Colloid Polym Sci (2002) 280: 624–629
DOI 10.1007/s00396-002-0657-4

ORIGINAL CONTRIBUTION

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A thermodynamic study of the aggregation process of oxacillin sodium salt in aqueous solution

Received: 25 May 2001
Accepted: 10 January 2002
Published online: 24 April 2002

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Abstract The aggregation characteristics of oxacillin in aqueous solutions have been examined by means of conductivity measurements over the temperature range 288.15–313.15 K and by static light scattering measurements at 298.15 K. Two critical concentrations were detected in conductivity and light scattering over the concentration range 0–0.35 mol kg^{-1}. Light scattering measurements indicate the formation of dimers at the first critical concentration (0.024 mol kg^{-1}) and the subsequent formation of aggregates with an aggregation number of 8 at the second critical concentration (0.104 $molkg^{-1}$). The thermodynamic parameters of aggregation were derived from the critical concentration data using a mass-action model that has been modified for application to systems of low aggregation number. Values for the enthalpy of aggregate formation calculated by this method showed that the aggregation became increasingly exothermic with increasing temperature. The values of the two critical concentrations show that this penicillin, oxacillin, is more hydrophobic than other molecules of similar structure.

Keywords Aggregation · Oxacillin · Critical micelle concentration · Thermodynamics · Conductivity · Light scattering

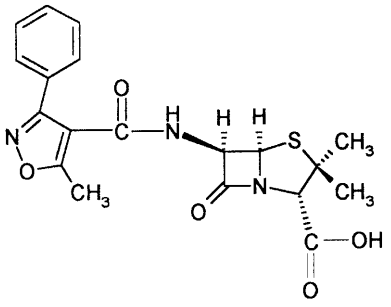
Introduction

The study of the colloidal properties of antibiotics is of interest because the pharmacological activity of these drugs appears at low concentrations where aggregation is negligible [1, 2]. However, it is possible that accidental accumulation of the antibiotics can take place at certain sites in the organism, giving rise to the formation of aggregates unable to pass through the membranes and consequently rendering the antibiotic ineffective for therapeutic use. Then, the study of the self-association of these drugs in aqueous solutions is important for their biological and pharmaceutical implications.

Different studies have examined the aggregation of penicillin drugs. Hauser et al. [3], Kumler and Alpen [4], McBain et al. [5], and Atwood and Argawal [6] analyzed the state of aggregation of the penicillin and established the structure–association relationship. A feature common to all these studies was the attempt to ascertain how particle size, electrical charge, and other properties, inherently colloidal, might affect the mode of action of these drugs. However, it is difficult to find studies on this type of compound that relate the thermodynamic properties with the mechanism of association.

Our most recent studies [7, 8, 9, 10, 11, 12] have reported the micellar properties of several synthetic penicillins in water and in the presence of electrolyte. We examined the relationship between molecular structure and self-aggregation characteristics.

The present study extends this work and considers the properties of oxacillin, whose structure is represented in Scheme 1, in aqueous solution using conductivity and



Scheme 1. Oxacillin

light scattering techniques. Furthermore, we present a thermodynamic study of the colloidal properties of oxacillin. The measurements were carried out over a wide concentration range, which enables the detection of the two critical concentrations to be made.

Experimental

Materials

Oxacillin sodium salt, [2*S*-(2α,5α,6*β*)]-3,3-dimethyl-6-[[(5-methyl-3-phenyl-4-isoxazolyl)carbonyl]amino]-7-oxo-4-thia-1-azabicyclo-[3.2.0]heptane-2-carboxylic acid, was obtained from the Sigma Chemical Company (catalogue ref. O-1002) and was used as received. The product conformed to the purity requirements of the British Pharmacopoeia and as such contained not less than 98.5% of the specified compound. Water was double-distilled, deionized, and degassed before use.

