Hydrophobic Polyampholytes

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ABSTRACT: Polyampholytes were prepared by amidation of various alternating maleic anhydride copolymers of methyl, propyl, and butyl vinyl ethers with *N,N*-dimethyl-1,3-propanediamine. Isoelectric points were determined from the minima in the reduced viscosity against pH plots. While the addition of a simple electrolyte decreased the steepness of these curves, it did not affect the position of the minima. The fluorescence of potassium 2-toluidinonaphthalene-6-sulfonate (TNS) and *N*-phenyl-1-naphthylamine (NPN) was enhanced little, if at all, by the methyl and propyl polyampholytes but very substantially by the butyl polyampholyte, indicating the presence of micellar hydrophobic clusters in the last. For anionic TNS the maximum fluorescence enhancement occurred on the acidic side of the isoelectric point, indicating that both hydrophobic and ionic forces affected the binding; for electroneutral NPN the maximum fluorescence enhancement occurred at the isoelectric point. Two very low molecular weight samples of butyl polyampholyte showed greatly reduced fluorescence enhancements, indicating that the degree of polymerization affected micelle formation.

Introduction

It has been shown that in aqueous solution the behavior of the polyacids that result from the hydrolysis of alternating copolymers of maleic anhydride and alkyl vinyl ethers depends critically on the size of the alkyl group. The copolymers containing methyl, ethyl, or propyl vinyl ether behave as ordinary polyacids, exhibiting normal random coil conformations. The butyl, pentyl, and hexyl copolymers, however, display compact micellar conformations at low degrees of ionization, due to hydrophobic interactions of the alkyl groups. As the ionization is increased by the addition of base, these latter copolymers undergo conformational transitions to random coil states.[1-3]

We thought it worthwhile to explore whether these differences in behavior would also appear in corresponding polyampholytes. Such polyampholytes may be prepared by the amidation of the maleic anhydride–alkyl vinyl ether copolymers with *N,N*-dimethyl-1,3-propanediamine [$(CH_3)_2NCH_2CH_2CH_2NH_2$]. We want to report here the results of viscosity and fluorescent probe experiments performed on the methyl, propyl, and butyl polyampholytes.

Experimental Section

The high molecular weight maleic anhydride copolymers of propyl and butyl vinyl ether were samples prepared by J. Andrechak and S. Marvasti, respectively, following a procedure described previously.[1] The methyl copolymer (Gantrez AN-139), obtained from GAF Corp., was purified by precipitation from a tetrahydrofuran (THF) solution into pentane. The intrinsic viscosities of these methyl, propyl, and butyl copolymers in THF at 25.0 °C were 1.18, 1.12, and 2.06 dL g^{-1}, respectively. Two butyl copolymer samples in the low molecular weight range were prepared by a previously described method.[4] Their intrinsic viscosities in THF at 25.0 °C were 0.050 and 0.136 dL g^{-1}, corresponding to number-average degrees of polymerization of 10 and 30, respectively.[4]

To prepare polyampholyte, a tenfold stoichiometric excess of *N,N*-dimethyl-1,3-propanediamine was added slowly with stirring to a 1% solution of maleic anhydride–alkyl vinyl ether copolymer in THF at room temperature. After 24 h the solution was added to 3 times its volume of pentane. The precipitated polyampholyte was centrifuged, vacuum-dried, and dissolved in water. After dialysis, first against HCl at pH 3 to remove any unreacted diamine and then against neutral distilled water to remove all chloride ion, the solution was freeze-dried. Elemental analyses indicated that the conversions for the high molecular weight methyl, propyl, and butyl copolymers were 74, 85, and 92 mol %, respectively. The conversions for the two low molecular weight butyl copolymer samples were 100 mol %. All polyampholytes were soluble in water over the whole pH range (2–12) studied.

Viscosities were measured at 25.0 °C with a Cannon–Ubbelohde viscometer in a bath thermostated to within 0.01 °C. Solutions of polyampholyte were made up to be 0.008 monomolar (moles of alkyl vinyl ether monomer groups per liter). The pH was adjusted with 0.10 M LiOH and HCl solutions and measured with a Radiometer Model PHM-26 pH meter.

