significant improvement over the penultimate model in fitting the experimental composition data. If the polymer compositions would be determined with greater accuracy, then the antepenultimate model might be found to provide a better fit.

The small, systematic deviations from the predictions of the penultimate model for the ASA and ASSA sequences shown in Figures 3 and 4 may arise from the quantitative analysis of the ^{13}C NMR spectra. Alternatively, they may indicate a small contribution from comonomer complexes. A decrease in the temperature of the comonomer mixture will favor complex formation. Evidence for a variation in the participation of comonomer complexes with copolymerization temperature based on various measurements of polymer microstructure has been reported for a number of systems.[32] We are presently investigating the copolymerization of styrene and acrylonitrile at lower temperatures, where complex participation should be enhanced.

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Binding of the Fluorescent Probes 2-*p*-Toluidinonaphthalene-6-sulfonate and 8-Anilinonaphthalene-1-sulfonate to Poly(vinylbenzo-18-crown-6) and Poly(vinylbenzoglyme)

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ABSTRACT: The fluorescent probes 2-*p*-toluidinonaphthalene-6-sulfonate (TNS) and 8-anilinonaphthalene-1-sulfonate (ANS) were used in the binding of solutes to poly(vinylbenzo-18-crown-6) (P18C6) and to poly(vinylbenzoglyme) (PVBG, a polystyrene with two $O(CH_2CH_2O)_2CH_3$ substituents per phenyl group) in aqueous media. The binding of these two fluorophores to the polysoap-type macromolecules causes a strong enhancement in their fluorescence intensity. From data obtained at either constant polymer or constant dye concentration, the first and intrinsic binding constants and the enthalpies and entropies of binding of TNS and ANS to P18C6 and PVBG were calculated. Addition of crown ether complexable cations converts the neutral polycrown ether into a polycation, resulting in an increase in the binding of the two anionic fluorophores. This increase is a measure of the binding capacity of the cations to P18C6 except for cations such as Tl^+ and Pb^{2+}, which bind to P18C6 but can quench the fluorescence of bound dye. Addition of dioxane or alcohol causes chain expansion of the tightly coiled macromolecules, and the penetrating water molecules quench the fluorescence of the bound dye.

Introduction

Recently reported preliminary data on the fluorescence of aqueous solutions of 2-*p*-toluidinonaphthalene-6-sulfonate (TNS) and of 8-anilinonaphthalene-1-sulfonate (ANS) in the presence of poly(vinylbenzo-18-crown-6) (P18C6) demonstrated the strong affinity of these fluorescent probes to this polymer.[1] The neutral macromolecule P18C6, its structure depicted in Chart I, acts in water as a polysoap and interacts with a variety of neutral and ionic solutes.[2,3] Addition of crown ether complexable

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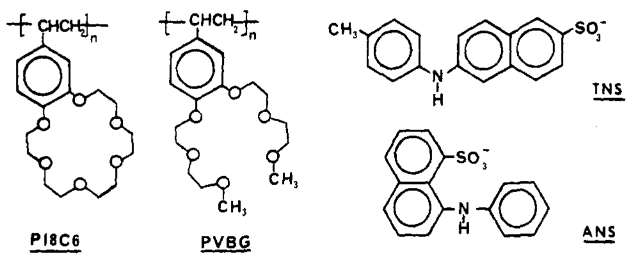
Chart I



cations (e.g., K+, Cs+, Tl+, Ba2+) converts P18C6 into a polycation of variable charge density. These cations can be used to regulate the binding of anionic (picrate, TNS, ANS, tetraphenylboride) and cationic (auramine O) solutes to P18C6, a procedure that we also applied recently in the cation-controlled decarboxylation of 6-nitrobenzisoxazole-3-carboxylate in aqueous solution and catalyzed by P18C6.[4]

Fluorophores have been used extensively in probing the binding capacity as well as polarity, conformation, and structural features of proteins, micelles, polysoaps, and other macromolecules, e.g., poly(vinylpyrrolidone).[5,6] Ionic probes are especially useful in studying the polysoap behavior of P18C6 since their interaction with this polymer can be controlled by crown-complexable cations. Moreover, cations such as Tl+ or Pb2+ are effective quenchers, and their use may provide information on the interaction between P18C6-bound cations and P18C6-bound neutral or ionic fluorophores.

In addition to the binding of ANS and TNS to P18C6, this paper also reports data on the binding of these probes to poly(vinylbenzoglyme) (PVBG, Chart I). This neutral polymer has polysoap properties similar to that of P18C6,[7] but in an aqueous medium the pendent benzoglyme ligands do not bind cations. Results obtained with the fluorophores pyrene and auramine O (preliminary data for the latter dye have been reported in ref 8) will be published in a subsequent paper.

Experimental Section

The synthesis of poly(vinylbenzo-18-crown-6) and of the poly(vinylbenzoglymes) has been previously reported.[7,9] The number-average molecular weights of the two polymers as determined by membrane osmometry were 63 000 and 58 000, respectively. 2-*p*-Toluidinonaphthalene-6-sulfonate and 8-anilinonaphthalene-1-sulfonate were obtained from Aldrich and purified by repeated recrystallization of the potassium salt from water. The dyes were eventually recovered in the acid form. Reagent grade inorganic salts were used without further purification.

Fluorescence spectra were measured by means of a Perkin-Elmer 650-10S spectrofluorimeter. Temperatures were controlled to within 0.1 °C. The excitation wavelengths were 326 nm for TNS and 380 nm for ANS. Dye concentrations were varied between 3×10^{-7} and 3×10^{-6} M, the optical densities of the solutions being less than 0.05. Inner cell effects in the 5-mm fluorescence cell were found to be negligibly small. Minor corrections for interference of polymer spectra were necessary only at the highest polymer concentrations of PVBG.