Conductivity measurements

The conductance was measured by using a specific conductivity meter (Kyoto Electronics, type C-117). The cell constant was determined with aqueous solutions of KCl over the appropriate concentration range using the molar conductivity data published by Shedlovsky [13] and Chambers et al. [14]. Water was progressively added to concentrated aqueous solutions of penicillin, of known molality, using an automatic pump (Metrhom, Dosimat, model 655). The measuring cell was immersed in a thermostatted bath, maintaining the temperature constant to within ±0.01 K. Temperature control was achieved using a Hewlett-Packard Vectra computer.

Static light scattering measurements

Static light scattering measurements were performed at 298.15 K using a Coherent DPSS 532 laser light scattering instrument equipped with a 0.5 W solid laser operating at 532 nm with vertically polarized light. The solutions were clarified by ultrafiltration through 0.45-μm filters with the ratio of light scattering at angles of 45° and 135° not exceeding 1.10. The refractive index increments of the oxacillin aggregates were measured at 298.15 K using a Mettler Toledo RA-510 M precision refractometer, giving a variation with concentration of 0.0787 kg mol^{-1}. No inflection in the refractive index was noted, and the same value of the refractive index was used for aggregates present over the entire concentration range.

Results and discussion

Critical concentrations

A representative plot of specific conductivity, κ, as a function of the molar concentration, c, for oxacillin in aqueous solution at 288.15 K is shown in Fig. 1. Similar plots were obtained at temperatures between 288.15 and 313.15 K. In all cases, the plots of κ against c show two breaks, corresponding to the first and second aggregation. According to the Williams method [15], the critical concentration is usually obtained from the intersection of the fitting lines of the conductivity–concentration plots above and below the breakpoint. For the criterion of the fit, the best correlation coefficient was chosen. The precision of the method depends on the width of the concentration range over which the change in the physical properties is observed. As seen in Fig. 1, where the specific conductivity (at 288.15 K) of oxacillin against concentration is plotted, this change is gradual. To obtain a value of the critical micellar concentration (cmc) by the Williams method, we used the plots of the specific conductivity versus the logarithm of the concentration shown in Fig. 2. Similar plots were obtained for the other temperatures.

The analysis of Figs. 1 and 2 shows the existence of two breakpoints in the conductivity, identified as critical concentrations and denoted by arrows. The first critical concentration represents a preassociation state, which can be associated with the formation of small primary aggregates. The second critical concentration may then self-associate the primary aggregate to form larger aggregates. In order to identify these two critical concentrations, we refer to the first concentration as the

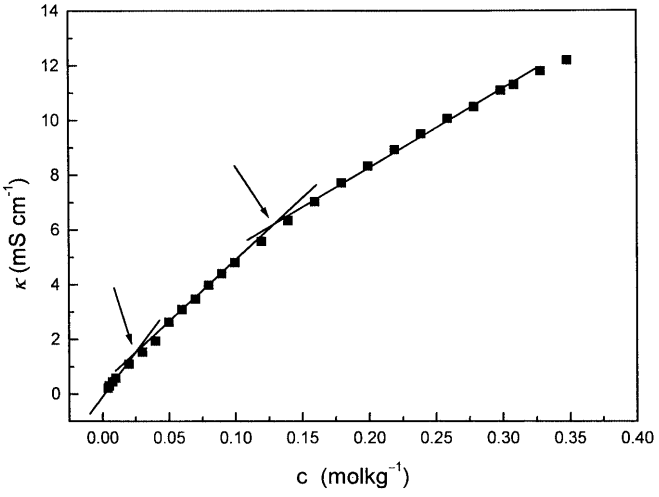


Fig. 1. Specific conductivity of oxacillin in water as a function of molal concentration at 288.15 K. The *arrows* denote the critical premicellar concentration (*cpc*) and the critical micellar concentration (*cmc*)