Fluorescence emission spectra were obtained at 25.0 °C with a Hitachi–Perkin-Elmer MPF-3L fluorescence spectrophotometer. The apparatus was adjusted to record corrected spectra by using the emission spectrum of Rhodamine B as standard. Excitation and emission slits were set at 6 nm. The fluorescent probes employed were potassium 2-toluidinonaphthalene-6-sulfonate (TNS) and *N*-phenyl-1-naphthylamine (NPN). Both TNS (Aldrich Co.) and NPN (Eastman Kodak Co.) were used without further purification.

Polyampholyte solutions employed in fluorescence measurements ranged in concentration from 2×10^{-4} to 2×10^{-2} monomolar. TNS concentrations were brought to 1.5×10^{-5} M by adding 10 μL aliquots of a 4.5×10^{-3} M stock solution to 3-mL portions of polyampholyte solutions. TNS concentrations were measured with a Cary 17 spectrophotometer by their absorbance at 317 nm, where the molar absorption coefficient, ε, equals 1.89×10^4 M^{-1} cm^{-1}.[5] The excitation wavelength was 366 nm, and the emission was measured covering the range 380–580 nm.

NPN concentrations were brought close to 5×10^{-6} M by adding 10-mL aliquots of freshly prepared stock solutions in methanol to 3-mL portions of polyampholyte solutions. The NPN concentrations of the stock solutions were obtained from the absorbance at 335 nm of solutions prepared by 300-fold dilution into a 1:9 ethanol–water mixture. The molar absorption coefficient of NPN is 8.7×10^3 M^{-1} cm^{-1} under these conditions.[6] The excitation wavelength was 340 nm, and the emission was measured over the range from 350 to 600 nm.

Results and Discussion

Viscosity. The reduced viscosity of 0.008 monomolar solutions of the high molecular weight polyampholytes in 0.04 M LiCl is shown in Figure 1. Each curve shows a minimum that is characteristic of polyampholytes and indicates the location of the isoelectric point. The isoelectric points are seen to lie at the pH values 4.8, 5.8, and 6.8 for the methyl, propyl, and butyl polyampholyte, respectively. The differences may be ascribed to the fact that the amino to carboxylic acid ratio is smallest for our methyl and largest for our butyl polyampholyte sample. These dissimilarities in the chemical composition, as well as differences in the molecular weights of the samples, make a quantitative comparison of the reduced viscosity values difficult to interpret. We merely wish to point out that at the isoelectric points the reduced viscosities of all three samples range from 0.6 to 0.7 times the corresponding intrinsic viscosities of the parent copolymers in

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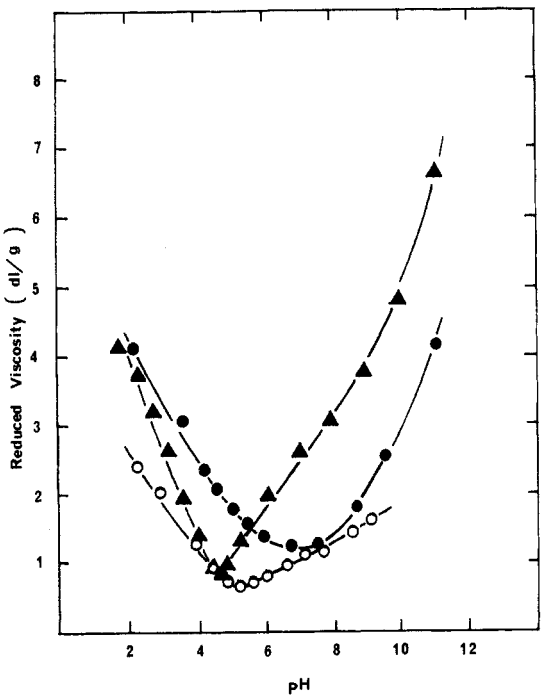

Figure 1. Reduced viscosity of 0.008 monomolar polyampholyte solutions as function of pH in 0.04 M LiCl: (▲) methyl polyampholyte; (○) propyl polyampholyte; (●) butyl polyampholyte.

