cording to which this quantity is treated as the tensor sum of contributions of constituent groups. These groups being fairly large, the distances between them are sufficient to lower mutual inductive effects which otherwise might vitiate this premise, as we have noted in the Introduction. The studies here reported demonstrate further the utility of optical anisotropies as sensitive indices of chain conformation and of molecular structure.[1,4]

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Notes

Static and Dynamical Properties of Polystyrene in Carbon Tetrachloride. 3. Effects of Freezing on Solution Properties[†]

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In a recent series of articles,[1–6] we reported our studies of static and dynamical properties of polystyrene in *trans*-decalin from around the Θ temperature to about 20 °C above the Θ temperature. We were interested in the effects of solvent quality on polymer solution properties and did a series of additional measurements using a high molecular weight polystyrene sample (TSK F-1500) and a fairly good solvent, carbon tetrachloride, as our model system.[7–10] In our preliminary investigation of the polystyrene/CCl_4 system, we observed an abnormal behavior in the intermediate concentration range ($C_1^* \sim 3M/N_A\rho_s 4\pi r_g^3$, with ρ_s, M, N_A, and r_g being the density, the molecular weight, Avogadro's number, and the radius of gyration, respectively) and suggested the possible presence of a pseudotransition behavior.[7,8] Subsequent studies[9,10] confirmed the existence of such an anomalous effect in terms of the characteristic frequency Ω (or $1/\tau$), the characteristic length L_{app}, and the osmotic compressibility $(\partial\pi/\partial C)_{P,T}$ in the neighborhood of C_1^*. However, there were difficulties in achieving quantitative results in the neighborhood of this anomaly because of the long equilibration time required to obtain reproducible results. In the intermediate concentration range, where the irregular behavior had occurred, we often encountered unpredictable difficulties. In this Note, we have carried out a range of experiments trying to find out the reasons for the anomaly. The observed strange behavior in both static and dynamical properties of polystyrene in carbon tetrachloride is related to the usually accepted freeze-drying procedures used in biopolymer preparations.

Experimental Methods

Polystyrene, TSK standard F-1500, was purchased from Toyo Soda Manufacturing Co. Ltd., Japan. We used the same sample as that reported previously.[5–10] Reagent grade carbon tetrachloride was dried over CaH_2, distilled under N_2, and filtered through a Teflon filter of 0.22-μm nominal pore diameter. As CCl_4 has an unusually high density, dust-free sample solutions were prepared by substituting the solvent from benzene to CCl_4. The sample cells were then prepared according to the following different procedures.

1. **Flame-Sealed Samples.** After addition of CCl_4, the solution portion of the light scattering cell was dipped in liquid nitrogen, a vacuum was pulled, and the top was sealed by flame. As we were dealing with a high molecular weight polystyrene sample, we permitted mixing of the polymer with the solvent only by mildly shaking the cell several times a day. Solutions at different concentrations were contained in individual sealed cells.

2. **Dilution Method.** Two types of cells were used to study the concentration dependence of polystyrene in CCl_4 by dilution: (i) a cell equipped with a Mininert valve and (ii) a cell with a ground-glass joint. The Mininert valve permits addition of solvent without exposing the solution in the light scattering cell to the atmosphere. We permitted about 7 days for the initial solution of the freeze-dried polystyrene to be dissolved in the dried, distilled, and filtered CCl_4 during each run.

Measurements. We used light scattering spectrometers operating at $\lambda_0 = 488$[11] and 632.8 nm. Temperature was controlled and measured to 0.01 °C in both spectrometers.

Results and Discussion

The TSK F-1500 polystyrene (PS) sample had a reported molecular weight of 1.4×10^7. During our PS/*trans*-decalin studies,[5,6] we determined a molecular weight of 1.2×10^7. A least-squares fit of $1/P(x) - [\lim_{C\to0} HC/R^{\mathrm{vv}}]M_w$ (with $P(x) = 2(e^{-x} - 1 + x)/x_2$, $x = K^2 r_g^2$, and $K = (4\pi/\lambda)\sin(\theta/2)$) yields $r_g = 1.65 \times 10^3$ Å and $M_w = 8.9 \times 10^6$, which agrees with a separate and independent determination of our present polystyrene sample using methyl acetate as the Θ solvent.

