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Self-Association of Penicillin V in Aqueous Solution

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The self-association of penicillin V in aqueous solution at 298.15 K has been examined by conductivity, density, ultrasound, static light scattering, and microcalorimetry techniques. Two critical concentrations were detected in conductivity, light scattering, and ultrasound data over the concentration range 0−0.35 mol kg^{-1}. Light scattering measurements indicate the formation of trimers at the first critical concentration (0.04 mol kg^{-1}) and subsequent formation of aggregates of aggregation number 12 at the second critical concentration (0.23 mol kg^{-1}). Analysis of the apparent molar volumes and isentropic apparent molar compressibilities has also provided evidence for limited association at concentrations below the first critical concentration. A model of the association process and possible structures of the primary and secondary aggregates have been proposed on the basis of an analysis of the thermodynamics of the self-association.

Introduction

Interest in the colloidal properties of the penicillin drugs dates from the work of McBain and co-workers,[1] Hauser et al.,[2] and Few and Schulman[3] in the late 1940s and early 1950s. These investigations, mainly on penicillin G, were strongly affected by surface active impurities which complicated the characterization of the self-association process. In recent years an interest in the properties of surface active drugs has been greatly renewed, particularly for the penicillin and phenothiazine drugs.[4−11] The colloidal properties of these amphiphilic compounds are largely determined by the nature of the aromatic ring systems of their hydrophobic moieties, and such compounds are useful in probing the relationship between molecular architecture and physicochemical properties. The importance of investigating the possibility of aggregation of bioactive molecules is demonstrated in the case of the penicillin drugs by the work of Funasaki et al.,[6] who have discussed the effect of self-association of these drugs on their bactericidal activity and chemical stability.

Light scattering studies of the micellar properties of several synthetic penicillins both in water and electrolyte solution[5] has shown limited association of penicillin V at high concentrations (0.24 mol kg^{-1}). In a study of the association of penicillin V by gel filtration chromatography a gradual increase in aggregation was reported in the presence of 0.15 mol dm^{-3} KCl with no apparent critical concentration.[6] In the present paper we present a more detailed study of the self-assembly of this penicillin in water using a range of experimental techniques. Particular attention has been paid to the possible existence of any second critical concentration which is a characteristic feature of some drugs, notably those based on a phenothiazine ring system.[9−11] Critical concentrations and aggregate size and charge have been determined by conductivity, ultrasound, and light scattering measurements; the apparent molal volume and adiabatic compressibilities of the aggregates have been calculated from the combination of density and ultrasound measurements. Values of the enthalpic gain on association predicted from a thermodynamic analysis of the self-assembly process using the mass action model have been compared with experimental values from microcalorimetry. On the basis of these studies, we have proposed a model for the self-association of this drug.

Experimental Section

Materials. Penicillin V (potassium salt) of at least 98.5% purity was obtained from Sigma Chemical Co. and was used as received. Water was doubled-distilled, deionized, and deaerated before use.

Conductivity Measurements. Conductivities were measured with a HP 4285A Precision LCR meter equipped with a HP E5050A colloid dielectric probe operating at a frequency of 200 kHz. The probe was specially designed to measure inductances and to avoid the polarization that occurs in normal condensor probes. The measurement cell design was conceived to obtain the highest degree of accuracy and consists of a cylinder of 8 cm diameter and 5 cm height with the probe entrance at the side. This geometry ensures the probe head to be always surrounded by at least 2 cm of solution during the measurement process, so avoiding possible interferences of the cell walls. The cell was immersed in a Techne model RB-12A water bath equipped with a Tempunit TU-16A thermostat. The temperature was monitored using an Anton Paar DT 100−30 thermometer

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and maintained at 298.15 ± 0.01 K. To homogenize the solution, a Variomag 20P shaker was used.

