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On the Heat Precipitation of Poly(L-proline)[1]

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ABSTRACT: The temperature dependence of the second virial coefficient of poly(L-proline) in water and in trifluoroethanol is analyzed. This analysis yields the entropy and enthalpy of dilution parameters which are in turn applied to the inverted solubility observed in water. These thermodynamic data together with a previous conformational analysis lead to the conclusion that this process represents a liquid–crystal transition, which is similar to the dilute solution crystallization of long-chain molecules, wherein the polymer undergoes a cooperative structural change from a statistical to an ordered conformation.

Dilute aqueous solutions of poly(L-proline) exhibit the unusual property of inverted solubility in that a precipitate, or concentrated polymer phase, is formed on heating while the mixture becomes homogeneous upon cooling.[3–8] It has also been shown[7,8] that the precipitated polymer is in the ordered crystalline state. Although inverted solubility is not a unique property of macromolecules in general, or poly(L-proline) in particular, it has only been observed in aqueous solutions for this polymer. In other solvents the more conventional type of solubility behavior is found. Moreover, it has also been shown that the optical properties of the homogeneous solution, such as optical rotatory dispersion and circular dichroism, do not sensibly change up to the point of precipitation.[7,8] These observations have led to discussion and argument in the literature with respect to the detailed nature of the precipitation process.[6,7]

In one point of view[6] it was concluded that the process was very similar to a liquid–crystal phase transformation wherein initially, in the dilute solution, the polymer molecules adopt a statistical or "random coil" conformation. By appropriate variations of the thermodynamic environment, crystallization occurs from the dilute solution with conformational and molecular ordering taking place. This process is thus identical with the dilute solution crystallization of synthetic long-chain molecules.[9] Alternatively, it has been argued,[7] based on theoretical conformational analyses[10] and experimental studies of relatively low molecular weight polymers,[7] that the molecule in solution is in a highly extended rodlike conformation. Consequently, it was proposed[7] that the precipitation process represented the kind of phase transition discussed theoretically by Onsager[11] and by Flory[12] which results from the geometrical asymmetry of the solute molecules. For this process there would be no molecular conformational change during the phase transition.

Although the latter point of view was supported by the interpretation of the optical rotatory dispersion measurements and the observation of a crystalline solid phase, a firm understanding of the phenomena requires quantitative thermodynamic data and an experimental determination of the chain conformation. With the recent availability[13] of high molecular weight samples of poly(L-proline), it has become possible to obtain the necessary experimental data. A conformational study, carried out in a standard manner, has indicated that the characteristic ratio for this polymer is in the range of 14–20, depending on the nature of the solvent.[13] It thus becomes clear that the anticipated highly extended chain conformation is not observed. Furthermore, since the high molecular weight sample also exhibits heat precipitation in water, a reexamination of this problem clearly becomes in order. In the present paper we analyze the temperature dependence of the second virial coefficient of poly(L-proline) in water and in organic solvents (where precipitation does not occur) in an effort to clarify the nature of the phase transition and to place it in the proper physical–chemical framework.

Results and Discussion

The determination of the second virial coefficient for a high molecular weight poly(L-proline) sample (M_n = 53,000, M_w = 99,000), as a function of temperature, in water and

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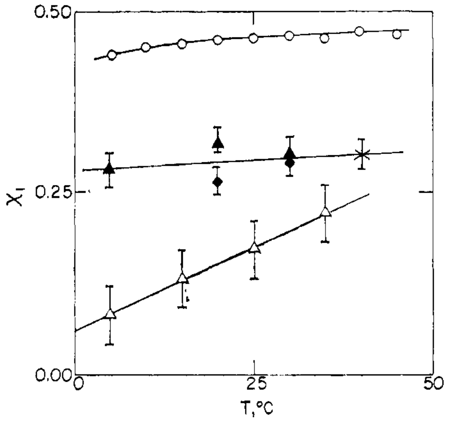

Figure 1. χ_1 in water (○), acetic acid (◆), propionic acid (▲), and trifluoroethanol (△).