The freeze-drying process has obviously broken up the high molecular weight polystyrene sample and decreased its molecular weight from 1.4×10^7 to 1.2×10^7 to finally 9×10^6. In the 0.01–0.02 wt % concentration range, sealed PS/CCl_4 samples showed anomalies of a maximum in $(\partial\pi/\partial C)_{P,T}$ and a minimum in L_{app} near 0.015 wt % polystyrene as shown in Figures 1 and 2, but the samples prepared by the dilution method showed no anomalous behavior over the entire concentration range. Results of the sealed PS/CCl_4 solutions fluctuate more over the entire concentration range when compared with those using the dilution method but are reproducible.

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The anomalous behavior in the intermediate concentration range was repeated after a 3-month period. It is interesting to note that the minimum in L_{app} is reproducible while the maximum in $(\partial\pi/\partial C)_{P,T}$ is not always reproducible. The values of $(\partial\pi/\partial C)_{P,T}$ differ by a factor of 10 as shown in Figure 2, but those values at concentrations away from the anomaly remain essentially the same. We also repeated measurements on a sample, at ~0.01 wt %, that was prepared about 2 years ago[8] and obtained the same anomalous behavior. The present study is essentially in agreement with previous findings.[9,10] The method of preparation has a profound effect on the static properties of polymer solutions in terms of the osmotic compressibility $(\partial\pi/\partial C)_{P,T}$ and the apparent characteristic length in the intermediate concentration range ($0.01 < C^* < 0.02$ wt %). The anomaly is not a pseudotransition behavior since it appeared only when we used the freeze-dry method, which includes freezing the partially dissolved polymer solution before flame sealing.

In order to check the method of solution preparation involving flame sealing, we took two samples (H33 and H55 in Figures 1 and 2) at concentrations near the minimum in L_{app} and the maximum in $(\partial\pi/\partial C)_{P,T}$. Sample H33 was subject to a quick freeze–thaw cycle four times, and the results show a dramatic decrease in $(\partial\pi/\partial C)_{P,T}$ and an increase in L_{app}. The apparent characteristic length, L_{app}, has increased to a value beyond the normal curve expected for solutions prepared by the dilution method while $(\partial\pi/\partial C)_{P,T}$ has retained a portion of its anomaly. The freeze–thaw cycle has no effect at other concentrations. It appears that the flame-sealed samples in the intermediate concentration range are in a metastable state that can remain unchanged for periods of years. However, in the concentration range where the large anomaly occurs, the freeze–thaw cycle is able to disrupt the metastable state. Furthermore, at those concentrations, large changes in the values of $(\partial\pi/\partial C)_{P,T}$ take place over periods of several weeks. In the 0.01–0.02 wt % concentration range, $(\partial\pi/\partial C)_{P,T}$ increases as a function of time, signifying a decrease in the absolute scattered intensity. Therefore, the observed anomaly is not due directly to undissolved polymers in solution.

We have tested the short-term stability of sealed polymer solutions in the intermediate concentration range by heating them at 55 °C for periods of 48–72 h. The anomaly remains. Thus the metastable state cannot be disrupted easily by warming the solution.

The suggestion of a metastable state for the flame-sealed polymer solutions in the intermediate concentration range came from differences in the static properties of the samples prepared by the two independent methods. The freeze–thaw cycle can change the values of a flame-sealed sample (H33) toward its equilibrium value. We broke the seals of two samples (H33 and H55), performed a series of studies by the dilution method, and obtained an apparent $A_2 \sim 1 \times 10^{-2}$ (cm^3·mol)/g and an apparent molecular weight $M_w \sim 5 \times 10^4$ for the H55 sample. The breaking of the seal has no effect on the values of $(\partial\pi/\partial C)_{P,T}$ and L_{app}. For the H33 sample that had gone through four freeze–thaw cycles, we obtained an apparent $A_2 \sim 2 \times 10^{-2}$ (cm^3·mol)/g and an apparent $M_w \sim 9 \times 10^6$. Furthermore, $\lim_{C\to 0} L_{\text{app}} \sim 3 \times 10^3$ Å, signifying the presence of larger size particles or molecules. The large values of A_2 cannot be accounted for because of low molecular weight. As $A_2 \sim 2.0 \times 10^{-4}$ (cm^3·mol)/g, the apparent A_2 (~10^{-2} (cm^3·mol)/g) obtained by dilution of the originally sealed solutions suggests that we have overestimated the concentrations of the polymers in solution in those sealed samples exhibiting an anomaly.

