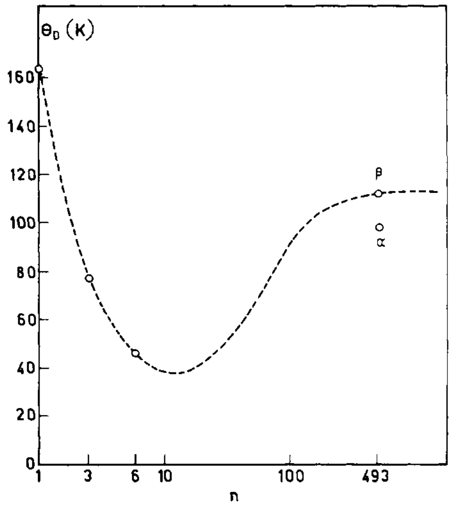

Figure 2. Debye temperatures θ_D (for $r = 1$) vs. n (the chain length) for the L-alanine series. α and β refer, respectively, to the α-helical structure and the β-pleated-sheet form of poly(L-alanine). The dashed line is simply an interpolation.

is found (Figure 1), whereas the Debye–Tarasov model, which introduces a vibrational anisotropy, does not work, as demonstrated in polypeptides.[2,3]

Now we can make a comparison of the characteristic vibrational temperatures found for the two series of compounds, namely, the alanine and the valine amino acids and peptides.

The ratio of the Debye temperatures $k = \theta_{D_{Val}}/\theta_{D_{Ala}}$ gives the following: amino acids,[3] $k = 0.84$; hexapeptides, $k = 0.84$; polypeptides (β sheets),[3] $k = 0.74$.

Thus, the hexapeptides are homologous with the amino acids in that they are isomorphous molecular solids with a Debye temperature smaller for the valine series because of a steric effect due to the bulky isopropyl group. This result has to be compared with those from vacuum-ultraviolet circular dichroism[14] and infrared absorption[13,15] studies which have already shown that in the *t*-Boc-(L-X)$_n$-OMe (X = Ala and Val) series in the solid state the β conformation is well developed at the alanine tripeptide ($n = 3$) and its development at the pentapeptide ($n = 5$) is essentially complete; conversely, the β conformation is fully obtained in the valine series only at the heptapeptide ($n = 7$).

This apparent discrepancy can be explained in the following way: the spectroscopic observations on small homopeptides give information about the local order in these molecular solids, whereas in specific heat experiments only the long-range order is detected. At very low temperatures only the long-wavelength acoustic phonons are excited and we detect structural details of the order of these wavelengths. Conversely, spectroscopic experiments[15] indicate the presence of new internal vibrations in the hexapeptides. Since the number of vibrational degrees of freedom is constant for a given solid, we have a progressive change from the molecular acoustical modes to those of polymerically associated forms.

This fact is confirmed by the Debye temperature variation vs. the number of peptide units for the L-alanine series (Figure 2). The θ_D values have been calculated by taking into consideration the acoustical phonons only. On the basis of all the results that we have collected on this series (see also ref 2 and 3), we conclude that there exists a minimum in the Debye temperature for an oligopeptide of higher rank. This means that the unit entropy goes through a maximum value on going from the molecular solids to the polymerically associated states because a more disordered state exists between them, around the hexapeptide level. To observe a new increasing order between peptide chains (characterized by a slightly different value of the given ratio) one should examine an oligopeptide with $n > 12$. It is of interest to mention that $n \simeq 12$ is the critical chain length for α-helix formation in the solid state for homopeptides derived from L-alanine, as shown by Fujie et al. by infrared absorption and X-ray diffraction techniques.[16] As pointed out above, the normal modes associated with the polymerically associated form become predominant around that stage of chain length.

In conclusion, the present specific heat measurements at low temperatures have confirmed the influence of a bulky lateral group on the cohesive energy of small peptides together with a similar vibrational behavior of these homopeptides. Furthermore, by analyzing the Debye temperatures variation vs. chain length in the alanine series we have shown that a polymerically associated structure may be present for the dodecapeptide: such a prediction has to be verified experimentally.