Calorimetric Measurements. The calorimetric measurements were performed at 298.15 K with a Beckmann 190B microcalorimeter. This is a twin differential calorimeter in which the heat produced in the reaction vessels is rapidly conducted through two surrounding thermopiles to an aluminum heat sink in which they are encased. The thermopiles surrounding each reaction vessel are wired in opposition in order that the thermoelectric response is a measure of the heat flux from the two vessels. The entire system is held in position by a yoke which can be rotated to mix the components in the reaction vessels, heat effects due to the rotation and friction in each vessel canceling. The calorimeter was calibrated as described by Pilcher et al.[12] The reaction vessel was charged with 1 g of solution of known molality and 1 g of water; the reference vessel contained 2 g of water. Hence, on mixing the final molality was reduced to half of its original value.

Static Light Scattering Measurements. Static light scattering measurements were made at 298.15 K with a Malvern PCS 100 light scattering instrument with vertically polarized light of wavelength 488 nm supplied by a 2 W argon ion laser (Coherent Innova 90). Solutions were clarified by ultrafiltration through 0.1 μm filters until the ratio of light scattering at angles of 45 and 135° did not exceed 1.10. A refractive index increment of 0.082 kg mol^{-1} was measured for the aggregates using an Abbé 60/ED precision refractometer (Bellingham and Stanley Ltd.). No inflection in the refractive index data was noted, and the same value of the refractive index increment was used for the aggregates present over the entire concentration range.

Density Measurements. Density measurements were made at 298.15 K using an Anton Paar DMA 60/602 vibration densimeter with a resolution of 10^{-6} g cm^{-3}. Temperature was controlled using a HETO Hetofrig CB 7 bath and a HETO DBT Hetotherm thermostat to within ±0.005 K. The uncertainty on the density measurements with this setup is about $\pm 3 \times 10^{-6}$ g cm^{-3}.

Ultrasound Velocity Measurements. Ultrasound velocity measurements were performed at 298.15 K at a frequency of 2 MHz using a Nusonic model 6380 concentration analyzer (Nusonics Inc.), with a temperature transducer connected to a Hewlett-Packard digital microvoltimeter 3455A. The sound velocity transducer was connected to a Hewlett-Packard multimeter 3437A giving an accuracy in the sound velocity of ±0.01 m s^{-1}. Each experimental point is the mean of 100 measurements of sound velocity. Temperature control was achieved using the same equipment as for the density measurements. The uncertainty on the sound velocity measurements with this setup is about ±0.05 m s^{-1}.

Results and Discussion

Critical Concentrations. Conductometric measurements on aqueous solutions of penicillin V were analyzed to detect the presence of any critical concentrations using the Phillips[13] definition of the critical concentration (cc) as the concentration corresponding to the maximum change in gradient in plots of the solution conductivity (κ) versus concentration (m):

$$\left(\frac{d^3\kappa}{dm^3}\right)_{m=cc} = 0 \qquad (1)$$

The numerical analysis of the data was made by means of a recently developed algorithm based on the Runge–Kutta numerical integration method and the Levenverg–Marquardt least-squares fitting algorithm which allows the determination of precise values of the critical concentrations of drugs and surfactants of low aggregation number.[14] The results of the analysis are shown in Figure 1, which plots the measured conductivity of penicillin V and a Gaussian fit of its second derivative obtained by the previously mentioned algorithm against molal concentration. This figure shows the existence of two minima of the second derivative of conductivity and therefore of two critical concentrations for penicillin V. The value obtained for the second critical concentration (cc_2) was 0.233 mol kg^{-1} which is similar to the previously reported[5] value (0.240 mol kg^{-1}) for the critical micelle concentration of penicillin V from light scattering measurements. In addition, the conductivity plots of Figure 1 show a clear inflection at 0.045 mol kg^{-1} which represents a previously unreported lower critical concentration cc_1.