Table I
Height Dependence of the Diffusion Constant, Apparent Molecular Weight, and Apparent Radius of Gyration of a PS Sample (0.0147 wt %) at 25 °C

parameter	lower	upper	upper (after 4 freeze–thaw cycles)
$10^7\bar{D}$,[a] cm^2/s	5.96	3.70	0.955
M_w	5×10^5	11×10^5	10×10^6
L_{app}, Å	297	506	

[a] $\bar{D}$ is the z-average translational diffusion coefficient.

Finally, we took a flame-sealed sample at a PS concentration of 0.0147 wt % and waited for 2 weeks *without* shaking. We carefully inserted the solution into the light scattering spectrometer and measured both the angular distribution of scattered intensity and the time correlation function. Then we broke the seal, carefully removed the bottom two-thirds (by volume) of the solution, and repeated the measurements on the remaining solution before and after the solution was further subjected to four freeze–thaw cycles. The results are tabulated in Table I. The lower portion of the polymer solution has a high diffusion coefficient, suggesting the presence of lower molecular weight fractions, while the upper portion indicates the presence of higher molecular weight fractions, as $\bar{D}$ has decreased substantially. Upon four additional freeze–thaw cycles, $\bar{D}$ is further lowered, which signifies the possible dissolution of higher molecular weight fractions during the freeze–thaw cycle.

Conclusions

Freeze-drying of polystyrene tends to break up the polymer. In our studies, we have started with a TSK F-1500 polystyrene standard having a molecular weight of ~1.4×10^7. Over the past few years, the molecular weight has been reduced from 1.4×10^7 to 1.2×10^7 to finally 8.9×10^6 after several freeze-drying cycles.

By freezing the partially dissolved polymer solution before flame sealing the sample, we have created an anomalous state for polystyrene in CCl_4 in the intermediate concentration range. It is not a pseudotransition phenomenon since the anomaly is completely absent when we prepare the solutions by the dilution method, which requires no freezing step. The freeze has produced a new polystyrene/CCl_4 state, perhaps in gel form, that becomes essentially insoluble in CCl_4. As most of the polymer remains insoluble in the intermediate concentration range, the soluble portion of the polymer has a much lower molecular weight, and, in fact, the solution has a fairly dilute effective concentration. This conclusion is based on the fact that $(\partial\pi/\partial C)_{P,T}$ is unusually high, signifying a low concentration. Furthermore, the unusually high translational diffusion coefficient and the very small characteristic length suggest a low molecular weight polystyrene fraction. Therefore, the "metastable" state formed possibly by the entangled polystyrene coils with CCl_4 is composed of higher molecular weight polystyrene fractions. The larger dispersion, which cannot be detected by visual observation, is relatively stable and can remain separated from CCl_4 for periods of years and will not dissolve at 55 °C. Nevertheless, smaller dispersions could be soluble after periods of several weeks because the solution yields more reproducible results after it has been equilibrated over long periods of time.

The creation of this metastable (and perhaps gel) state by freezing the polymer solution has led us to suspect that

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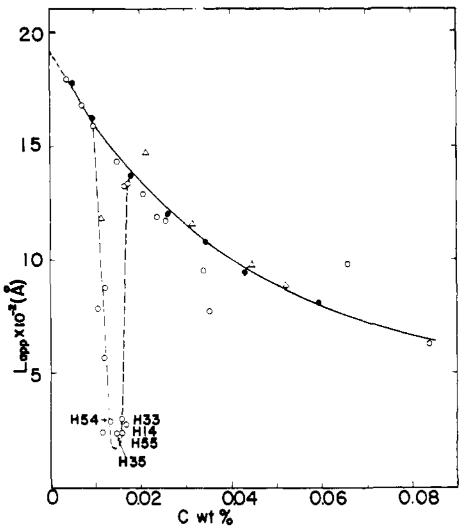

Figure 1. Plot of L_{app} vs. concentration for PS (TSK F-1500) in CCl_4 at 25 °C: (○) sealed samples prepared separately over a period of several months and measured at λ_0 = 632.8 nm; (●) solutions at various concentrations prepared by dilution and measured at λ_0 = 632.8 nm; (△) results from ref 10.