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Thin-Layer Chromatographic Comparison between Deuterated and Hydrogenous Polystyrenes

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Thin-layer chromatography (TLC) is a powerful method for separating molecules by the small structural differences which are often difficult to detect by other methods. Thus far, it has been successfully applied to macromolecules having differences in molecular weight, stereoregularity, microstructure, terminal group, and composition and sequential arrangement in copolymers.[1] It is then tempting to ask whether TLC is capable of separating isotopically different polymers.

Recently, extensive use of the deuterium-labeling techniques has been made in neutron scattering experiments.[2-4] In most cases, the scattering data are explained by assuming deuteration to give no thermodynamic effects on the system. On the other hand, there have already been several observations showing that deuterio and protio systems are not identical. Strazielle and Benoit[5] have

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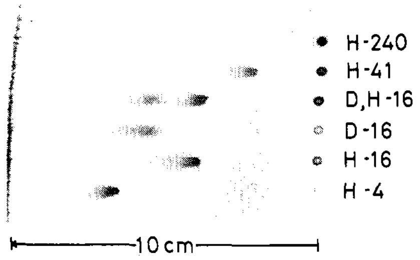

Figure 1. Phase separation TLC of D- and H-PS's. Developer, 2-butanone/acetone (45/55 by volume).

reported that the Θ temperatures of deuterated (D) polystyrene (PS) in hydrogenous (H) cyclohexane (CH) and of H-PS in D-CH are both different from that of the ordinary (i.e., H-PS/H-CH) system. Stehling et al.[6] have noticed differences in the melting temperatures of D- and H-polyethylenes (PE). In certain cases with crystalline polymers such as PE[7] and poly(ethylene oxide),[8] isotopic effects have manifested themselves as clustering of solute molecules dispersed in the matrix of isotopically unlike molecules. In this context also, TLC studies may be interesting.

Generally, TLC separation is effected either by the phase-separation mechanism or by the adsorption–desorption mechanism.[1] In the former, separation is controlled essentially by the solubility of a sample polymer in a developer solvent. The Θ temperature problem quoted above may be tested by the TLC of this type. In the latter mechanism, separation occurs through the competition between solvent and polymer molecules for active sites of the stationary phase, and thus it depends on the details of the energy fields of the molecules. In what follows, we will test PS systems by the TLC of the two types.

An anionically polymerized fully deuterated PS (herein coded D-16) was obtained through the kindness of Professor G. Allen. A gel permeation chromatographic analysis showed the weight-average molecular weight M_w to be 1.6 × 10^5 and the polydispersity index M_w/M_n to be about 1.25. Four Pressure Chemical standard polystyrenes (H-4, H-16, H-41, H-260) were used as protonous specimens, their molecular weights ($M_w \sim M_n$) being 4.0 × 10^4, 1.6 × 10^5, 4.1 × 10^5, and 2.6 × 10^6, respectively.

The TLC substrate used was silica gel 60H (E. Merck) coated on a glass plate. It was activated by heating to about 110 °C for 1 h.

Phase-Separation TLC. The mechanism of this type of TLC separation is not necessarily clear in the details. The following picture has been envisaged.[1] As a sample polymer is carried up the chromatoplate by the developer, the effective fraction of solvent in the sample zone becomes smaller and smaller for reasons such as solvent evaporation and trapping by the adsorbent. With a developer of poor solvency suitably chosen, thermodynamic phase separation will take place, even at a constant temperature, when the zone concentration arrives at a certain level, and the polymer molecules will be distributed into the gel and sol phases according to the molecular weight or solubility. The molecules in the sol phase will migrate at the same rate as the developer, while those in the gel phase will have a retarded migration, and thus a difference in the rate-of-flow R_f results. In order for this mechanism to work effectively, the developer should be higher in polarity than the sample so that the preferential solvation of the sample onto the stationary phase may be suppressed.