Results

The fluorescence intensity of aqueous solutions of ANS or TNS is very weak, but strong enhancement of the fluorescence is observed on addition of small quantities of P18C6 or PVBG. The emission maxima of the bound dyes are 462 nm for ANS and 423 nm for TNS and are identical for the two polymers. An example of the effect of added P18C6 on the fluorescence intensity, F, of a 2.72×10^{-7} M TNS solution is depicted in Figure 1, including the effect of added cesium chloride. Defining the intrinsic binding constant, K, as the equilibrium constant for the reaction dye + site $\rightleftharpoons$ bound dye, it can be easily verified that at constant polymer concentration the observed fluorescence intensity, F, in arbitrary units, is given by

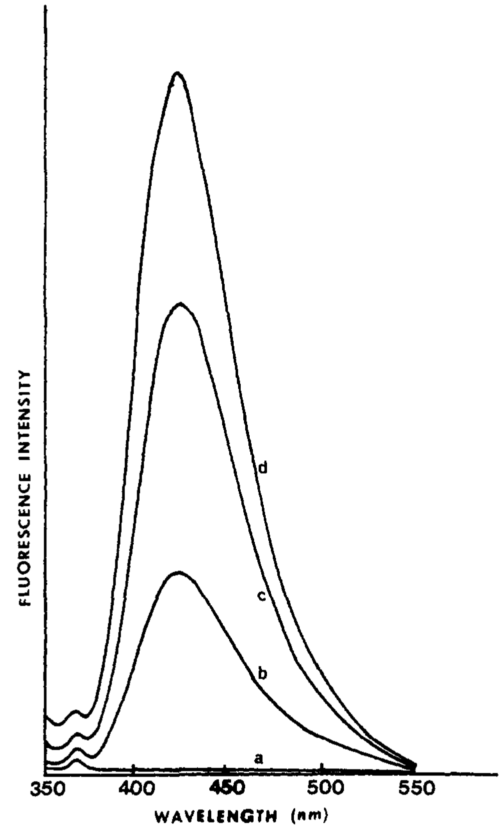


Figure 1. Fluorescence spectra of a 2.72×10^{-7} M solution of TNS in water in the presence of P18C6: (a) no P18C6 present; (b) 2×10^{-4} M P18C6; (c) 4×10^{-4} M P18C6; (d) 4×10^{-4} M P18C6 + 5×10^{-4} M CsCl.

$$1/F = 1/F_m + 1/KF_mD \qquad (1)$$

where D is the free dye concentration and F_m is the fluorescence intensity at high dye concentration, i.e., under saturation conditions when all "sites" are occupied. The binding "site" refers to the minimum number, N, of crown monomer base units needed to bind one dye molecule. Alternatively, when experiments are carried out at constant dye concentration, the fluorescence intensity, F, is given by

$$1/F = 1/F_m' + 1/K_1F_m'P \qquad (2)$$

where P is the free polymer concentration in terms of crown monomer base units and F_m' denotes the fluorescence under conditions when all dye molecules are bound, i.e., at high polymer concentration. K_1 is the first binding constant and is equal to K/N.

We chose conditions such that for eq 1 $D \approx D_0$ (only a small fraction of dye is bound) and for eq 2 $P \approx P_0$ (only a small fraction of sites are occupied). Plots of $1/F$ vs. $1/D_0$ and $1/F$ vs. $1/P_0$ for interaction of TNS with P18C6 in water at four temperatures are depicted in Figures 2 and 3. Similar plots, not shown here, were obtained for ANS. Binding of TNS to PVBG is shown in Figures 4 and 5. The K, K_1, and N values computed by least-squares analysis from the respective plots are collected in Table I (see Discussion). van't Hoff plots for the three systems are depicted in Figure 6, and the respective binding enthalpies and entropies calculated from these lines are also listed in Table I. One series of experiments was carried

Table I
Intrinsic (K) and First (K_1) Binding Constants and Binding Enthalpies and Entropies of TNS and ANS to P18C6 and PVBG

polymer	dye	temp, °C	$K \times 10^{-4}$, M^{-1}	$K_1 \times 10^{-2}$, M^{-1}	N	ΔH,[a] kcal/mol	ΔS,[a] eu
P18C6	TNS	35	4.16	15.6	27		
		25	6.18	24.1	26		
		15	8.09	41.3	20		
		5	13.9	81.3	17	-6.7	-0.64
PVBG		35	10.0	17.7	56		
		25	11.9	27.3	44		
		15	16.0	41.1	39		
		5	22.3	62.4	36	-4.6	7.7
P15C5		25	3.64				
P18C6	ANS	35	4.44	4.63	96		
		25	5.38	6.73	80		
		15	5.99	7.57	79		
		5	7.60	11.0	69	-2.7	12.4

[a] Enthalpies and entropies were calculated from plots of ln K (intrinsic binding constant) vs. $1/T$.

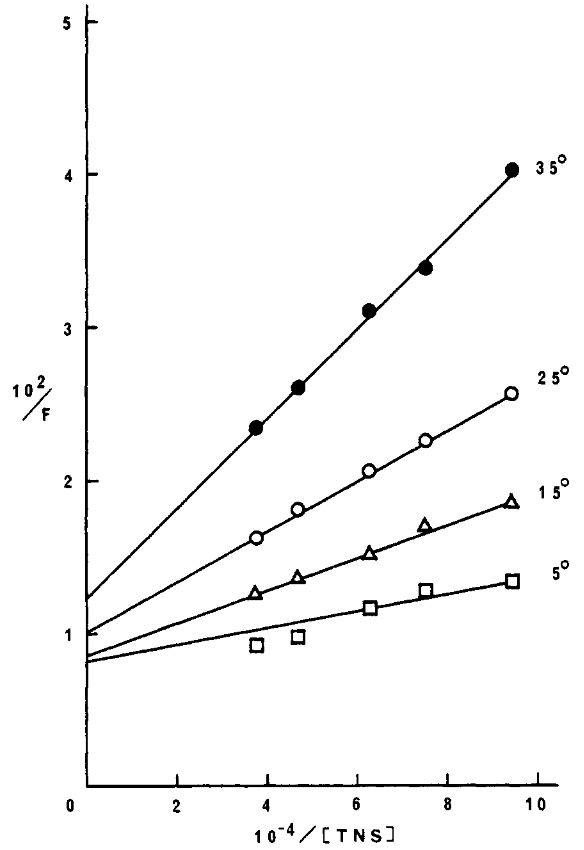


Figure 2. Plots of $1/F$ (in arbitrary units) vs. $1/[\mathrm{TNS}]_0$ for TNS binding to P18C6 at 5 (□), 15 (△), 25 (○), and 35 °C (●). $[\mathrm{P18C6}]_0 = 9.32 \times 10^{-6}$ M; $[\mathrm{TNS}]_0 = (1.06\text{–}4.26) \times 10^{-5}$ M.