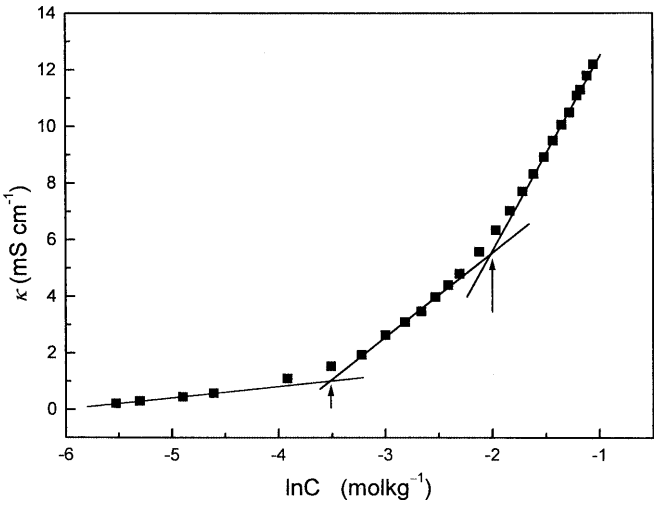


Fig. 2. Specific conductivity of oxacillin in water as a function of the natural logarithm of molal concentration at 288.15 K. The *arrows* denote the cpc and the cmc

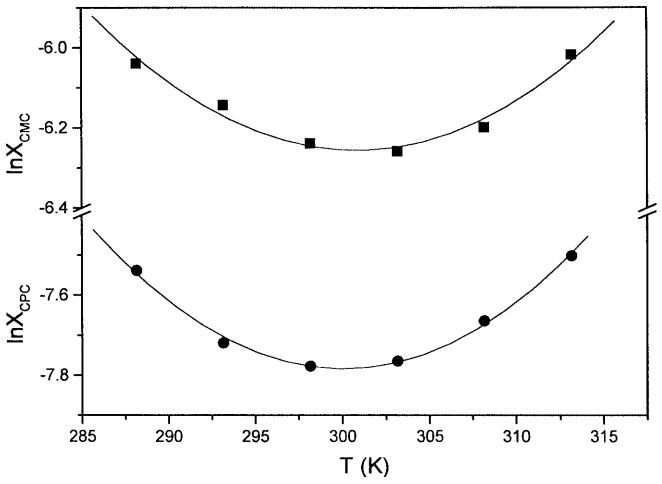


Fig. 3. Natural logarithm of cpc and cmc of oxacillin in water as a function of temperature. The *solid line* was calculated from Eq. (1)

critical premicellar concentration (cpc) and to the second as the cmc. The values obtained for the cpc are shown in Table 1, and we can see that these results are similar to those of other penicillins previously reported [9, 15]. In some drugs with similar characteristic features of association a model has been proposed [7, 12]. In this model, the drugs form trimers or tetramers at the cpc and, by self-assembly, side-to-side stacking results in micelles being formed at the cmc. The cmc values at each temperature are shown in Table 1.

The variation in the two critical concentrations of oxacillin with temperature is shown in Fig. 3. This plot was fitted to the equation

$$\ln X_{CC} = aT^2 + bT + c, \quad (1)$$

where X_{cc} is the cpc or the cmc expressed as a mole fraction and a, b, and c are the fitting constants of the best fit obtainable. These values are shown in Table 2. The curves pass through a minimum at 300 K at both critical concentrations.

As seen in Table 1, the values of the cpc and the cmc are lower than the same critical concentrations for the other penicillins. We can therefore deduce that oxacillin is more hydrophobic than other penicillins, such as cloxacillin, dicloxacillin, or flucoxacillin [7].