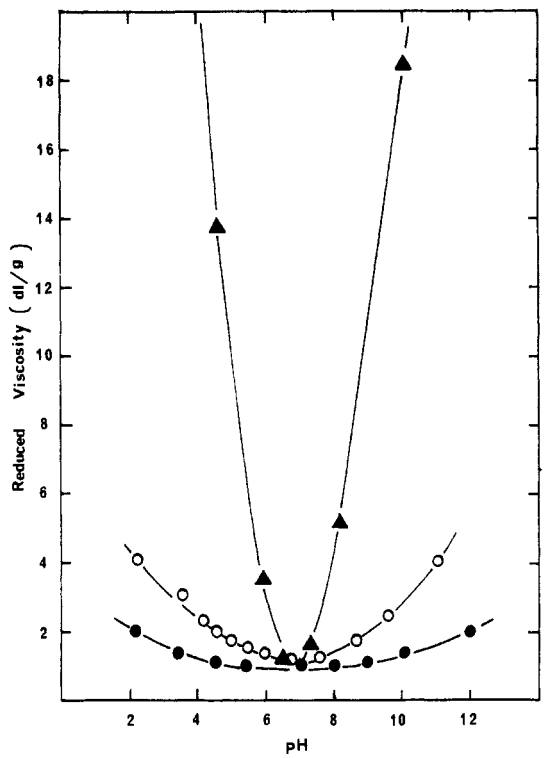

Figure 2. The effect of ionic strength on the reduced viscosity of 0.008 monomolar butyl polyampholyte solutions. LiCl molarity: (▲) 0.00 M; (○) 0.04 M; (●) 0.20 M.

THF and that the rise of the curves on both the acidic and the basic sides of the isoelectric points, caused by ionic repulsion, is relatively steeper for the methyl than for the other two polyampholytes.

The effect of a simple electrolyte on the reduced viscosity of the high molecular weight butyl polyampholyte is shown in Figure 2. The addition of electrolyte reduces the ionic repulsion and lowers the reduced viscosity on both the acidic and the basic sides. It is noteworthy that the three curves almost coincide at their minima, indicating

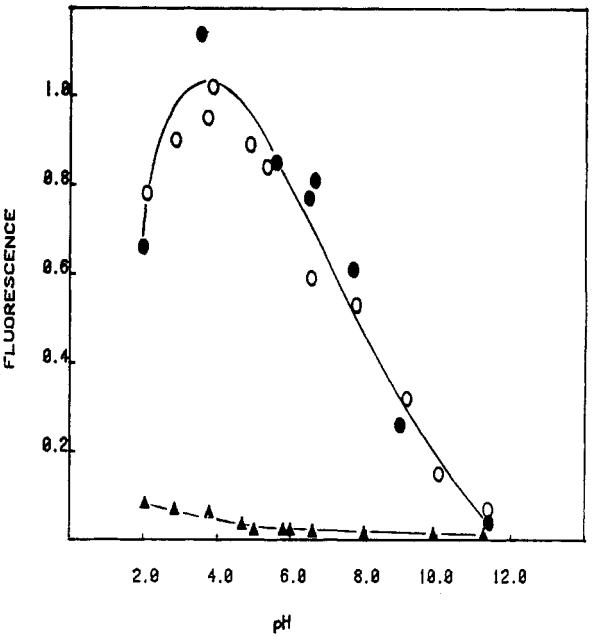

Figure 3. Relative fluorescence intensity at λ = 440 nm of 1.5 × 10^{-5} M TNS in 0.008 monomolar polyampholyte solutions as a function of pH: (▲) propyl polyampholyte in 0.04 M LiCl; (●) butyl polyampholyte in 0.04 M LiCl; (○) butyl polyampholyte in 0.10 M LiCl.

little, if any, interaction of the polyampholyte with the lithium chloride at the isoelectric point.

Fluorescence. TNS has a very low fluorescence in water. However, it exhibits substantial fluorescence in organic solvents. It has therefore been useful as a hydrophobic probe in protein studies. The results of the application of this probe to the high molecular weight butyl and propyl polyampholytes are summarized in Figure 3, where the relative emission intensity at 440 nm, representing the wavelength of maximum emission, is shown as a function of pH. The fluorescence from the propyl polyampholyte solutions is seen to be very small, while a substantial fluorescence enhancement of TNS is produced by the butyl polyampholyte. This finding suggests that, just as in the case of their respective parent copolymers, the butyl polyampholyte does, and the propyl polyampholyte does not, form micelle-like clusters of its alkyl side chains that bind TNS. One would expect this micelle formation to have its optimum at the isoelectric point, which occurs at pH 6.8 for the butyl polyampholyte. The fact that the fluorescence has a maximum at a lower pH may be due to the electrostatic attraction between the anionic TNS and the polyampholyte, which carries a net positive charge on the acidic side of the isoelectric point. However, it can be seen that electrostatic attraction is not necessary to bind the TNS. The fluorescence enhancement is very pronounced at the isoelectric point and persists even quite far on the basic side of the isoelectric point, where the electrostatic interaction is one of repulsion. A change in ionic strength from 0.04 to 0.10 M LiCl, shown in Figure 3, is apparently too small to produce any significant effect.