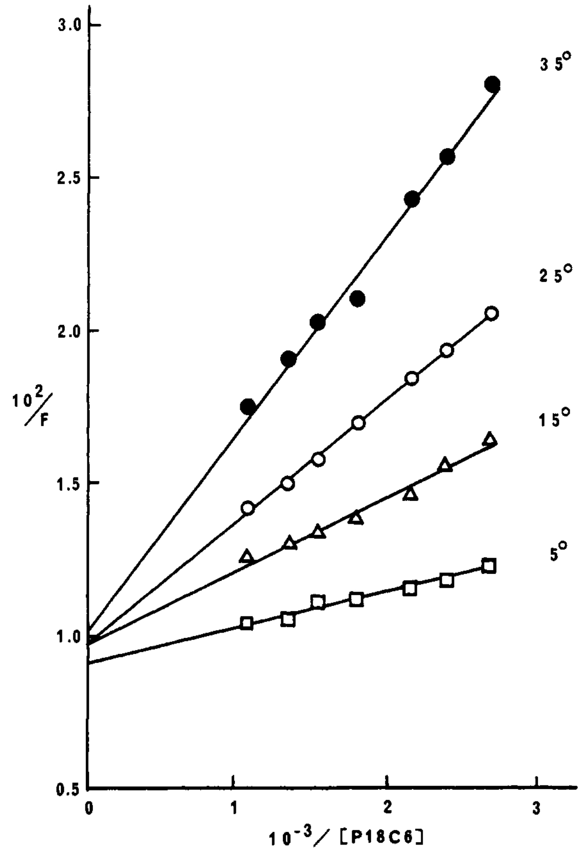


Figure 3. Plots of $1/F$ vs. $1/[\mathrm{P18C6}]_0$ for 2.72×10^{-7} M TNS at 5 (□), 15 (△), 25 (○), and 35 °C (●). $[\mathrm{P18C6}]_0 = (3.73\text{–}9.32) \times 10^{-4}$ M.

out with the polymer poly(vinylbenzo-15-crown-5) (P15C5, see ref 9), using a constant polymer concentration and varying the TNS content. The intrinsic binding constant for this system calculated from eq 1 is also given in Table I. The low water solubility of this polymer prevented us from varying the polymer concentration sufficiently to determine K_1 and N.

The effects of added cation on the binding of TNS and ANS to P18C6 are shown in Figures 7 and 8. To avoid saturation, concentrations of polymer and dye were chosen such that the total site concentration, P_0/N, exceeded the total dye concentration, D_0. For TNS the fraction of dye bound to neutral P18C6 in the starting solution was about 0.5, while for ANS the fraction was close to 0.2 (see Figures 7 and 8). The value F_m' used in the calculation of the ratios F/F_m' plotted in Figures 7 and 8 was obtained by carrying out simultaneously with the cation addition experiment a series of runs in the absence of salt at constant D_0 by varying P_0 and then plotting the data according to eq 2. It was ascertained that the fluorescence intensity of TNS or ANS in the absence of P18C6 was not affected by salt addition. The ratio F/F_m' should not exceed unity (i.e., when all dye is bound) at high salt concentration if it is assumed that the crown-bound cation only aids in attracting the dye to the P18C6 domain but does not affect the fluorescence of the bound dye. Note, however, that in the ANS–P18C6 system the ratio F/F_m' exceeds unity for a number of cations, approaching the value 2 for CsCl.

A few experiments were carried out to determine K_1 values for the TNS–P18C6 system as a function of the concentration of added cation. A constant K_1 value can only be obtained when the electrostatic force created by the P18C6-bound cations to attract more negative TNS ions to the charged P18C6 domain remains constant on

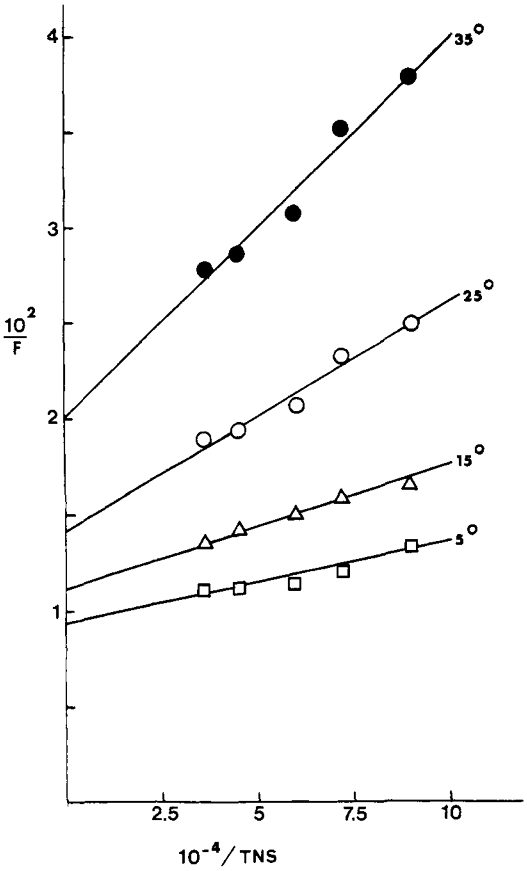


Figure 4. Plots of $1/F$ vs. $1/[\text{TNS}]_0$ for TNS binding to PVBG at 5 (□), 15 (△), 25 (○), and 35 °C (●). $[\text{PVBG}]_0 = 1.9 \times 10^{-5}$ M; $[\text{TNS}]_0 = (1.12\text{–}4.48) \times 10^{-5}$ M.