Aggregation characteristics

The plot of the scattering intensity, S_{90}, of the solution relative to that of toluene against the oxacillin concentration at 298.15 K is shown in Fig. 4. This plot shows discontinuities at two well-defined critical concentrations. The first critical concentration, cpc, with a value of 0.024 mol kg^{-1}, was determined as the intersection of the scattering curve and the theoretical monomer line (shown as a dashed line in Fig. 4), and represents the scattering from unassociated molecules. The second critical concentration, cmc, was taken at the inflection point of the scattering curve at higher concentration. A second inflection point has been reported for a wide range of surfactants, and has generally been interpreted

Table 1. Critical premicellar concentrations (*cpc*), critical micellar concentrations (*cmc*), standard Gibbs energies, enthalpies, and entropies of premicellization (ΔG_p^0, ΔH_p^0, ΔS_p^0) and micellization (ΔG_m^0, ΔH_m^0, ΔS_m^0), respectively, of oxacillin sodium salt at different temperatures

T (K)	cpc[a] (molkg^{-1})	cmc[a] (molkg^{-1})	ΔG_p^0 (kJmol^{-1})	ΔH_p^0 (kJmol^{-1})	ΔS_p^0 (Jmol^{-1}K^{-1})	ΔG_m^0 (kJmol^{-1})	ΔH_m^0 (kJmol^{-1})	ΔS_m^0 (Jmol^{-1}K^{-1})
288.15	0.0296	0.1327	–8.1 ± 0.4	19.7 ± 0.5	96.6 ± 0.9	–18.4 ± 0.4	38.0 ± 0.7	195.8 ± 10.2
293.15	0.0247	0.1196	–8.6 ± 0.3	12.0 ± 0.5	70.3 ± 0.8	–19.1 ± 0.4	23.9 ± 0.5	146.7 ± 9.2
298.15	0.0233	0.1087	–8.8 ± 0.3	3.7 ± 0.2	42.2 ± 0.5	–19.8 ± 0.4	8.7 ± 0.2	95.8 ± 5.4
303.15	0.0236	0.1066	–9.0 ± 0.5	–5.1 ± 0.2	12.7 ± 0.2	–20.2 ± 0.5	–7.5 ± 0.2	42.1 ± 2.3
308.15	0.0261	0.1132	–8.9 ± 0.4	–14.5 ± 0.4	–18.2 ± 0.3	–20.3 ± 0.5	–24.7 ± 0.5	–14.3 ± 0.8
313.15	0.0307	0.1357	–8.8 ± 0.4	–24.6 ± 0.5	–50.5 ± 0.6	–20.0 ± 0.5	–43.2 ± 0.8	–74.1 ± 6.1

[a]Estimated errors ± 0.0005

Table 2. Values of the parameters a, b, and c corresponding to Eq. (1) for the first critical concentration (cpc) and the second critical concentration (cmc)

Parameter	cpc	cmc
a (K^{-2})	143.4 ± 7.1	125.8 ± 14.9
b (K^{-1})	-1.01 ± 0.05	-0.878 ± 0.099
c	$1.7\times10^{-3} \pm 7.8\times10^{-5}$	$1.5\times10^{-3} \pm 1.6\times10^{-4}$

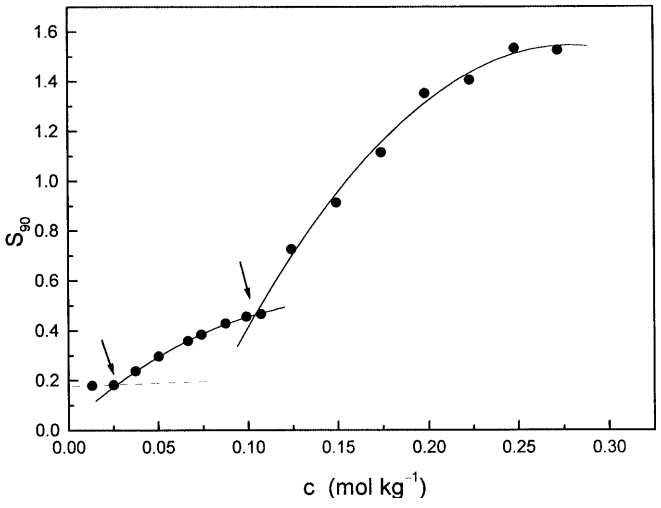


Fig. 4. Variation of the scattering ratio, S_{90}, with concentration, c, of oxacillin in water at 298.15 K. The *dashed line* represents the monomer. The *arrows* denote the cpc and the cmc

in terms of a transition from spherical to cylindrical micelles [1]. Amphiphilic drugs, notably those based on a phenothiazine ring system, also have two or three critical concentrations [16, 17], although, with such drugs, the additional critical concentration is thought to denote a restructuring of the stacked aggregates rather than a sphere-to-rod transition. The second distinct inflection in the scattering curve, occurring at a concentration of 0.104 mol kg^{-1}, was identified as the cmc. These critical concentrations are in good agreement with the values obtained from conductivity measurements.

