Table 1 and plotted in Figure 3. Our value at pH 10.0 coincides with that reported by Eastoe et al.[16] who give a value of 65 Å^2/molecule in water at 313.15 K. Discrepancies exist with the data of Bian and Roberts[12] (51.4 Å^2/molecule at 298.15 K in water). The existence of discrepancies is usual in this type of measurement because, fundamentally, of (i)the method used to determine the limiting area and (ii) the appearance of a minimum in the surface tension versus concentration curves (because of the existence of impurities in the product). Indeed, in the cited work of Bian and Roberts, we can find differences of 12.4 Å^2/molecule for the limiting area of diC$_6$PC in the temperature range of (288.15−313.15) K. Regarding other lecithins, for diC$_4$PC a value of 64.4 Å^2/molecule in water at 298.15 K has been reported[12] and for diC$_{14}$PC a value of about 80 Å^2/molecule in a pH range of 1 to 7.[17] Our variation of A_{min} with the pH confirm the analysis made with the cmc−pH variation.

For the association equilibrium $M_1 \leftrightarrow (1/g)M_g$ between monomers (M_1) and monodispersed micelles (M_g) with aggregation numbers (numbers of diC$_8$PC molecules per micelle), g, the standard free energy per mole for micelle formation ΔG^0_m is given by

$$\Delta G^0_m = -RT \ln K = -(RT/g) \ln X_{M_g} + RT \ln X_{M_1} \qquad (2)$$

where R is the universal gas constant, T is the temperature in Kelvin, K is the association constant, and X symbols represent mole fractions. For large association numbers the term containing the micellar concentration will vanish and the free energy change per monomer may be approximated by

$$\Delta G^0_m = RT \ln X_{M_1} = RT \ln X_{CMC} \qquad (3)$$

In Table 2 the change in standard free energy of the monomers, when associating in micelles, is given for diC$_8$PC at different pHs. In Figure 2 we have plotted ΔG^0_m against pH. The negative values of ΔG^0_m indicates a spontaneous process at all pHs and the increase of ΔG^0_m with the pH shows that micelles at low pH are more easily formed and more stable.

(B) Influence of the Electrolyte Concentration. To study the effect of the addition of an inert electrolyte on the critical micelle concentration of diC$_8$PC, we have chosen the pH 1.2 where the cmc is lowest and, most likely, the influence of the salt will be bigger. Table 3 shows the experimentally obtained values. In Figure 4 the surface tension as a function of the logarithm of the concentration is shown for different concentrations of KCl. The cmc values obtained by the intersection of straight lines of fit of $\gamma-\ln c$ curves are summarized in Table 4. The addition of electrolyte has a strong influence on the micellization of diC$_8$PC. Added electrolyte favors micellar growth and may be responsible for changes in the micellar shape from a minimum-size micelle into a more elongated structure.

To estimate the most favored structure of the diC$_8$PC micelles, we have used the dimensionless packing parameter or shape factor (N), defined as[18]

$$N = \frac{v}{A_{\min} l_c} \qquad (4)$$

where v is the hydrocarbon volume of the lipid and l_c the length of hydrophobic chain. The value of N determines whether the micelles are spherical ($N < 1/3$), nonspherical ($1/3 < N < 1/2$), vesicles or bilayers ($1/2 < N < 1$), or "inverted" structures ($N > 1$). Each of these structures correspond to the minimum-sized aggregate in which all the PCs have minimum free energy. To have information on N of our systems, a cylindrical shape for diC$_8$PC monomer with a characteristic length $L = l_c + h$ is assumed. The length of the hydrophobic chain (l_c) of the phospholipid is estimated by Tanford's[19] expression:

$$l_c \approx 1.54 + 1.265 \times n \text{ (Å)} \qquad (5)$$

In our case ($n = 8$) $l_c = 11.6$ Å. h is the radial distance occupied by the phosphatidylcholine polar head that we have estimated to be 8.4 Å. This value has been reported by Huang[20] who used the data on the thickness of the polar region of diC$_{14}$PC and the thickness of the bilayer of diC$_{16}$PC.[21] Consequently, L is about 20 Å. Taking a value of 780 Å^3 for the molecular volume of diC$_8$PC,[8] 3.5 Å is obtained for the radius of the cylinder, so the hydrophobic