varying P_0 at constant KCl concentrations. This will be the case when the ratio $P_0/[\text{bound K}^+]$ remains constant. This ratio equals $1 + 1/K[\text{free K}^+]$ for 1:1 crown–K$^+$ complexes, K being the binding constant of K$^+$ to P18C6 in water. This expression will not change on varying P_0 when [free K$^+$] ≈ total KCl concentration, i.e., when the fraction of bound K$^+$ is very small. Taking $K = 110$ M^{-1},[3] it could easily be shown that under the conditions of our experiments (see Figure 9) the fraction of bound K$^+$ ranges from 0.04 at the lowest polymer concentration to 0.1 at the highest value of P_0. Plots of $1/F$ vs. $1/P_0$ at three KCl concentrations are shown in Figure 9. The linearity of the plots implies that in the range of P_0 concentrations used, the ratio $P_0/[\text{bound K}^+]$ remains essentially constant, its value being dependent on the amount of KCl used. The respective K_1 values were found to be 2.41×10^3 M^{-1} (no KCl), 5.70×10^3 M (5×10^{-4} M KCl), 6.31×10^3 M (10^{-3} M KCl), and 7.20×10^3 M (2×10^{-3} M KCl).

The effect of some *organic* ions on the fluorescence of TNS is shown in Figure 10. Addition of BPh$_4^-$ to a solution in which initially 50% TNS is bound to P18C6 leads to a rapid decrease in the ratio F/F_m. The decrease on addition of dodecyl sulfate is more gradual, while cetyltrimethylammonium bromide first decreases and then appears to increase the observed fluorescence.

Finally, addition of an organic solvent to an aqueous P18C6 solution is expected to decrease the polysoap character of P18C6 and its capacity to bind dye molecules. The change of F with the volume fraction of added ethanol and dioxane in the TNS–P18C6 system is shown in Figure 11. The fluorescence intensity passes through a minimum at about 30 vol % dioxane and 40 vol % ethanol and then rises again since the dye itself, in the absence of P18C6, is known to increase its fluorescence in water on adding dioxane or ethanol. The dashed lines between 0 and 40 vol % organic solvent represent the observed fluorescence intensity of TNS in the absence of P18C6. Figure 11 also shows the corresponding shift in the emission maximum of the TNS fluorescence peak as a function of the content of the organic solvent.

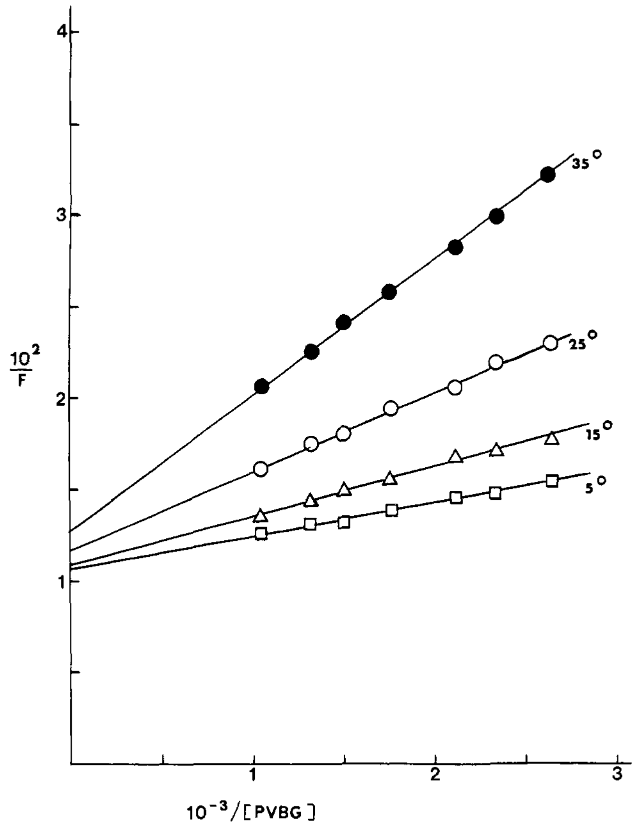


Figure 5. Plots of $1/F$ vs. $1/[\text{PVBG}]_0$ for 3.60×10^{-7} M TNS at 5 (□), 15 (△), 25 (○), and 35 °C (●). $[\text{PVBG}]_0 = (3.8\text{–}9.5) \times 10^{-4}$ M.

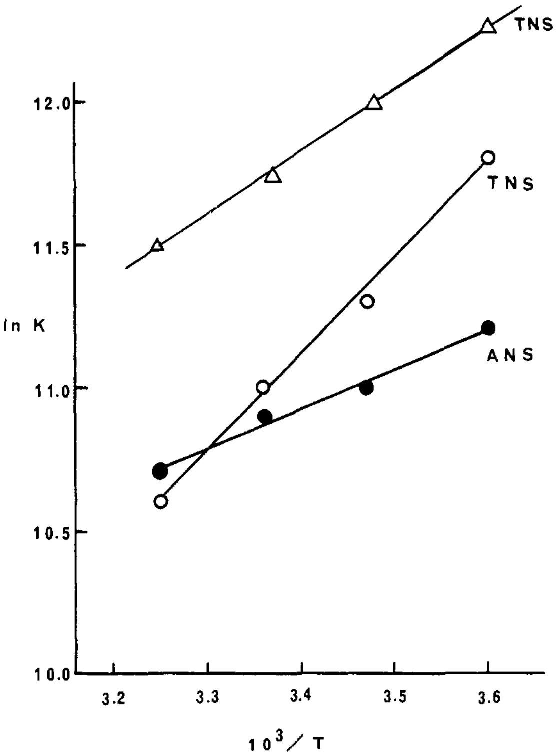


Figure 6. Plots of ln K vs. $1/T$ for binding of TNS (○) and ANS (●) to P18C6 and for TNS (△) to PVBG.