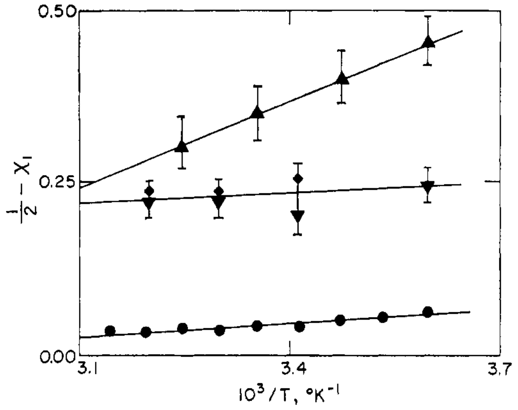

Figure 2. $1/2 - \chi_1$ *vs.* $1/T$ in water (●), acetic acid (◆), propionic acid (▼), and trifluoroethanol (▲).

in trifluoroethanol, acetic acid, and propionic acid has recently been reported.[13] An analysis of these data allows for a discussion and delineation of the factors influencing the heat precipitation in water. The polymer–solvent interaction parameter χ_1 can be obtained from the second virial coefficient, by utilizing the Orifino–Flory theory[14] as is summarized in

$$A_2 = \frac{2^{5/2}\pi N}{3^3\Phi}\frac{[\eta]}{M}\ln\left[1+\frac{\pi^{1/2}}{4}X_1\right] \qquad (1)$$

where

$$X_1 = \frac{3^3\Phi}{2^{1/2}\pi^{3/2}N}\frac{\bar{v}^2}{V_1}M\left(\frac{1}{2}-\chi_1\right) \qquad (2)$$

A_2 is the second virial coefficient, N is Avogadro's number, Φ is a universal constant, $\bar{v}$ is the partial specific volume of poly(L-proline), and V_1 is the molar volume of the solvent. The constant Φ was taken to be 2.1×10^{21} with $[\eta]$ expressed in dl/g, and $\bar{v}$ was taken to be 0.758 cm^3/g in all solvents.[5]

The calculated values for χ_1, for the four solvents studied, are presented as a function of temperature in Figure 1. The values for χ_1 are significantly greater in water as compared with the organic solvents. The two organic acids yield essentially the same results, while much lower values are obtained in trifluoroethanol. In the acidic solvents, χ_1 is essentially independent of temperature. On the other hand, in water, there is a slight increase of χ_1 with increasing temperature. This temperature coefficient is much more marked in trifluoroethanol. If χ_1 were calculated from the expression for the virial coefficient obtained from the Flory–Huggins theory,[15] namely

$$A_2 = \frac{\bar{v}^2}{V_1}\left(\frac{1}{2}-\chi_1\right) \qquad (3)$$

the results for water would be indistinguishable from those in Figure 1. Those for the organic solvents would be within the experimental error. This unusual agreement between the two methods results from the fact that the expansion coefficients are very close to unity for poly(L-proline) in the solvents studied.[13]

We note from Figure 1 that the value of χ_1 is less than 0.50 for all the solvents and temperatures studied. This value represents the θ condition, or the critical miscibility temperature for liquid–liquid phase temperature for a solution containing an infinite molecular weight chain in a statistical conformation. For a sample of a degree of polymerization of 1000, which is close to the weight-average degree of polymerization of the sample studied here, the critical value of χ_1 must be equal to or greater than 0.53.[15] Since the equilibrium dissolution temperature for poly(L-proline) is at least 10°,[6,8] and probably lower, it is clear that the thermodynamic criteria for liquid–liquid phase separation are not satisfied for this polymer in water. For phase separation in a dilute solution, involving highly geometrically asymmetric macromolecules, the value of χ_1 is expected to be much smaller.[12]