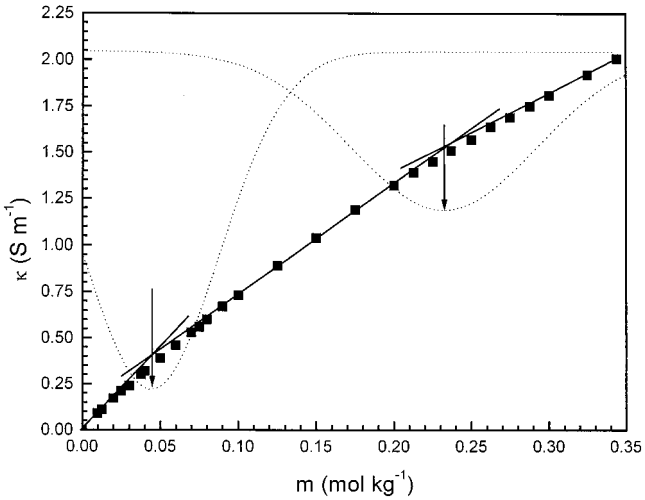

Figure 1. Specific conductivity of penicillin V in water as a function of molal concentration at 298.15 K. The dotted line corresponds to the Gaussian fit of the second derivative. Arrows denote the critical concentrations.

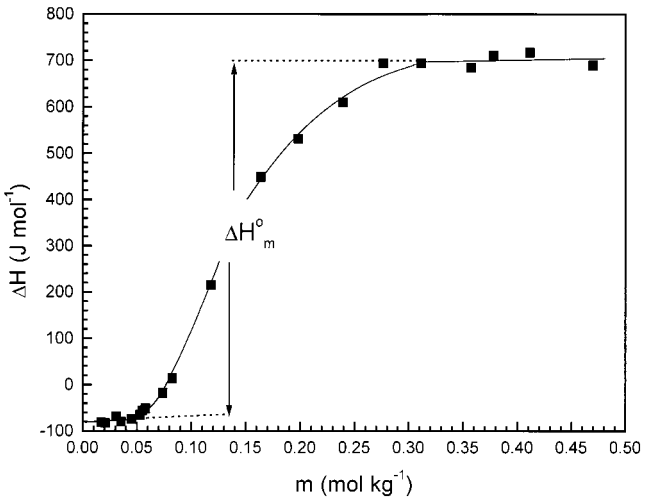

Figure 2. Enthalpies of dilution of penicillin V in water as a function of molal concentration at 298.15 K.

Thermodynamics of Association

Figure 2 shows the experimentally measured enthalpies of dilution of penicillin V in water at 298.15 K. Three concentration regions can be distinguished. For $m < 0.05$ mol kg^{-1} a slight linear increase of ΔH is observed suggesting only very limited association at these concentrations; the transition zone extending over the concen-

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tration range $0.05 < m < 0.3$ is indicative of amphiphilic association; for concentrations higher than 0.3 mol kg^{-1} linear behavior is restored suggesting the existence of stable aggregates. The broad transition region commenced at the lower critical concentration and covered the concentration region between the two inflection points detected in the conductivity data. As a consequence of the broad width of this region, which is a typical feature of micellar systems of low aggregation number,[15] it was not possible to distinguish two aggregation processes by means of microcalorimetric measurements. The enthalpy of the association processes of penicillin V from the difference of the values of the standard enthalpy of dilution of the micellar and monomeric states[16] is $\Delta H^\circ_m = 0.76 \pm 0.02$ kJ mol^{-1}.

The thermodynamic properties of the association process at the lower critical concentration of penicillin V were derived by the application of the mass action model as follows. The equilibrium constant K_m for the formation of the aggregates may be written:[13,17]

$$\frac{1}{K_m} = N\frac{(2N-z)(4N-2z-1)}{2N-z-2} \times \left[\frac{(2N-z)(4N-2z-1)}{(2N-z-1)(4N-2z+2)} X_{cc_1}\right]^{2N-z-1} \quad (2)$$