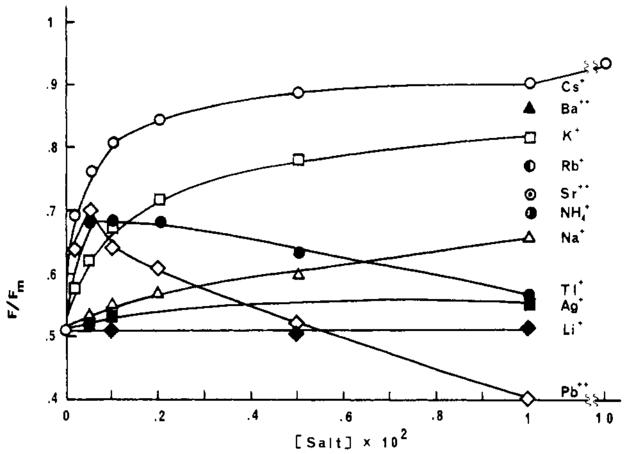


Figure 7. Effect of cation on the ratio F/F_m for TNS binding to P18C6 in water at 25 °C. [TNS] = 2.72×10^{-7} M; [P18C6] = 3.73×10^{-4} M.

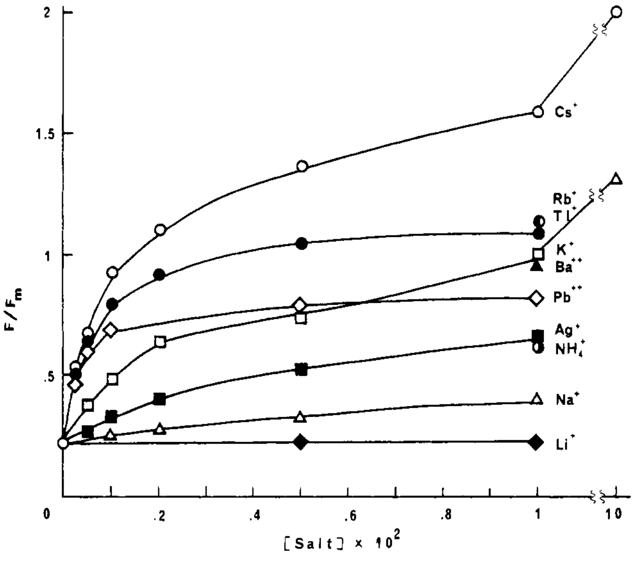


Figure 8. Effect of cation on the ratio F/F_m for ANS binding to P18C6 in water at 25 °C. [ANS] = 3.13×10^{-7} M; [P18C6] = 3.73×10^{-4} M.

Discussion

Previous studies have demonstrated that in water the polymer chains of P18C6 and PVBG assume tightly coiled conformations.[2,3] The polymers, which exhibit low intrinsic viscosities and inverse temperature solubilities, resemble in their binding of organic solutes such as methyl orange and picrate anions the properties of polysoaps, micelles, and globular proteins. Therefore, it is not surprising that the fluorophores ANS and TNS, which have been extensively employed in studies of solute binding to proteins,[10,11] polysaccharides,[12,13] poly(vinylpyrrolidone),[14,15] hydrophobic polycations,[16,17] and micelles,[18] give enhanced fluorescence in water on adding P18C6 or PVBG. The increased quantum yield is accompanied by a blue shift in the maximum, λ_{em}, of the emission wavelength. For TNS the shift for both polymers is from 500 nm in water to 423 nm for the bound dye and from 515 to 462 nm for ANS. Similar shifts and enhanced quantum yields have been reported for the two fluorophores in the absence of polymers when water is replaced by an organic solvent, the effects being more pronounced in less polar media. The extent of the blue shift and the increase in quantum yield (frequently offset by quenching) have often been used in describing the hydrophobic character of the sites of macromolecules to which the fluorescent probes are bound.

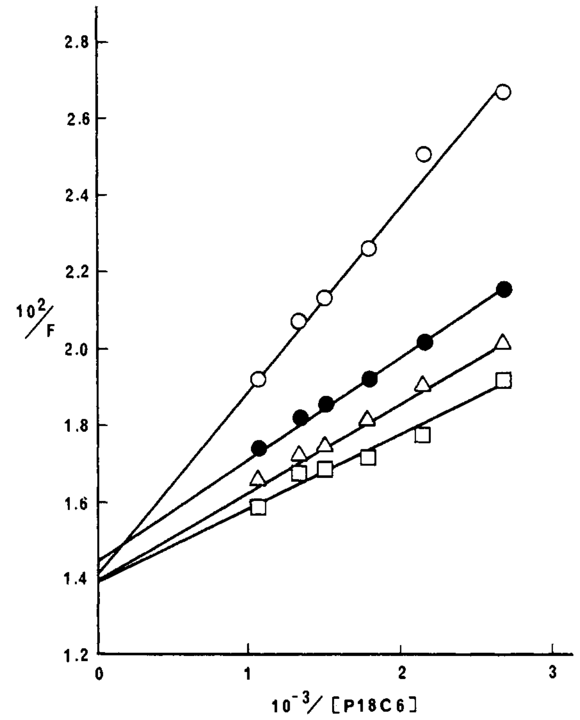


Figure 9. Plots of $1/F$ vs. $1/[\mathrm{P18C6}]_0$ for TNS binding to P18C6 in the presence of KCl: (○) no KCl added; (●) 5×10^{-4} M KCl; (△) 10^{-3} M KCl; (□) 2×10^{-3} M KCl. [TNS] = 2.72×10^{-7} M; [P18C6] = $(3.73\text{–}9.32) \times 10^{-4}$ M.