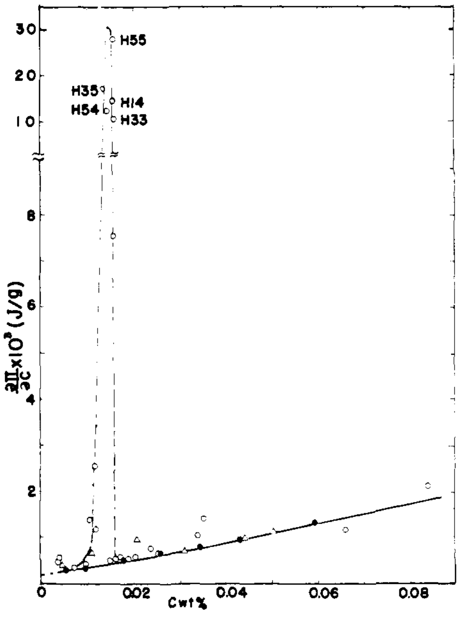

Figure 2. Plot of $(\partial\pi/\partial C)_{P,T}$ vs. concentration for two different methods of sample preparation at 25 °C: (○) sealed polymer solutions; (●) polymer solutions prepared by the dilution method; (△) results from ref 10. λ_0 = 632.8 nm.

we have to reexamine some of the accepted methods of polymer solution preparation. We must always be cautious with respect to possible gel formation, and freezing should surely be avoided.

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Measurement of the Specific Refractive Index Increment of Polyelectrolytes in Aqueous Salt Solutions with the Chromatix KMX-16 Differential Refractometer

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The absolute value of the molar mass of polyelectrolytes can be determined from the Rayleigh light scattering intensity of their aqueous solutions with additional low molar mass electrolytes but an accurate value of the limiting specific refractive index increment at constant chemical potential of the electrolyte, $(\Delta n/\Delta C_p)_{\mu_s}{}^{\circ}$, is required.[1,2] Usually a series of polyelectrolyte solutions of different macromolecular concentrations C_p are equilibrated through a semipermeable membrane with the same electrolyte solution of equilibrium concentration C_s. This electrolyte solution is also used as the reference solvent for the measurement of the refractive index (RI) increment, Δn, in a differential refractometer. The manufacturer of the Chromatix KMX-16 laser differential refractometer recommends[3] for the determination of Δn to multiply ΔX, the difference between the corrected instrument reading for solution and solvent, by the instrumental constant k obtained from calibration with different NaCl solutions. The refractive index increments of those solutions with respect to pure water can be established through the accurate RI measurements of NaCl solutions by Kruis.[4] A linear relation between Δn and ΔX is a prerequisite for such a procedure. We have found that this linearity cannot be established over the total range of this refractometer, however, and therefore the specific RI increment of polyelectrolyte solutions should be determined in a slightly different way than usually performed.

We have used 33 different NaCl solutions (prepared from NaCl (Merck, Urtitersubstanz) dissolved in conductivity water) over a broad range of concentrations ($0.46 < C_s < 3.6$ g/100 g of H_2O) for the calibration of the refractometer. Some of the solutions were measured again after a few days. The Δn values were calculated from a cubic equation in C_s fitted to the data of Kruis (after interpolation to λ_v = 633 nm, the wavelength of the laser used) corresponding to seven different concentrations of NaCl ($0.33 < C_s < 6.9$ g/100 g of H_2O).

$$\Delta n/C_s = (1.735 \pm 0.002) \times 10^{-3} - (2.7 \pm 0.2) \times 10^{-5}C_s + (1 \pm 2) \times 10^{-6}C_s^2 \quad (1)$$

This equation differs from that given by Chromatix[3] as we did not use the two lowest NaCl concentrations measured by Kruis, the Δn of which may be less accurate. The difference between the two equations is not significant, however, and our least-squares fit turns out to be slightly better. The result of the calibration is shown in Figure 1, where $\Delta n/\Delta X$ is plotted against ΔX. In the same figure have also been represented the values of $\Delta n/\Delta X$ measured for several NaCl concentrations, $C_s < 0.4$ g/100 g of H_2O ($\Delta X < 5000$). It is clear that ΔX is *not* a linear function of Δn over the total range investigated. For $\Delta X < 5000$ the value of $\Delta n/\Delta X$ increases with ΔX, contrary to what is observed for $\Delta X > 5000$. The reproducibility for $\Delta X < 10000$ is poor. The data for $10000 < \Delta X < 45000$ could be fitted to a cubic equation in ΔX

$$\Delta n/\Delta X = (1.3858 \pm 0.0009) \times 10^{-7} - (3.2 \pm 0.8) \times 10^{-14}\Delta X + (8 \pm 15) \times 10^{-20}\Delta X^2 \quad (2)$$

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