Here z is the net charge of the aggregate, which may be expressed in terms of the degree of ionization (α) and the aggregation number (N) as $z = N\alpha$, and X_{cc_1} is the first critical concentration as a mole fraction. In the calculation of the equilibrium constant of aggregation, values of $N = 3$ (from light scattering; see below) and $\alpha = 0.6$, calculated as the ratio of the mean gradients of conductivity against concentration above and below the critical concentration,[18] were used. A standard Gibbs energy change on aggregation, ΔG°_m, of -12.7 kJ mol^{-1} was calculated from the experimental data from microcalorimetry and the mass action equation:

$$\Delta G^\circ_m = -\frac{RT}{N}\ln K_m \quad (3)$$

This low value of the Gibbs free energy of aggregation is of the same order of those derived for short-chain surfactants[15] and indicates a relatively weak amphiphilic character. The positive ΔH°_m value suggests that the association processes of penicillin V are driven by hydrophobic interactions[16] which are associated with the release of structured water from hydrophobic hydration around the aromatic rings of the molecule and which are thought to be a major driving force for micelle formation. However, the low absolute value of the enthalpic gain indicates a partial compensation of the caloric fluxes in the two aggregation processes, which may be explained in terms of the existence of a mixed hydrophobic−hydrophilic dehydration mechanism involved in the association of penicillin V. Because of the inability of the microcalorimetric technique to distinguish two association processes, it was necessary to use techniques of higher sensitivity to further characterize the association of this drug.

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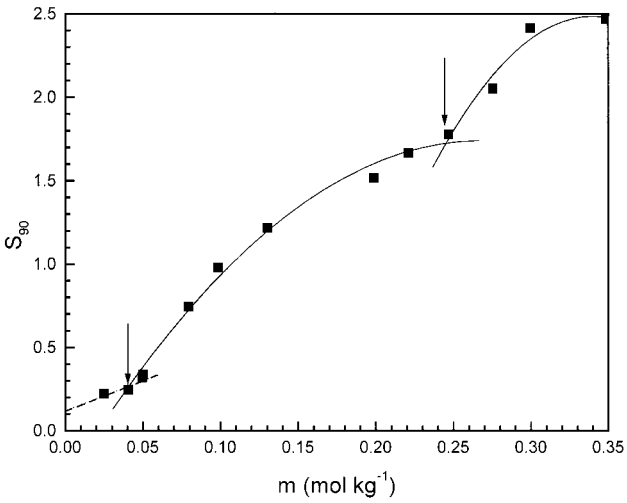

Figure 3. Variation of the scattering ratio, S_{90}, with molality, m, for penicillin V in water at 298.15 K. - - - indicates the theoretical line for unassociated monomers. Arrows denote the critical concentrations.

Aggregation Numbers. The concentration dependence of the light-scattering ratio, S_{90} (intensity of light scattered by the solution relative to that obtained from benzene), shows abrupt discontinuities at two well-defined critical concentrations as shown in Figure 3. The first critical concentration (cc_1) was determined as the intersection of the scattering curves and the theoretical monomer line (represented by a dashed line in Figure 3) giving a value was 0.04 mol kg^{-1}. The second distinct inflection in the scattering curve occurring at a concentration of 0.246 mol kg^{-1} was identified as the second critical concentration cc_2. These critical concentrations are in good agreement with the values from conductivity.

The aggregation number, N_1, and the effective charge, z_1, corresponding to the first critical concentration were calculated according to the Anacker and Westwell[19] treatment in which the light scattering from solutions of ionic aggregates is represented by

$$\frac{K'm_2}{\Delta R_{90}} = \frac{2m_3 + N_1^{-1}(z_1 + z_1^2)m_2}{[2N_1 + (2N_1)^{-1}(z_1 + z_1^2)f^2 - 2fz_1]m_3 + z_1 m_2} \quad (4)$$

where ΔR_{90} is the Rayleigh ratio of the solution in excess of 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 $f = (dn/dm_3)_{m_2}/(dn/dm_2)_{m_3}$. K' is defined by

$$K' = 4\pi^2 n_0^2 (dn/dm_2)_{m_3} V^0/L\lambda^4 \quad (5)$$

with n_0 being the refractive index of the solvent, V^0 the volume of solution containing 1 kg of water, L the Avogadro number, and λ the wavelength of the incident light (488 nm). Expansion of eq 5 in powers of m_2 leads to