To eliminate the effect of electrostatic interactions, a neutral probe, *N*-phenylnaphthylamine (NPN), was used. In Figure 4 the emission spectra of 5 × 10^{-6} M NPN solutions in water, methanol, and 1.2 × 10^{-2} monomolar aqueous solutions of our three high molecular weight polyampholytes, each at its isoelectric pH, are compared. The figure shows the typically very weak fluorescence in water and the strong fluorescence in organic solvents, exemplified here by methanol. The characteristic shift of the emission maximum to lower wavelengths with de-

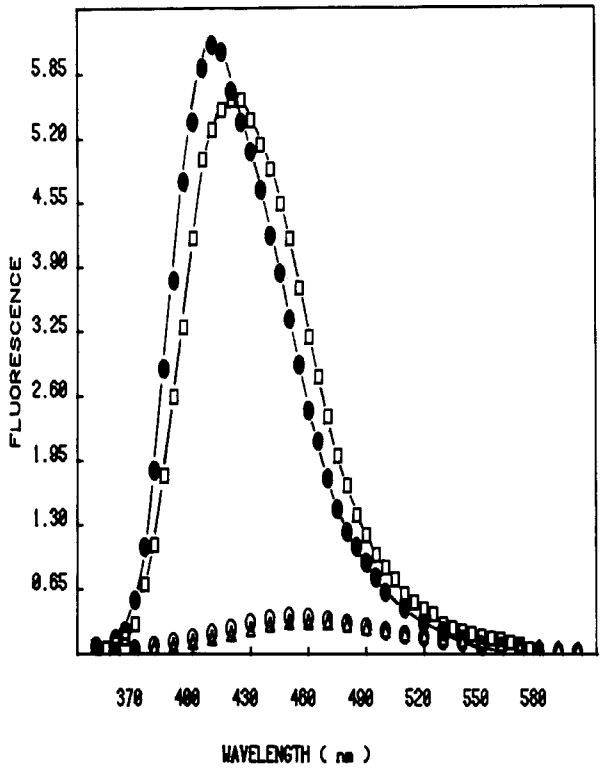

Figure 4. Relative fluorescence spectra of 5.6×10^{-6} NPN in various media: (□) in methanol; (△) in water and also in methyl polyampholyte solution; (○) in propyl polyampholyte solution; (●) in butyl polyampholyte solution. Polyampholytes are 1.2×10^{-2} monomolar in aqueous 0.004 M LiCl, each at this isoelectric pH.

creasing solvent polarity is also apparent. Turning our attention now to the polyampholytes, the fluorescence of the methyl and propyl polyampholyte solutions is hardly distinguishable from that of water. However, the butyl polyampholyte produces a large increase in the emission and also a blue shift of the emission maximum. On the basis of the known quantum yield of 0.22 for methanol,[7] the quantum yield for the butyl polyampholyte is 0.38. This value, together with the 410-nm emission maximum, indicates an effective local solvent dielectric constant smaller than 10.[7] This result clearly indicates that the probe molecules are bound in hydrophobic pockets, presumably consisting of micelle-like clusters formed by the butyl side chains of the macromolecules.

In Figure 5 we show the fluorescence of NPN in solutions of butyl polyampholyte in 0.004 M LiCl as a function of pH. The curves corresponding to three different polymer concentrations, with the NPN concentration being constant at 6.7×10^{-6} M, have their maxima at the isoelectric point, where the micelle content is expected to be optimal. With increasing distance from the isoelectric pH in either direction, the micelle content is seen to decrease, presumably due to the intramolecular electrostatic repulsion that also caused the rise of the reduced viscosity. In the pH region above 3.5, where NPN is electroneutral, one would expect its binding to follow a mass action law of the form

$$\frac{[\mathrm{NPN_b}]}{[\mathrm{NPN_f}](C_p - t[\mathrm{NPN_b}])} = K_b \qquad (1)$$

where $[\mathrm{NPN_f}]$ and $[\mathrm{NPN_b}]$ are the molarities of free and bound NPN, respectively, C_p is the polyampholyte monomolarity, t is the number of monomers per binding site, and K_b is the binding constant per unit monomolarity of polyampholyte. Control experiments have shown that the