The interaction parameter χ_1 can be resolved into its enthalpy and entropy components by means of the relations[15]

$$\frac{1}{2} - \chi_1 = \psi_1 - \kappa_1 = \psi_1(1 - \theta/T) \qquad (4)$$

Here κ_1 and ψ_1 are the conventional enthalpy and entropy parameters.[15] These latter two parameters can thus be obtained from an analysis of the temperature coefficient of χ_1. In Figure 2, $(1/2 - \chi_1)$ is plotted as a function of $1/T$ in accordance with eq 4. It is found that propionic and acetic acid are essentially athermal solvents for poly(L-proline). The values of ψ_1, κ_1, and θ in water and in trifluoroethanol can be obtained, in principle, by the linear extrapolation of the data in Figure 2 to $(1/2 - \chi_1) = 0$. It is clear from the figure that a rather lengthy extrapolation is involved. Despite the fact that these parameters cannot be precisely evaluated, their signs and magnitudes can be obtained. This information is adequate for the problem at hand. The results obtained from this extrapolation are listed in Table I. For both solvents the θ temperature is predicted to be at or above their normal boiling points. In addition, both ψ and κ_1 are negative in each of the solvents with the absolute magnitude of each of these quantities being significantly larger in trifluoroethanol. Nonpolar polymers usually exhibit positive values for ψ_1 and κ_1 in nonpolar solvents.[16]

The quantitative requirements for the inverted solubility of a crystalline polymer, in terms of these parameters, can be obtained from the theoretical analysis of the problem due to Flory[17] which follows. The partition function for a mixture of n_1 monomeric solvent molecules and n_2 polymer

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TABLE I
THERMODYNAMIC PARAMETERS FOR POLY(L-PROLINE) IN WATER AND IN TRIFLUOROETHANOL

Solvent	θ, °C	ψ_1	κ_1 at 30°
Water	100	-0.16	-0.19
Trifluoroethanol	130	-0.9	-1.2

molecules comprising x segments per chain can be expressed as[12,18]

$$Q_M = \frac{q_1^{n_1} q_2^{n_2} [\epsilon(\epsilon - 1)]^{n_2} (n_1 + xn_2)!(n_1 + xn_2)^{-n_2(x-1)} Z^{n_2}}{n_1! n_2!} \times \exp\left\{\frac{-\chi_1 x n_1 n_2}{n_1 + xn_2}\right\} \qquad (5)$$

Here ϵ is the lattice coordination number, q_1 and q_2 are the respective internal partition functions, and Z is the chain configurational partition function. The number of segments per chain molecule is defined in the usual manner as the ratio of the molar volume of the polymer to that of the solvent. For present purposes, for high molecular weights, the chain configurational partition function can be expressed in completely general form as[19]

$$Z = z^{m-2} \qquad (6)$$

where m is the number of rotatable bonds per chain.[20]

The free energy change accompanying the process of dissolving n_2 moles of crystalline polymer in n_1 moles of solvent can be written as

$$\Delta G = RT\left(n_2 x \frac{V_1}{V_u} g - \ln Q_M\right) \qquad (7)$$

where RTg represents the free energy change per repeating unit that is associated with the disruption of the crystalline structure. The effects of chain disorientation and subsequent dilution are not included in this expression. These latter contributions are included in the partition function Q_M. The number of repeating units per chain is related to the number of segments per chain by the quantity xV_1/V_u. At equilibrium, the difference in chemical potential between dissolved and crystalline polymer, $\mu_2 - \mu_2^c$, must equal zero. By performing the necessary differentiation of eq 7 to obtain this difference in chemical potential, and setting the resultant equal to zero for phase equilibrium, there is obtained in the limit of high molecular weight ($1/x = 0$)

$$\left(\frac{V_u}{V_1}\right)[v_2 + \chi_1(1 - v_2)^2] + g = \beta \ln z \qquad (8)$$