$$\frac{K'm_2}{\Delta R_{90}} = A + Bm_2 + \ldots \quad (6)$$

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where

$$A = 4N_1\,[(2N_1 - fz_1)^2 + z_1 f^2]^{-1} \tag{7}$$

and

$$B = z_1 A(2m_3)^{-1}[(1 + z_1)N_1{}^{-1} - A] \tag{8}$$

The properties of the aggregates formed at the second critical concentration were determined by application of the general fluctuation theory of light scattering by multicomponent system to surfactant solutions following the method of Anacker and Jacobs.[20] This method of treatment of the light scattering data, which we have previously applied to other systems exhibiting this type of secondary association,[4] gives only an approximate indication of the size of the aggregates formed at cc_2 and assumes that the aggregates formed at the first critical concentration are particles with charge z_1, which aggregate at the second critical concentration. According to Stockmayer[21] the excess turbidity due to a micellar component of a three-component system with regard to the solvent is given by

$$\tau = \frac{C_3 + C_4 m_2'}{C_1 + C_2 m_2'}\,(H n_2'^{\,2} V^0 m_2') \tag{9}$$

where $H = 16\pi K'/3$ and m_2' is the molality of the micellar species at concentrations above cc_2,

$$n_2' = (\partial n/\partial m_2')_{T,P,w_1,w_3} \qquad C_1 = \theta(\theta + \phi)m_3$$

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

$$C_3 = [\theta(\theta + \phi)\,N_2 + z_2(z_2 + 1) \times (f^2/N_2)(\theta/(\theta + \phi)) - 2fz_2\theta]m_3$$

$$C_4 = z_2\phi$$

T is the absolute temperature, P is the pressure, w_1 is the number of water molecules, w_2 is the number of micelles, $zw_2 + \theta w_3$ is the number of counterions, ϕw_3 is the number of surfactant ions and co-ions, $f = N_2 n_3/n_2$, N_2 is the aggregation number, and z_2 is the charge of the aggregate. Expansion of eq 9 in powers of m_2' leads to

$$\frac{H n_2'^{\,2} V^0 m_2'}{\tau} = A + B m_2' + \ldots \tag{10}$$

where

$$A = \frac{C_1}{C_3} = \frac{\theta\,(\theta + \phi^2)N_2}{\theta\,(\theta + \phi)^2\,N_2{}^2 + \theta\,(z_2 + z_2{}^2)\,f^2 - 2\theta\,(\theta + \phi)\,z_2 f N_2} \tag{11}$$

$$B = \frac{(C_2 - C_4 A)A}{C_1} = \frac{\phi A(z_2 + z_2{}^2 - z_2 A N_2)}{\theta\,(\theta + \phi)m_3 N_2} \tag{12}$$

A and B can be determined experimentally as the intercept and limiting slope, respectively, of the $H n_2'^{\,2} V^0 m_2'/\tau$ versus m_2' plot. By solving equations (11) and (12) simultaneously the following expresions for z_2 and N_2 are obtained:

$$z_2 = \frac{[\theta\phi\,(\theta + \phi)^3 m_3 B]^{1/2} + \theta(\theta + \phi)\,f m_3 B}{(\theta + \phi - fA)\phi A} \tag{13}$$

$$N_2 = \frac{(z_2 + z_2{}^2)\phi A}{\theta(\theta + \phi)m_3 B + z_2\phi A^2} \tag{14}$$

The approximate mean aggregation number of the aggregates of penicillin V in the first association process from eqs 4−8 was $N_1 = 3$. Although the curvature of the light scattering plots in the concentration region $cc_1 < m < cc_2$ 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 eq 9−14 to the second aggregation process gave $N_2 = 4$. The indications therefore from the analysis of the light scattering data are of the formation of trimers at $cc_1 = 0.04$ mol kg^{-1} with a low charge due to counterion binding. In our data analysis we speculate that the aggregates formed at cc_2 result from the association of four of these primary aggregates and therefore have a mean aggregation number $N = N_1 N_2 = 12$. There is a similarity between the aggregation characteristics of this penicillin drug and those of the phenothiazine drugs, the aggregates of which are thought to form from tetrameric units in dilute solution.[7]

Apparent Molar Volumes. The concentration dependence of the apparent molar volume was calculated from experimental density measurements by means of the equation

$$\phi_v = \frac{M}{\rho} - \frac{1000(\rho - \rho_0)}{m\rho\rho_0} \tag{15}$$

where M is the molecular weight of penicillin V, ρ the density of the solution, m the molality, and ρ_0 the density of pure water (0.997043 g cm^{-3} at 298.15 K).