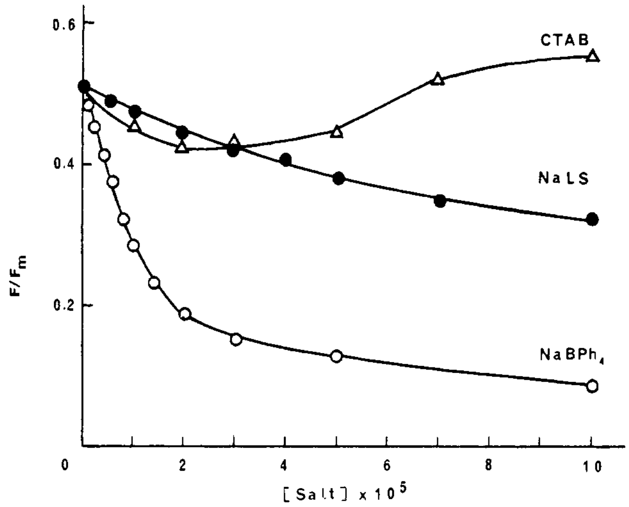


Figure 10. Effect of organic solutes on the fraction of P18C6-bound TNS in water at 25 C: (○) sodium tetraphenylboride; (●) sodium dodecyl sulfate; (△) cetyltrimethylammonium bromide. [TNS] = 3.13×10^{-7} M; [P18C6] = 3.73×10^{-4} M.

The 423-nm λ_{em} value for P18C6- or PVBG-bound TNS is identical with that found for TNS dissolved in 1-butanol[10] and close to the lowest value (λ_{em} 426 nm for β-lactoglobulin) reported for a series of proteins.[10] The 462-nm value for polycrown- or polyglyme-bound ANS is lower than that reported for ANS in butanol or octanol (464 nm, ref 19) or for ANS bound to bovine serum albumin (469 nm) but higher than the 454-nm value reported for apomyoglobin, a protein with a strongly hydrophobic site.[19] These data suggest, therefore, that the cavity formed by a number of benzocrown ether or benzoglyme monomer units as a site for binding TNS or ANS has considerable hydrophobic character. This is not surprising considering the polystyrene-type backbone of the two polymers and their contracted conformation. Similar conclusions were arrived at from the observed shifts in the optical absorp-

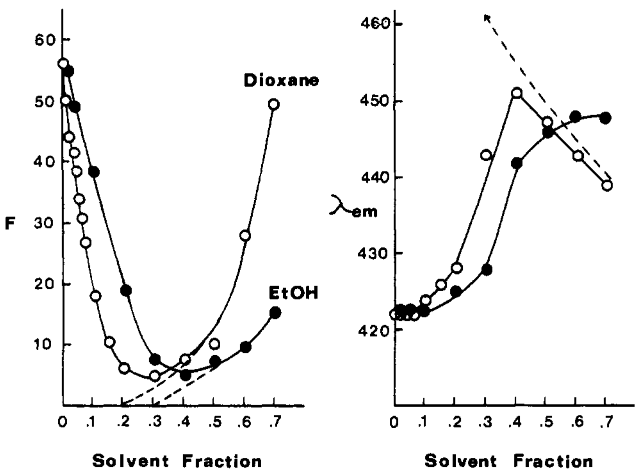


Figure 11. Effect of increasing volume fractions of ethanol (○) and dioxane (●) on the fluorescence intensity, F (in arbitrary units), and the emission maximum, λ_{em}, of TNS in water at 25 °C in the presence of P18C6. $[TNS]_0 = 2.5 \times 10^{-7}$ M; $[P18C6]_0 = 4 \times 10^{-4}$ M. Dashed lines represent the change in F and λ_{em} of TNS as a function of alcohol or dioxane content in the *absence* of P18C6.

tion maxima of picrate anions, methyl orange, and the dye 2-[(4-hydroxyphenyl)azo)]benzoate bound to P18C6[2,3] and PVBG.[7]

The binding constants and thermodynamic parameters for the respective systems are shown in Table I. The approximations $[D] \approx D_0$ and $[P] \approx P_0$ applied in plotting 1 and 2, respectively, can be justified from the data of Table I. For example, under the least favorable conditions the fraction of sites occupied at constant dye concentration ($D_0 \approx 3 \times 10^{-7}$ M) is less than 0.04 since at the lowest polymer concentration ($\approx 3.8 \times 10^{-4}$ M) the total concentration of available sites, P_0/N, at the highest N value equals 7×10^{-6} M for TNS and 4×10^{-6} M for ANS. In most cases the fraction of occupied sites is less than 0.02, justifying the approximation $[P] \approx P_0$. In the experiments at constant polymer concentration ($P_0 = 9.4 \times 10^{-6}$ M), the fraction of bound dye will be less than the maximum fraction, P_0/ND_0 that can be bound. Choosing again the least favorable of our experimental conditions ($D_0 = 1 \times 10^{-5}$ M, $N = 17$ for TNS; see Table I), we calculate a value 0.055 for this fraction. In nearly all cases it varies between 0.01 and 0.04, justifying the approximation $[D] = D_0$ for eq 1. While the free dye and polymer concentrations D and P can be quite easily calculated, the errors introduced as a result of the approximations appear to fall within the experimental errors of our measurements.