Here, $v_2 = xn_2/n_1 + xn_2$ represents the volume fraction of polymer. The quantity β is equal to m/x', where x' is the number of repeating units per molecule. β is thus a constant for a given polymer chain independent of the solvent, and will have the value unity as a lower limit. Equation 8 can now be examined in more detail for the condition of phase equilibrium. In the limit of infinite dilution, we obtain

$$\left[\frac{(V_u/V_1)\psi_1\theta + b}{T}\right] = \beta \ln z - \left[\left(\frac{V_u}{V_1}\right)(^1/_2 - \psi_1) - a\right] \qquad (9)$$

where eq 4 has been substituted for χ_1, and g has been resolved into its entropic and enthalpic components so that

$$g = -a + b/T \qquad (10)$$

Equation 9 thus represents the condition for phase equilibria between the crystalline polymer and the solution, in the limit of infinite molecular weight and infinite dilution. We next examine this equation for physically meaningful solutions of T ($T > 0$) under the assumption that the quantities ψ_1, θ, a, and b are independent of temperature. We distinguish two cases depending upon whether the quantity $(V_u/V_1)\cdot\psi_1\theta + b$ is positive or negative.[21]

The quantity z is a monotonically decreasing function of $1/T$, varying from a maximum value at $1/T = 0$ to an asymptotic limit of unity as $1/T \to \infty$. Thus when $(V_u/V_1)\cdot\psi_1\theta + b$ is positive a single solution for T is possible only if $[(V_u/V_1)(^1/_2 - \psi_1) - a] \leq \beta \ln z_{\max}$. This case corresponds to conventional solubility. For the other case of interest where $[(V_u/V_1)\psi_1\theta + b]$ is negative, a single solution for T can be obtained only if $[(V_u/V_1)(^1/_2 - \psi_1) - a] > \beta \ln z_{\max}$. This again corresponds to the conventional solubility. However, if $[(V_u/V_1)\psi_1\theta + b]$ is still negative, but if $[(V_u/V_1)\cdot(^1/_2 - \psi_1) - a] \leq \beta \ln z_{\max}$ there is the possibility for the existence of two real solutions of T. If these two such solutions exist, then the lowest temperature will represent the one for inverted solubility.

With the foregoing analysis as a basis, we can examine the thermodynamic parameters that have been obtained to see if the solubility properties are explicable. The molar volume of an L-proline residue, V_u, is approximately 72. The molar volume for trifluoroethanol is also about 72, while it is about 18 for water. The first condition for inverted solubility, namely

$$(V_u/V_1)\psi_1\theta + b < 0 \qquad (11)$$

requires, utilizing the parameters of Table I, that b be less than 240 cal/repeating unit for water and less than 364 cal/repeating unit for trifluoroethanol. The value for the parameter b is not known, but it would be expected to be positive and small since it represents the intermolecular contribution to the enthalpy of fusion. We note that if this criterion is satisfied by water as a solvent it must also be satisfied by trifluoroethanol. The other requirement for inverted solubility that

$$(V_u/V_1)(^1/_2 - \psi_1) \leq \beta \ln z_{\max} + a \equiv K \qquad (12)$$

requires that K be ≥ 2.64 for H_2O and ≥ 1.40 for trifluoroethanol. Again if this condition is satisfied by water it must also be automatically satisfied by trifluoroethanol. Thus, although the necessary condition for inverted solubility (eq 11) can be established, the sufficiency criterion requires the independent determination of the parameters a, b, and $z_{\max}$.

If we wish to attribute the inverted solubility of poly(L-proline) in water to a liquid to crystal phase transition, we must then explain why a similar phenomenon is not observed in trifluoroethanol, since the same criteria are apparently satisfied. An insight into this apparent dilemma can be

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(20) For present purposes only the form of eq 6 is necessary. For chains with independent rotational potentials z is equal to the bond rotational partition function. For very high molecular weight chains, with interdependent rotational potential functions, z is the largest eigenvalue of the statistical weight matrix describing this interdependence.[19]

(21) For the special case where $V_u = V_1$, this distinction will be recognized as corresponding to either a positive or negative enthalpy of solution of the ordered chain.