Assuming that solutions of surfactants behave as singly dispersed systems up to the critical concentration, the apparent molar volumes may be described in the first premicellar region by the equation[22]

$$\phi_V = \phi^0{}_V + A_V m^{1/2} + B_V m \tag{16}$$

where ϕ_V^0 is the standard (infinite dilution) apparent molar volume, A_V is the Debye−Hückel limiting law coefficient, and B_V is an adjustable parameter equivalent to the second virial coefficient that measures the deviation from the limiting law due to nonelectrostatic solute−solute interactions. For 1:1 electrolytes in water at 298.15 K, $A_V = 1.868$ cm^3 kg$^{1/2}$ mol$^{-3/2}$ [22] and B_V is generally negative except for hydrogen-bonding interactions.[23] The excess function $\phi_V^{EX} = \phi_V - A_V m^{1/2}$ is plotted in Figure 4 against molal concentration and shows two discontinuities. The first, at 0.03 mol kg^{-1}, is in agreement with the cc_1 values from light scattering and conductivity; the second, at $m \approx 0.11$ mol kg^{-1}, although at a lower concentration than the cc_2 values from these techniques, is nevertheless clearly associated with the second association process. A

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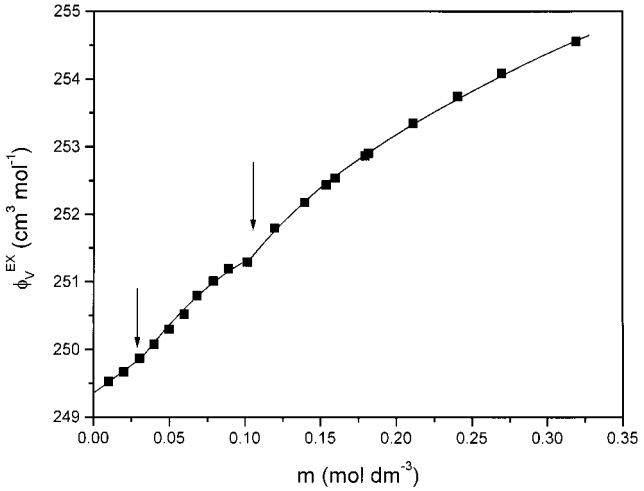

Figure 4. Excess apparent molar volume of penicillin V in water as a function of molal concentration (see text). (■) represents the calculated values of this function from the experimentally measured data. The arrows denote the critical concentrations.

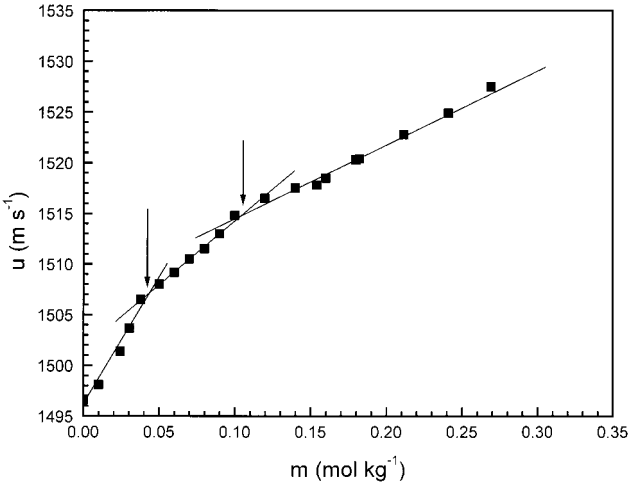

Figure 5. Variation of ultrasound velocity with molal concentration in aqueous solution of penicillin V at 298.15 K. The arrows denote the critical concentrations.