Table 3. Surface Tension, γ, as a Function of Concentration, c, of Dioctanoylphosphatidylcholine in Aqueous Electrolyte Solution, pH 1.2, and Temperature of 298.15 K

0.1 mol dm^{-3} KCl		0.5 mol dm^{-3} KCl		1.0 mol dm^{-3} KCl		1.5 mol dm^{-3} KCl		2.0 mol dm^{-3} KCl	
c	γ	c	γ	c	γ	c	γ	c	γ
mmol dm^{-3}	mN m^{-1}	mmol dm^{-3}	mN m^{-1}	mmol dm^{-3}	mN m^{-1}	mmol dm^{-3}	mN m^{-1}	mmol dm^{-3}	mN m^{-1}
1.702	24.61	1.686	24.22	1.711	23.96	1.372	24.13	1.322	24.31
1.501	24.51	1.489	23.90	1.502	24.17	1.155	24.13	1.120	24.07
1.302	24.38	1.290	24.08	1.296	24.07	0.936	24.10	0.914	24.04
1.104	24.32	1.093	24.27	1.098	23.98	0.729	24.16	0.709	24.12
0.903	24.49	0.896	23.87	0.903	24.09	0.519	24.04	0.509	24.09
0.701	24.54	0.698	24.05	0.705	24.19	0.310	24.27	0.305	24.91
0.502	24.49	0.496	24.16	0.505	24.21	0.258	24.11	0.254	24.27
0.301	24.47	0.297	24.15	0.300	24.04	0.206	24.42	0.203	24.19
0.251	24.47	0.247	24.18	0.249	24.26	0.154	24.43	0.153	24.32
0.201	24.49	0.198	24.43	0.200	24.18	0.102	24.39	0.102	24.45
0.151	24.83	0.149	24.39	0.149	24.16	0.082	24.71	0.082	24.61
0.100	27.00	0.099	26.48	0.099	24.99	0.061	25.69	0.061	24.71
0.080	28.43	0.079	27.53	0.079	25.89	0.041	27.24	0.041	25.72
0.060	29.77	0.059	29.51	0.060	27.54	0.020	30.56	0.020	28.88
0.040	31.99	0.039	31.04	0.040	29.10	0.015	33.15	0.015	30.03

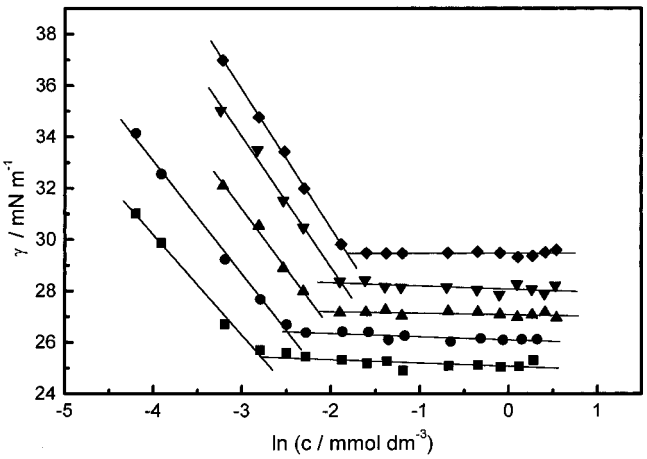

Figure 4. Logarithm of the cmc of aqueous solutions of dioctanoylphosphatidylcholine at 298.15 K, pH 1.2, as a function of electrolyte concentration: (◆) 0.1 mol dm^{-3} KCl; (▼) 0.5 mol dm^{-3} KCl; (▲) 1.0 mol dm^{-3} KCl; (●) 1.5 mol dm^{-3} KCl; (■) 2.0 mol dm^{-3} KCl.