The aggregation number, N_1, and the effective charge, z_1, corresponding to the cpc were calculated according to the Anacker and Westwell [18] treatment in which the light scattering from solutions of ionic aggregates is represented by

$$\frac{K'm_2}{\Delta R_{90}} = \frac{2m_3 + N_1^{-1}(z_1 + z_1^2)m_2}{\left[2N_1 + (2N_1)^{-1}(z_1 + z_1^2)f^2 - 2fz_1\right]m_3 + z_1 m_2}, \quad (2)$$

where ΔR_{90} is the Rayleigh ratio of the solution in excess to that of a solution at the critical concentration, m_2 is the molality of the micellar species in terms of monomer, m_3 is the molality of any supporting electrolyte, and $f = (\mathrm{d}n/\mathrm{d}m_3)_{m_2}/(\mathrm{d}n/\mathrm{d}m_2)_{m_3}$, with n the refractive index of solution. K' for vertically polarized light is defined by

$$K' = \frac{4\pi^2 n_0^2 (\mathrm{d}n/\mathrm{d}m_2)_{m_3}^2 V^0}{L\lambda^4}, \quad (3)$$

with n_0 being the refractive index of the solvent, V^0 the volume of solution containing 1 kg water, L is Avogadro's number, and λ the wavelength of the incident light (532 nm). Expansion of Eq. (2) in powers of m_2 leads to

$$\frac{K'm_2}{\Delta R_{90}} = A + Bm_2 + ..., \quad (4)$$

where

$$A = 4N_1\left[(2N_1 - fz_1)^2 + z_1 f^2\right]^{-1} \quad (5)$$

and

$$B = z_1 A(2m_3)^{-1}\left[(1 + z_1)N_1^{-1} - A\right]. \quad (6)$$

The properties of the aggregates formed at the cmc were determined by application of the general fluctuation theory of light scattering for a multicomponent system to surfactant solutions following the method of Anacker and Jacobs [19]. Estimation of the size of the aggregates formed at the cmc is more speculative. We assumed that these aggregates are formed by a single-step association of the aggregates present at the first critical concentration, and are in equilibrium with the primary aggregates. This method of treatment of the static light scattering data gives only an approximate indication of the size of the micelles formed at the cmc, assuming that the aggregates formed at the cpc are particles with charge z_1, which aggregate at the cmc, with an aggregation number N_2. We have previously applied this method to other systems exhibiting this type of association process, such as dicloxacillin and flucloxacillin [7], and penicillin V [9]. According to Stockmayer [20], the turbidity, τ, due to a micellar component of a three-component system with regard to the solvent is given by

$$\tau = \frac{C_3 + C_4 m_2'}{C_1 + C_2 m_2'}\left(Hn_2' 2V^0 m_2'\right), \quad (7)$$

where $H = 16\pi \mathrm{K}'/3$ and m_2' is the molality of the micellar species at concentrations above the cmc,

$$n_2' = (\partial n/\partial m_2')_{T,P,w_1,w_3},$$

$$C_2 = z_2(z_2+1)\phi m_2'/N_2,$$

$$C_3 = \{\theta(\theta+\phi)N_2 + z_2(z_2+1)$$

$$(f_1^2/N_2)[\theta/(\theta+\phi)] - 2f_1 z_2 \theta\}m_3,$$

$$C_4 = z_2\phi,$$

where T is the absolute temperature, P is the pressure, w_1

is the number of water molecules, w_2 is the number of micelles, $z_2w_2+\theta w_3$ is the number of counterions, θw_3 is the number of drug ions and coions, $f_1=N_2n_3/n_2$, is the aggregation number, and z_2 is the charge of the micelle. Expansion of Eq. (7) in powers of m_2' leads to