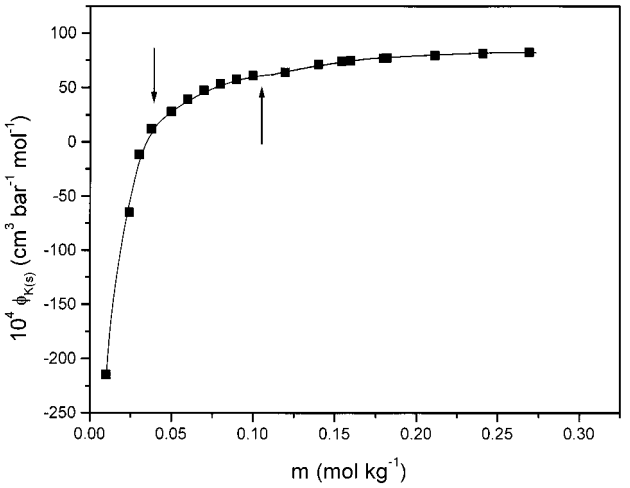

Figure 6. Isentropic apparent molar compressibility of penicillin V in water at 298.15 K. The arrows denote the critical concentrations. (N.B.: bar = 10^5 Pa.)

discrepancy between cc_2 values from the different experimental techniques has been noted previously[10] and possibly indicates that the second association process occurs over a wide concentration range, its first detection by a particular technique depending on the sensitivity of that technique and the solution property being measured. The fitted values of ϕ_V^0 and B_V in the preassociation region ($m < cc_1$) were 249.4 cm^3 mol^{-1} and 25.25 cm^3 kg mol^{-2}, respectively. The large positive value of the limiting law deviation parameter B_V is a possible consequence of hydrogen-bonding interactions in the preaggregation region which lead to the formation of trimers at cc_1 so establishing the role of nonelectrostatic solute−solute interactions in the aggregation of penicillin V. On the other hand, Figure 4 shows the characteristic behavior of the partial molar volume of a typical surfactant which increases above the critical concentration as a result of a decrease of the hydration shell around the hydrophobic part of the amphiphilic molecules. The increment is appreciably lower for the second association process at cc_2 suggesting a mixed dehydration mechanism including both polar dehydration of the side chains and hydrophobic dehydration of the aromatic ring. These results confirm the differing nature of the association processes at the two critical concentrations and support the interpretation of the low enthalpic gain obtained by microcalorimetric measurements given above.

Ultrasound Velocity Measurements and Isentropic Compressibilities. The association pattern of penicillin V was also studied using the ultrasound velocity technique. A plot of the ultrasound velocity against molality of penicillin V solution is shown in Figure 5. This plot shows three concentration regions which are clearly distinguished by two discontinuities. The three linear segments of Figure 5 correspond to the monomeric state, a primary aggregate state, and a secondary aggregate state. The first critical concentration, determined from the intersection of the monomer and primary aggregate state segments, occurred at 0.044 mol kg^{-1}, in good agreement with the cc_1 values from other techniques; a second critical concentration is observed at 0.104 mol kg^{-1}, a value similar to the cc_2 from Figure 4.

Density and ultrasound velocity measurements were combined to calculate adiabatic compressibilities of penicillin V solutions using the Laplace equation[22]

$$k_S = \frac{10^6}{\rho u^2} \tag{17}$$

where k_s is the adiabatic compressibility coefficient expressed in bar^{-1} when the ultrasound velocity u is expressed in cm s^{-1} and the density in g cm^{-3}. Figure 6 shows the isentropic apparent molar compressibilities calculated from the above compressibility values by means of the equation[22]

$$\phi_{K(S)} = \frac{1000}{m\rho_0}(k_S - k_S^0) + k_S\phi_V \tag{18}$$

where k_s^0 is the isentropic molar compressibility of the solvent. From this plot it is possible to determine the values of the apparent molar compressibilities of the associating species, $\phi_{K(S)}^{s,i}$, as the value of the apparent molar compressibility at the ith critical concentration, and of the corresponding aggregate, $\phi_{K(S)}^{m,i}$, as the saturation value of the compressibility[23,24] for each aggregation process. The