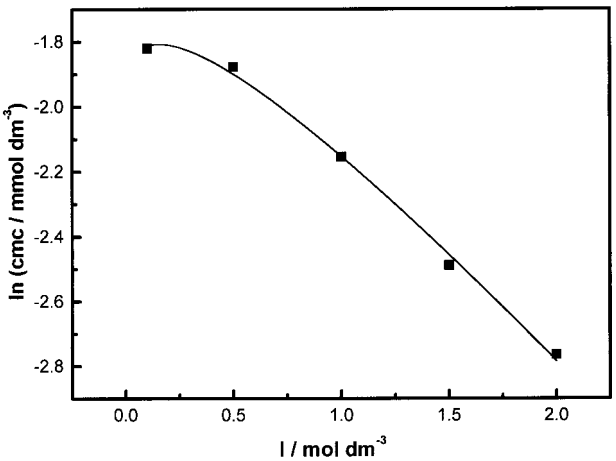

Figure 5. Logarithm of the cmc as a function of ionic strength of a aqueous solution of dioctanoylphosphatidylcholine with added KCl. The curve is the fit of experimental points.

Table 4. Critical Micelle Concentrations (cmc) and Standard Free Energies of Micellization (ΔG^0_m), for Dioctanoylphosphatidylcholine at 298.15 K, pH 1.2, and Different KCl Concentrations

KCl	cmc[a]	ΔG^0_m [b]	KCl	cmc[a]	ΔG^0_m [b]
mol dm^{-3}	mmol dm^{-3}	kJ mol^{-1}	mol dm^{-3}	mmol dm^{-3}	kJ mol^{-1}
0.1	0.16	−31.6	1.5	0.08	−33.3
0.5	0.15	−31.7	2.0	0.06	−34.1
1.0	0.12	−32.1			

[a] Deviations in cmc values are ±0.01. [b] Deviations in the ΔG^0_m are ±0.1.

portion of the molecule (v) is 450 Å^3. With these considerations the values of N were in the range 0.45 to 0.59, indicating a transition between nonspherical micelles to bilayers.

Standard free energies of micellization, calculated by eq 3, are given in Table 4. They are bigger than that corresponding to the absence of the added electrolyte. As has been studied,[22] the addition of a salt has the effect of increasing the Gibbs energy of micellization. This increment is determined by contributions from the ion−ion interactions and the ion−dipole interaction between the ions and the polar heads of the amphiphiles, between ions and water molecules, and so forth. From the Debye−Hückel theory, the contribution from ion−ion interaction is proportional to the square root of ionic strength,[23] while the contribution from ion−dipole interaction, as suggested by Kirkwood's model, is proportional to ionic strength.[24] Accordingly, the relation between cmc and the ionic strength, I, for a salt-added micellar solution is[25]

$$\ln(\mathrm{cmc})' = \ln(\mathrm{cmc}) + k_1 I^{1/2} + k_2 I \qquad (6)$$

where ln(cmc)′ and ln(cmc) represent the parameters in the solutions with and without salts, respectively; k_1 and k_2 are alternative expressions of the salt-effect parameters. The variation of the cmc with ionic strength is shown in Figure 5. Applying the least-squares method to the experimental data, the values of $k_1 = 0.7 \pm 0.2$ and $k_2 = -0.9 \pm 0.1$ were obtained. The values of k_1 and k_2 give information about the relative influence of the ion−dipole and ion−ion interactions on the Gibbs energy of micellization.[25] Obviously, the k_i values are salt concentration dependent and for each system different values will be obtained but the relative contribution of each interaction must be specific for each salt type. The similar values of k_1 and k_2 (in absolute terms) obtained by us indicate that the contribution of ion−ion and ion−dipole interactions to the Gibbs energy of micelle formation are similar.

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