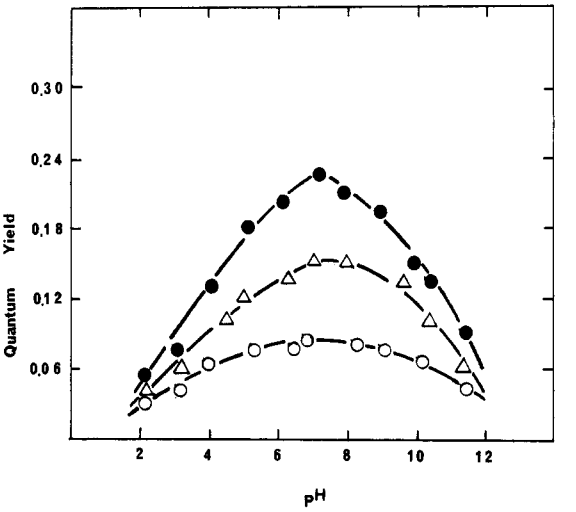

Figure 5. Effect of butyl polyampholyte concentration on the quantum yield of NPN as a function of pH. Concentration of NPN = 6.7×10^{-6} M. Butyl polyampholyte monomolarity in 0.004 M LiCl: (○) 8.5×10^{-4}; (△) 1.5×10^{-3}; (●) 2.3×10^{-3}.

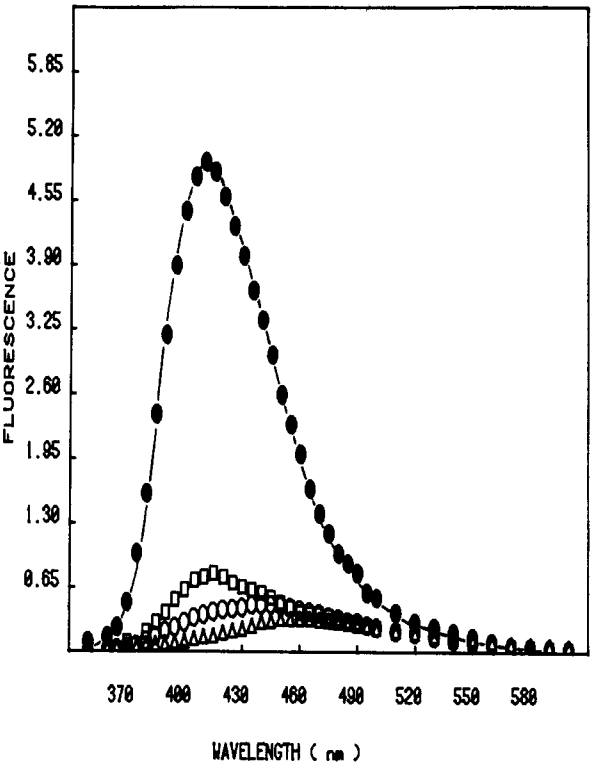

Figure 6. Effect of molecular weight of butyl polyampholyte on the fluorescence spectra of 5.5×10^{-6} M NPN in 0.004 M LiCl at pH 6.8. (△) NPN in water. NPN in 5.8×10^{-3} monomolar solutions of butyl polyampholytes with values of (○) 0.050, (□) 0.136, and (●) 2.06 dL/g for the reduced viscosity of their parent copolymers in THF.

NPN fluorescence in water is proportional to its concentration up to 6.0×10^{-6} M. Furthermore, from experiments carried out at constant NPN concentration and constant pH, the fluorescence of the bound NPN species may be obtained by extrapolation to infinite polymer concentration. One can then use fluorescence data to test eq 1. Our results turned out to fit a simplified version of the equation that did not include the $t[\mathrm{NPN_b}]$ term in the denominator. This finding indicates that in our experiments only a very small fraction of binding sites was occupied by NPN molecules. Since our free NPN concentration range came very close to the solubility limit, it appears to be impossible

to increase the occupation fraction significantly. Consequently, we were unable to determine t, but only K_b, which for the butyl polyampholyte in 0.004 M LiCl at pH values of 4.2, 6.8, and 8.2 amounted to 1.6×10^2, 2.5×10^2, and 2.0×10^2 M^{-1}, respectively.