Figures 2–5 for TNS, as well as those for ANS, all show a good linear relationship, implying that under the chosen experimental conditions the binding of the two fluorophores can be described by a Langmuir adsorption isotherm. This means that even under conditions where the fraction of occupied binding sites reaches 0.7 (i.e., in the $1/F$ vs. $1/D_0$ plots), with about five dye molecules present in the polymer domain (DP $\approx$ 200, $N \approx 30$), no cooperative effects are found. A similar conclusion was arrived at from binding studies with picrate and methyl orange.[2,3]

At room temperature the intrinsic binding constants for TNS and ANS to P18C6 and P15C5 range from 3.6×10^4 to 6.2×10^4 M^{-1}. These values compare favorably with those found for bovine serum albumin[10] and poly(vinylpyrrolidone)[14,15] and are slightly below those reported for picrate and methyl orange binding to P18C6.[2,3] The higher affinity of TNS for poly(vinylbenzoglyme) is consistent with results obtained for pyrene,[20] picrate, and methyl orange.[2,3,7]

At 25 °C the polycrown can bind on the average 1 TNS molecule per 26 monomer units. The N value of this system is lower than found for picrate–P18C6, where $N = 42$. The latter value is nearly identical with that found for TNS–PVBG (Table I). At 25 °C the system ANS–P18C6, with $N = 80$, resembles more the binding behavior of methyl orange to P18C6, where $N = 85$.[2] The decrease of N with temperature (Table I) is found in all systems and is consistent with data obtained for methyl orange and picrate. The average value of N will depend on the nature of the monomer unit, the conformational structure of the polymer coil, and the structure of the dye, but the available data are not sufficient to draw specific conclusions as to their interrelationship. The polymer chains of P18C6 are known to slightly expand in aqueous solution on lowering the temperature, and this could account for the lower N values.[2]

In the spectrophotometric studies of picrate binding to P18C6, the N values were confirmed by measuring the limiting value of P_0/[bound dye] under saturation conditions, i.e., at high dye concentration.[2] However, in the fluorescence experiments with TNS–P18C6, inner filter effects cause the fluorescence to decrease significantly above 5×10^{-5} M TNS. It is also noteworthy that the $1/F$ vs. $1/D_0$ plots remain linear even under conditions where as many as five dye molecules are bound to a P18C6 molecule. In spite of their close proximity (i.e., high local concentration), no dynamic concentration quenching occurs, apparently because the probability of encounters is decreased by translational constraints imposed on the bound dye molecule by the tightly coiled P18C6 chain.

Table I reveals that binding of both TNS and ANS is an exothermic process. The unitary entropy change (i.e., ΔS corrected for the cratic term of 7.9 eu) is positive for all systems, which suggests that the interactions are largely of hydrophobic origins. ANS binding to P18C6 is the least exothermic and yields the largest gain in entropy. The ΔH and ΔS values for this dye are not much different from those found for methyl orange, i.e., −3.9 kcal/mol and 9.7 eu, respectively.[2] The SO_3^- substituent in ANS is more shielded by the anilino group than in TNS, and this may make it a more hydrophobic molecule, resulting in a lower exothermic binding enthalpy and a higher entropy. For the same reason, ANS may require a more hydrophobic site for effective binding, i.e., more in the interior of the polymer particle, and this in turn could lead to the higher N value. However, a systematic study is needed to determine with more certainty the effect of solute structure on N and on the thermodynamic binding parameters.

Effect of Cation. Cations that form stable complexes with the polymer-bound crown ligands in water will convert the neutral P18C6 polysoap into a polycation. Most cations form 1:1 complexes with benzo-18-crown-6, but Cs^+ forms a sandwich type 2:1 ligand–cation complex with this crown ether. Potentiometric measurements yielded intrinsic binding constants to P18C6 in water of 300 M^{-1} for Cs^+, 110 M^{-1} for K^+, and 2.4 M^{-1} for Na^+.[3] Depending on the type and concentration of the cation, salt addition is expected to enhance the binding of an anionic solute to P18C6 by augmenting the hydrophobic interaction with an electrostatic component. Except for Tl^+, Ag^+, and Pb^{2+}, the cation dependence of TNS and ANS binding to P18C6 as depicted in Figures 7 and 8 follows a pattern similar to that found for picrate anions.[3] When compared at 10^{-2} M salt concentration, the effect of alkali ions is found to decrease in the same order as their binding constants to P18C6, i.e., $Cs^+ > Rb^+ \approx K^+ > Na^+ \gg Li^+$, Li^+ being entirely ineffective even at 0.1 M LiCl. The same sequence was found in the binding of picrate and 2-[(4-hydroxyphenyl)azo]benzoate, although for picrate, Rb^+ appears to be slightly more effective than K^+.[3] The similarity between the picrate and TNS curves suggests that quenching of TNS or ANS fluorescence by larger cations such as Cs^+ cannot be a significant factor in spite of the fact that at 10^{-2} M CsCl the P18C6 chain is nearly saturated with 2:1 crown–Cs^+ complexes. Viscosity measurements have shown that the highly charged particle remains tightly coiled, and water penetration into the polymer domain remains difficult.

The results for Tl^+ and Pb^{2+} are distinctly different. Both cations are known to have an affinity for P18C6 comparable to that of K^+, and their addition to a P18C6–TNS mixture first enhances the fluorescence, but then it decreases at higher cation concentration. Both cations are known to be effective fluorescence quenchers. For example, Tl^+ binding to benzocrown ether quenches the fluorescence of this ligand,[21] and this observation was recently applied in measuring the binding constant of Tl^+ to P18C6 in ethanol.[20] Hence, the binding of Tl^+ or Pb^{2+} to P18C6 will first enhance the fluorescence intensity of TNS as a result of increased dye binding. But this will eventually be offset due to quenching by the same crown-bound cation when at higher cation density on the polymer the average distance between quencher and dye diminishes. Using for Tl^+ the same binding constant as found for K^+ to P18C6, one can easily calculate that under our conditions at 10^{-3} M $TlNO_3$ a polycrown of DP $\approx$ 200 contains about 20–30 Tl^+–crown complexes. At this concentration of $TlNO_3$, quenching is still rather weak. Most of the cation complexes are probably situated on the periphery of the P18C6 particle close to the polymer–water interface, since the polymer is kept in solution through hydrogen bonding of water molecules with crown ligands.[2] The dye is probably located more in the inner core of the P18C6 domain. It is possible that as the cation population on P18C6 increases, the anionic dye moves closer to the periphery of the particle and, therefore, closer to the quenching cations, but in our studies with picrate anions there was never any evidence of formation of tight ion pairs at high salt concentration. Ag^+ ions bound to P18C6 probably also cause quenching. In picrate binding, Ag^+ was found to be more effective than NH_4^+ and Na^+ ions.[3] With TNS, Ag^+ appears to be less effective, at least when based on the increase in F/F_m (Figure 7). Most likely, as with Tl^+ or Pb^{2+}, quenching by bound Ag^+ ions leads to an incorrect value of the fraction of bound TNS if calculated from the increase in F/F_m.