$$\frac{Hn_2'V^0m_2'}{\tau}=A+Bm_2', \tag{8}$$

where

$$A=\frac{C_1}{C_2}=\frac{\theta(\theta+\phi^2)N_2}{\theta(\theta+\phi)^2N_2^2+\theta(z_2+z_2^2)f^2-2\theta(\theta+\phi)z_2fN_2}, \tag{9}$$

$$B=\frac{(C_2-C_4A)A}{C_1}=\frac{\phi A\left(z_2+z_2^2-z_2AN_2\right)}{\theta(\theta+\phi)m_3N_2}. \tag{10}$$

A and B can be determined experimentally as the intercept and limiting slope, respectively, of the $Hn_2'V^0m_2'/t$ versus m_2' plot. By solving Eqs. (9) and (10) simultaneously, the following expressions for z_2 and N_2 are obtained:

$$z_2=\frac{\left[\theta\phi(\theta+\phi)^3m_3B\right]^{1/2}+\theta(\theta+\phi)fm_3B}{(\theta+\phi-fA)\phi A}, \tag{11}$$

$$N_2=\frac{(z_2+z_2^2)\phi A}{\theta(\theta+\phi)m_3B+z_2\phi A^2}. \tag{12}$$

The approximate mean aggregation number of the aggregates of oxacillin in the first association process from Eqs. (3), (4), (5), (6), and (7) was $N_1=2$. Although the curvature of the light scattering plot in the concentration region cpc < oxacillin concentration < cmc is indicative of aggregate charge, the precision of the data was not sufficient to permit a meaningful estimation of the effective charge, z_1, of these small aggregates. Application of Eqs. (8), (9), (10), (11), and (12) to the second aggregation process gave $n=4$. The analysis of the light scattering data indicates the formation of dimers at cpc = 0.024 molkg^{-1}, with a low charge due to counterion binding. In our data analysis, we speculate that the aggregates formed at the cmc result from the association of four of these primary aggregates and therefore have a mean aggregation number of $n=N_1N_2=8$. There is a similarity between the aggregation characteristics of oxacillin and other penicillins [7, 21].

Thermodynamics of self-association

The thermodynamic properties of the association process at the lower critical concentration (cpc) of oxacillin were derived by the application of the mass-action model, as shown in the following. The equilibrium constant, K_m, for the formation of aggregates may be written as [22]

$$K_m=\frac{[M^{p+}]}{[G^-]^{nv-p}[S^{v+}]^n}, \tag{13}$$

where G^-, S^{v+}, and M^{p+} represent the counterion, the surfactant ion, and micelles of aggregation number n and net charge p. In our case, for low aggregation numbers, we can use the expression [22, 23]

$$\frac{1}{K_m}=nv^{nv-p}\frac{[n(v+1)-p][2n(v+1)-2p-1]}{n(v+1)-p-2}\times\left\{\frac{[n(v+1)-p][2n(v+1)-2p-1]}{[n(v+1)-p-1][2n(v+1)-2p+2]}X_{CPC}\right\}^{n(v+1)-p-1}, \tag{14}$$

where v is the valence of the monomer and X_{cpc} corresponds to cpc as a mole fraction. To apply this equation, p/vn, the degree of ionization, α, of the aggregation must be calculated as the ratio of the gradient of conductivity against concentration above and below the critical concentrations [24]. The value obtained for α was 0.80. The value of the net charge of the aggregate may be expressed in terms of the degree of ionization and the aggregation number as $p=N\alpha$. N corresponds to the