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associating species of the second association process are assumed to be the primary aggregates, and hence, $\phi_{K(S)}^{s,2} = \phi_{K(S)}^{m,1}$. Previous studies have shown that $\phi_{K(S)}$ is large and negative for ionic compounds in water, positive for mainly hydrophobic solutes, and intermediate (small and negative) for uncharged hydrophilic solutes such as sugars.[25−28] Therefore, the isentropic apparent molar compressibilities of penicillin V shown in Figure 6 confirm (a) the initial ionic character of the free molecules, since the singly dispersed phase is characterized by large and negative values ($\phi_{K(S)}^{0} \approx -250 \times 10^{-4}$ cm^3 bar^{-1} mol^{-1}), (b) the slightly hydrophobic character of the aggregating species involved in the formation of the primary aggregate ($\phi_{K(S)}^{s,1} \approx 10 \times 10^{-4}$ cm^3 bar^{-1} mol^{-1}), (c) the clearly hydrophobic character of the primary aggregate itself ($\phi_{K(S)}^{m,1} = \phi_{K(S)}^{s,2} \approx 65 \times 10^{-4}$ cm^3 bar^{-1} mol^{-1}), which we consider as the associating species of the second association process, and (d) the hydrophobic character of the aggregates resulting from the second association process, where the compressibilities reached the value $\phi_{K(S)}^{m,2} \approx 80 \times 10^{-4}$ cm^3 bar^{-1} mol^{-1}. Similar high and positive values of the isentropic apparent molar compressibilities, previously encountered for alcohols in nonaqueous solvents,[25] have been explained in terms of van der Waals interactions between solute and solvent and imply that the micellar interior resembles a bulk liquid phase.[29]

As in the case of the apparent molar volumes, the changes in the apparent molar compressibilities of the two association processes are both positive, confirming the predominant role of the decrease of hydrophobic hydration in the association of the monomers of this drug. The large increment in the compressibility of the aggregates which is observed in dilute solution suggests appreciable dehydration of the aromatic rings of the penicillin V molecule during the first association process such as might occur as a consequence of vertical stacking of the molecules in the aggregate with the polar groups and the side chains arranged around the periphery of the stack. This type of association has been suggested for phenothiazine drugs and other penicillins on the evidence from high-field NMR studies.[4,8] The increment is much lower in the second association process (≈33% of the first one) suggesting that in this aggregation process both hydrophobic and polar dehydration occurs. This might occur for example as a result of a horizontal stacking of the primary aggregates, where the involvement of the side chains would result in the simultaneous decrease of both polar and hydrophobic hydration shells around the chain.

Summary

This study has provided experimental evidence of a two-stage self-association process for penicillin V in aqueous solution, an initial association occurring in dilute solution in addition to the aggregation at higher concentrations previously reported in the literature.[5] This initial association stage leads to the formation of small primary aggregates which may then self-associate to form larger aggregates at a second critical concentration, corresponding to the reported CMC. These association processes are mainly driven by hydrophobic interactions as shown by thermodynamic analysis of the increments of Gibbs energy and enthalpy of aggregation. The hydrophobic origin of both processes has been confirmed from changes in the apparent molar volume and the isentropic apparent molar compressibility derived from experimental density and ultrasound velocity measurements. Large incremental changes in these quantities during the initial association stage suggest that hydrophobic dehydration is mainly responsible for the formation of the primary aggregates, whereas the much smaller increments during the second association step imply that hydrophobic dehydration is partially compensated by a polar dehydration of the side chains. On the basis of this evidence and from comparisons with the behavior of similar drugs, we have proposed a model of the association process in which four vertically stacked trimers, formed at the first critical concentration, self-assemble by side-to-side stacking to form larger aggregates at the second critical concentration.

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