obtained by examining the ratio of the two equilibrium temperatures by means of eq 9. We find that

$$\frac{T_1}{T_2} = \left(\frac{b - 364}{b - 240}\right)\left(\frac{K_2 - 2.64}{K_1 \quad 1.40}\right) \tag{13}$$

where the subscripts 1 and 2 refer to trifluoroethanol and water, respectively. The equilibrium temperature in H_2O can be taken to be approximately 273°K.[6,8] The ratio T_1/T_2 thus depends on the values of b, K_1, and K_2. If it is assumed that K_1 and K_2 do not vary with temperature, then certain conditions can be established. If $K \gg 2.64$ and b is restricted to the physically sensible range $0 < b < 240$, then T_1/T_2 will be much greater than unity and the equilibrium solubility temperature in trifluoroethanol could easily exceed its boiling point. Only if K approaches 2.64 does the ratio T_1/T_2 approach or become less than unity. Under these circumstances heat precipitation would be expected in trifluoroethanol. Although a rationale can be established for the difference in solubility behavior of poly(L-proline) in trifluoroethanol and in water, a quantitative basis cannot be established without the independent determination of the other parameters that are involved.

The thermodynamic data reported here rule out the possibility of liquid–liquid phase separation (with subsequent crystallization of the polymer in the concentrated phase) as the nature of the phase transition involved. Furthermore, since the molecules of high molecular weight do not adopt an extended rodlike conformation, phase separation by the process described theoretically by Onsager[11] and by Flory[12] cannot be operative either. The only other known process that could describe the phenomena is a liquid to crystal transformation, in dilute solution, wherein the polymer undergoes a cooperative structural change from a statistical to an ordered conformation. The above analysis indicates that the thermodynamic data are consistent with the requirements for this type of transition and reasons can be postulated as to why it is not observed in trifluoroethanol.

A phase separation, thermodynamically similar to the heat precipitation of poly(L-proline) in water, is exhibited by cellulose nitrate in dilute ethanol solution.[22] In this case, phase separation also occurs upon heating, at temperatures below θ, and ψ_1 is negative (in the range -2 to -4). A liquid to crystal phase transition is occurring in this case also. The rate of phase separation is strongly temperature dependent here as it is in the heat precipitation of poly(L-proline).[7,8]

The previous conclusion that the origin of the heat precipitation phenomena resides in the Onsager–Flory theories is clearly in error.[7] At that time, investigations were limited to theoretical conformational analysis and to hydrodynamic studies of low molecular weight polymers. These led to the conclusion of an extended molecular conformation in solution. Optical rotatory dispersion studies[3,7] and the observation of a maximum in the circular dichroism spectra at 228 mμ were taken to support this conclusion. The fact that these spectra were maintained up to the point of precipitation appeared to give further support to this mechanism. However, more recent detailed studies have indicated similar optical properties for high molecular weight samples in water[13] and in concentrated $CaCl_2$[23] solutions, where a statistical conformation is clearly established for the macromolecule. It was concluded, therefore, that the optical properties in dilute solution reflect a locally ordered structure rather than an extended, highly ordered molecular conformation. The molecule as a whole thus adopts a statistical conformation. Therefore, the overall molecular conformational change, required for the crystallization process, could take place but would not necessarily manifest itself in any changes in the optical properties.

Acknowledgment. We wish to thank Professor P. J. Flory for making available to us, many years ago, his calculation for the inverted solubility of crystalline long-chain molecules and granting us permission to include it in the present paper.

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Thermodynamic Properties of Liquids, Including Solutions. V. Poly(propylene oxide) in Carbon Tetrachloride

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ABSTRACT: The author's new theory of the thermodynamic properties of solutions is applied to poly(propylene oxide) + CCl_4 solutions, using experimental data by Kershaw and Malcolm. Good agreement is obtained for excess volumes and excess entropies and, for volume fractions up to one-half, for the interaction parameter χ. Certain entropy factors, not allowed for in the elementary theory used, are discussed as possible causes of the deviations at higher concentrations.

The writer's new theory[1–5] of the thermodynamic properties of solutions can reasonably be expected to be applicable, with some modifications, to many polymer solutions. Several systems for which appropriate data are in the literature are now being studied to test this applicability. In a preliminary report[2] the rubber + benzene and poly(propylene oxide) + carbon tetrachloride systems were dealt with. The present paper gives more details of the application of the theory to the latter system, with some minor changes in

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