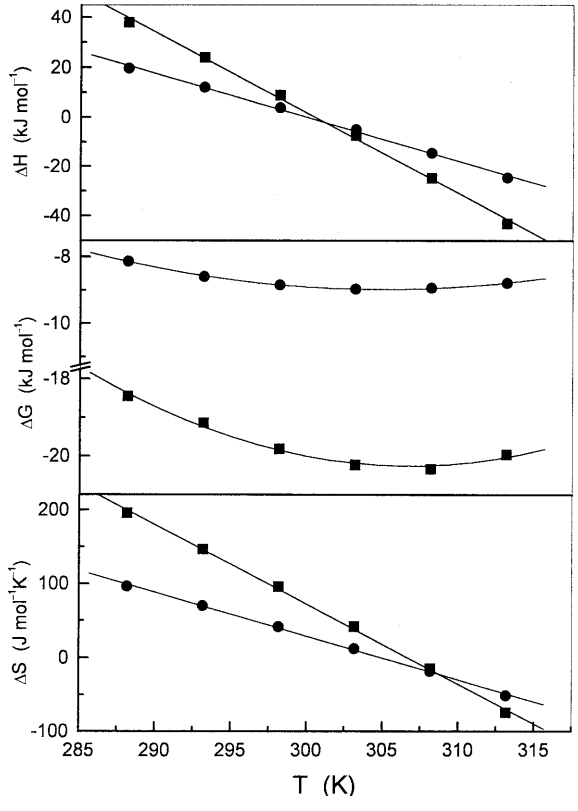


Fig. 5. Standard free energy, standard enthalpies, and standard entropies of aggregate formation per mole of monomer for oxacillin in water at different temperatures. The thermodynamic parameters for the two aggregations considered are represented: cpc (*circles*), cmc (*squares*)

value obtained by static light scattering measurements. In the second aggregation (we assume that this is micellization), the micellization constant is written as Eq. (14) with values for $n = N_1N_2 = 8$ and $\alpha = 0.67$, and X_{cpc} will be X_{cmc}, which is the cmc as a mole fraction.

The standard Gibbs energy change per mole of monomer, derived from the mass-action model, is given by

$$\Delta G_m^0 = -\frac{RT}{N}\ln K_m \tag{15}$$

and the standard enthalpy of aggregation follows from the application of the Gibbs–Helmholtz equation to Eq. (15), giving

$$\Delta H_m^0 = \left(\frac{\partial \Delta G_m^0/T}{\partial(1/T)}\right)_p = -\frac{RT^2}{N}\left(\frac{\partial \ln K_m}{\partial T}\right)_p. \tag{16}$$

The entropy of aggregation can hence be obtained from

$$\Delta G_m^0 = \Delta H_m^0 - T\Delta S_m^0. \tag{17}$$

The values of ΔG_m^0, ΔH_m^0, and $T\Delta S_m^0$ are given in Table 1 and plotted in Fig. 5. The use of Eqs. (15), (16), and (17) for thermodynamic parameters of micellization implies that the size, shape, and degree of ionization of the aggregates do not change with temperature and pressure. The values of the Gibbs free energy corresponding to the first aggregation (premicellization) are smaller than the Gibbs free energy of the second aggregation (micellization). These low values of the Gibbs free energy of aggregation in the two critical concentrations are of the same order as those derived for both short-chain surfactants [25] and for drugs [26], and indicates relatively weak amphiphilic character. For the premicellar concentration, this effect is more important, as seen in the lower value of the Gibbs free energy. The higher values of the Gibbs free energy at the second aggregation are consistent with the higher aggregation number in the micellization.

The change from positive to negative values of both ΔH_m^0 and ΔS_m^0 with increasing temperature suggests that at low temperatures the hydrophobic interactions are the major driving force in the aggregation and are associated with the release of structured water from hydrophobic hydration around the aromatic rings of the molecule. From a temperature of 300 K, the London dispersion is the dominant interaction. We can observe that ΔH_m^0 and ΔS_m^0 are higher than ΔH_p^0 and ΔS_p^0, which is in agreement with the behavior of the aggregation number.

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