The effect of lowering the molecular weight of the butyl polyampholyte on the NPN fluorescence is shown in Figure 6. The two low molecular weight samples are seen to display greatly reduced fluorescence compared to the high molecular weight sample, reflecting the relative abilities of these samples to bind NPN. This result corresponds to a similar molecular weight dependency found for the micelle content of the hydrolyzed parent butyl copolymers by means of potentiometric titrations.[4]

In summary, the fluorescence results show that the micelle formation of our polyampholytes depends strongly on the size of the alkyl group and also on the degree of polymerization. For the high molecular weight samples the break appears to lie between the propyl polyampholyte, which shows little, if any, tendency to micellize, and the butyl polyampholyte, which shows substantial micelle formation. A similar break was observed previously between the corresponding hydrolyzed parent copolymers. However, no break was detected in the molecular dimensions, as observed by means of the viscosity, between the propyl and butyl polyampholytes. This finding suggests that even at its isoelectric point the butyl polyampholyte is not a single hypercoiled micelle but consists of a randomly coiled assembly of small micelles.

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Static Investigation of the Influence of Polymer Molecular Weight and Loading in the Gas Chromatographic Determination of Poly(dimethylsiloxane) Interaction Parameters

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ABSTRACT: Activity coefficients and interaction parameters determined by a static method are reported for benzene and hexane at infinite dilution in five samples of poly(dimethylsiloxane) in the molecular weight range 3350–89 000 at 303 K. A small but significant variation of these properties is shown to occur with benzene if the polymer loading on a relatively inert solid support is reduced below ca. 20 wt %. The variation is attributed to adsorption at the surface of the support and the gas–liquid interface. An appreciable variation of the solution properties with polymer molecular weight is found. However, this variation is not sufficient to explain the differences between results of other workers, which are probably best explained by difficulties in determining the amount of polymer used in the gas–liquid chromatographic measurements.

The technique of gas–liquid chromatography (GLC) has proved useful in the determination of thermodynamic properties of nonelectrolyte liquid mixtures[1] and has been shown to be capable of providing information on a wide range of polymer properties including solution parameters.[2] Difficulties were experienced in dealing with a polymer stationary phase when the thermodynamic equations developed for GLC were applied to determine the activity coefficient of a volatile solute at infinite dilution in the stationary liquid phase, but ways of overcoming these have been described.[3] Summers, Tewari, and Schreiber[4] found good agreement between their infinite dilution activity coefficients and derived interaction parameters for various hydrocarbons in poly(dimethylsiloxane) (PDMS) measured by GLC and those from the static measurements of Chahal, Kao, and Patterson.[5] However, Lichtenthaler et al.[6] reported significant differences between static and GLC results and also between GLC determinations on the same polymer samples in an interlaboratory comparison.[7] A previous publication[8] showed that infinite dilution activity coefficients and interaction parameters measured by static and gas chromatographic methods for a number of hydrocarbon solutes with the same PDMS sample did agree within experimental error and suggested that differences in the results of other workers might well have arisen from difficulties in determining the amount of polymer present, as had been suggested earlier.[9] Two other factors that could have contributed to these differences are the polymer molecular weight and the ratio of polymer to solid support used in the GLC determinations.

The question of whether results obtained on a thin film of supported polymer represent the properties of bulk polymer has been examined by several workers. Lichtenthaler et al.[10] found significant differences between results obtained using a capillary column, with greater film thicknesses, and those on a packed column and doubted whether the latter represented interactions in the bulk polymer. Braun and Guillet[11] ascribed the differences to kinetic factors rather than to the presence of different sorption processes. The polymer loading, that is the amount of sample that is polymer, has been shown to be important in a number of studies,[12,13] and this paper describes its influence on the solution properties determined for benzene in PDMS.

Although there have been many studies of the thermodynamic properties of PDMS solutions, few of these have considered the effect of polymer molecular weight. Using GLC, Deshpande et al.[14] found appreciable differences in interaction parameters for two samples of PDMS, which they ascribed to molecular weight. Similarly, Galin[15] found

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