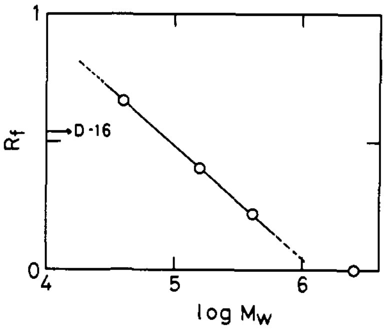

Figure 2. R_f vs. molecular weight for H-PS's (data as in Figure 1).

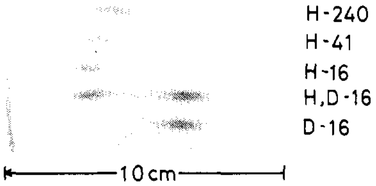

Figure 3. Adsorption TLC of D- and H-PS's. Developer, cyclohexane/benzene (32/68 by volume).

The D- and H-PS samples were tested by using as developer a mixture of 2-butanone (solvent for PS) and acetone (nonsolvent) of volume ratio 45:55. Figure 1 shows the chromatogram obtained. Clearly, sample D-16 has a larger R_f than the protonous equivalent H-16. This indicates that D-PS has a slightly better solubility than H-PS in this particular solvent.[9] We also note that a mixture of D-16 and H-16 has been separated satisfactorily into two discrete spots corresponding to the components.

The result seems to agree qualitatively with the observation of Strazielle and Benoit[5] that the deuteration of PS lowers the Θ temperatures in both H- and D-cyclohexanes. Interestingly, they also report data of the critical miscibility temperature T_c for the same systems, which indicate that in the H solvent the T_c of a D-PS corresponding to sample D-16 will be identified with the T_c of a H-PS of molecular weight about 7.7 × 10^4. In Figure 2, we have plotted R_f of the H-PS against log M. The data points for the three samples showing nonzero values of R_f fall on a straight line, as is usually the case with phase-separation TLC.[1] From the value of R_f of sample D-16, we find that the D-PS behaves in this TLC condition as a H-PS of molecular weight about 7.5 × 10^4. This implies a close relationship between the two independent experiments. Strazielle and Benoit[5] suggested that the solubility parameter of PS is lowered by deuteration. However, since the polymer solubility in a mixed solvent is not known, we are not sure that our result may be explained along these lines. Incidentally, the solubility parameters of cyclohexane, PS, 2-butanone, and acetone referred to from the literature[10] are 8.2, 9.1, 9.3, and 9.9 cal/mL$^{1/2}$, respectively. If a simple additivity rule holds as to the solubility parameter of a solvent mixture, D-PS should have a poorer solubility and hence smaller R_f than H-PS in the 2-butanone–acetone system.

Adsorption TLC. For this type of TLC, it has been established[1] that separation is controlled basically by the polarity of a sample relative to that of a developer, and the retention behavior of a polymer homologue depends little on the molecular weight if it is sufficiently large, say, $M > 10^4$.

Figure 3 shows the chromatograms obtained for the PS samples developed by a mixture[11] of cyclohexane (Θ solvent) and benzene (solvent) of volume ratio 32:68, which works as a good solvent for PS. Again, a marked difference is found between the chromatograms of the D and H samples. Contrarily to the phase-separation TLC, the D-PS shows a smaller R_f value than those of the H-PS's, the latter being nearly independent of molecular weight.

Clearly, the molecular weight is not an important factor in this range of M. We have to consider that the adsorbing power of a D-PS segment onto silica gel is slightly higher than that of an H segment. This suggests an intrinsic energetic difference between deuterium and proton, although we are unable to explain why it is so. This point should be confirmed by experiments for other polymers as well as low-molecular-weight compounds.

In summary, the TLC experiments of both the types have shown that isotopically different PS's are not identical thermodynamically. In practice, the differences between them may not be large enough to urge any modification for the interpretation of neutron scattering experiments[5] but are still large enough for one polymer to be isolated almost perfectly from the other. For separation purposes, adsorption TLC may be especially suitable since it is independent of molecular weight. We have recently constructed a rotating-disk-type TLC apparatus, in which a centrifugal field is employed to shorten the developing time, for the purpose of polymer fractionation on a semipreparative scale.[12] In a preliminary experiment with this apparatus, approximately 100 mg of a mixture of D- and H-PS's was nearly completely separated into the pure components within an hour when the same developer as used in the adsorption TLC experiments reported above was used.

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Linear Relation between Preferential Solvation and Intrinsic Viscosity of a Polymer in a Binary Solvent Mixture

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In previous papers[1-3] we proposed a relation between the coefficient of preferential solvation of a linear polymer and the segment density existing in the interior of the polymer coil. In this note we exhibit a linear relation between the coefficient of preferential solvation λ' and the segment density of samples dissolved in a solvent mixture of a given composition for the entire domain of molecular weights. Moreover, a better correlation of experimental results is achieved with a plot covering not only the entire domain of molecular weights but also the entire domain of compositions of the solvent mixture.

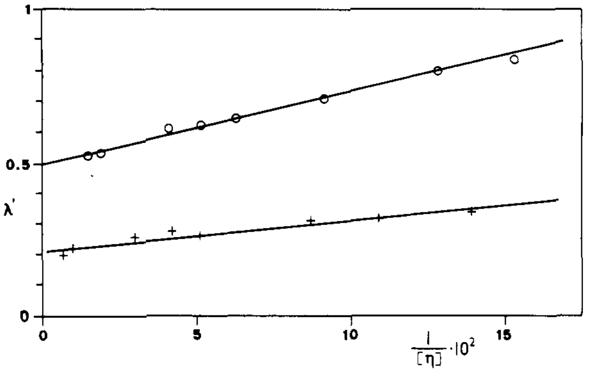

Figure 1. Application of eq 4 to linear PS samples in the mixture CCl_4 (solvent 1)/methanol (solvent 2): (+) $u_2 = 0.10$; (○) $u_2 = 0.21$.

A unique curve was obtained with linear and branched samples, even for the entire domain of compositions of the solvent mixture, plotting λ' as a function of a difference of segment density.[4-6] However, this relation was relatively complicated and knowledge of the intrinsic viscosity of each sample in a pure solvent of reference was necessary. The experimental results in this note, obtained by the light scattering technique, are taken mainly from our preceding papers. The method of treatment of these results is new.

Here the composition of the solvent mixture will be always expressed by the volume percentage of the second solvent (u_2) and the coefficient of preferential solvation λ' will be expressed, as in our preceding papers,[1,2] by the relation $\lambda' = (x_1/M)\bar{V}_1$, where x_1 is the number of molecules of solvent 1 absorbed in excess per macromolecular chain, M is the molecular weight of the chain, and $\bar{V}_1$ is the partial molar volume of solvent 1. The intrinsic viscosity will be expressed in cm^3/g.

Starting from the idea that the mean coefficient of preferential solvation λ' of a macromolecular chain is the normalized sum of the coefficients of each monomer unit and that the distribution of the monomers in the interior of the coil is Gaussian, we obtained[1,2]

$$\lambda' = \lambda'_\infty + a\left(\frac{3}{4\pi}\right)^{3/2}\left(\frac{N}{\langle R^2\rangle^{3/2}}\right) \tag{1}$$

and knowing that $\langle R^2\rangle = b^2N\alpha^2$, we have

$$\lambda' = \lambda'_\infty + a\left(\frac{3}{4\pi b^2}\right)^{3/2}\left(\frac{1}{N^{1/2}\alpha^3}\right) \tag{2}$$

In the above relations, λ'_∞ represents the value of the preferential solvation coefficient for an infinite chain, N the number of monomer units of the chain, b the length of monomer unit, R the radius of gyration, α the expansion coefficient, and a a constant.

Since it was known that N is proportional to the molecular weight M of the chain, relation 2 was fitted[1-3,7,8] to the experimental results, plotting $\lambda' M^{1/2}$ against $M^{1/2}$. This application is most nearly correct near the Θ point, where we have $\alpha \simeq 1$. If now we plot λ' as a function of $M^{-1/2}$, we observe that the points obtained with the samples of low molecular weight ($M < 15000$) do not fit on the

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