The cation effect on ANS binding to P18C6 shows one noteworthy phenomenon not found with TNS. The ratio F/F_m exceeds unity in some cases, reaching a value of 2 for Cs^+ (Figure 8). Also, unlike in TNS, the F/F_m ratio for Tl^+ and Pb^{2+} does not decrease at high salt concentration, although the curves appear to level off. Note also that the fluorescence enhancement by Ag^+ now exceeds that of NH_4^+ and Na^+. These results suggest that the quantum yield of ANS bound to charged P18C6 exceeds that of ANS bound to neutral P18C6, the effect being most pronounced for Cs^+.

Rotational restriction of the anilino moiety relative to the naphthalene ring is known to enhance the fluorescence of anilinonaphthalenesulfonates.[22] If binding of cations to P18C6 results in ion pairing with the anionic solute, it may well affect the rotation of the anilino group in 1,8-ANS because of its close proximity to the SO_3^- anion. In 2,6-TNS, a cation close to the SO_3^- group is not likely to affect to any extent the rotation of the remote toluidino substituent, and for this dye the ratio F/F_m in Figure 7 does not exceed unity. However, we have not found solid evidence for ion pairing between P18C6-bound cations and bound anionic solutes in any of our previous systems. If it did occur, we would expect Tl^+ and Pb^{2+} to be more effective quenchers in the ANS system. It also is not clear why Cs^+ would be so much more effective than K^+. An alternative explanation is that the rotational diffusion coefficients of TNS and ANS are affected differently when neutral P18C6 is converted into a cation charged particle. In this respect, Cs^+ may especially be effective since its 2:1 crown–cation complexation could result in the formation of cross-links within the polymer domain. This in turn could influence the rotational motions of the bound dye. If, as argued, ANS is located deeper in the inner core of the particle and requires more monomer units per site, such cross-linking may well have a more pronounced effect on the rotation of this dye than on TNS. Fluorescence polarization studies could possibly shed more light on this problem.

The linear plots of $1/F$ vs. $1/P_0$ obtained at constant KCl concentrations (Figure 9) demonstrate that conditions can be chosen such that binding constants of a fluorophore to charged P18C6 can be measured at a constant fraction of cation-complexed crown units, i.e., as a function of the number of cations bound per chain. For picrate binding to P18C6, a linear relationship was found between the log of the intrinsic binding constant, K, and the number of charges, Z, on the P18C6 chain as predicted from simple theoretical considerations.[2] As expected, K for TNS also increases with [KCl], but more data are needed to confirm any linearity between ln K and Z.

Effect of Anion and Solvent. Binding of nonfluorescent organic solutes to P18C6 or PVBG in water can be studied by fluorimetry using a fluorescent probe such as TNS as a competing solute. Three examples are depicted in Figure 10, showing the change in F/F_m for bound TNS as a function of added organic solute. Concentrations of P18C6 and TNS were such that less than 2% of the available binding sites, P_0/N, were occupied. Even under these conditions, bound TNS is replaced by tetraphenylboride at BPh_4^- concentrations as low as 10^{-5} M. This suggests a high intrinsic binding constant for this hydrophobic anion. A similar conclusion was arrived at from experiments with picrate anions using optical spectroscopy.[2] The less hydrophobic sodium dodecyl sulfate (SDS) is not as effective as BPh_4^-. In principle, the data of Figure 10 can be used to obtain the intrinsic binding constants of the organic solutes, since the TNS binding constant is known, assuming the same N value for the two competing solutes. However, as in the case of picrate,[2] no constant K value for BPh_4^- or dodecyl sulfate could be computed from the competitive equilibria, probably because the N values of the two solutes differ considerably.

With cetyltrimethylammonium bromide (CTAB), the ratio F/F_m first decreases and then increases again at higher CTAB concentrations. For both CTAB and SDS, concentrations in the P18C6–TNS solutions were well below their critical micelle concentration (CMC $\approx 10^{-3}$ M for CTAB, CMC $\approx 10^{-2}$ M for SDS[23]). Hence, formation of micelles, which are known to bind TNS and cause fluorescence enhancement, can be excluded. It is also unlikely that P18C6-bound CTAB, like bound alkali ions, will electrostatically attract more TNS to the P18C6 domain. The number of bound cations would be too low to attract enough TNS to compensate for displaced TNS. A more likely explanation can be found in the reported observation that ANS fluorescence in water is enhanced in the presence of CTAB at concentrations well below the CMC of this soap, due to formation of a 1:1 association complex.[24] In our system, the two oppositely charged hydrophobic ions TNS and CTAB form an ion pair even at low concentration, and the new hydrophobic environment for TNS causes enhancement of the fluorescence.

Addition of solvents such as *dioxane* or *ethanol* to an aqueous solution of P18C6 is expected to result in chain expansion and would allow water penetration into the polymer domain. Hence, the rapid decrease in the fluorescence of a P18C6–TNS solution on addition of these solvents (Figure 11) is not surprising. This decrease in F does not necessarily imply that TNS molecules are released. Quenching may well occur as a result of water penetration into the expanded coil while at least initially most of the TNS remains bound. Dialysis measurements could provide information on the actual release of bound TNS in the presence of ethanol or dioxane. Note that initially F decreases without an accompanying change in λ_{em}. For example, F (in arbitrary units) decreases from 55 to about 15 on addition of 10% dioxane or 20% ethanol while λ_{em} remains essentially at 423 nm, the value for P18C6-bound TNS in pure water. Apparently, tightly coiled regions of P18C6, where water penetration is difficult, continue to exist up to at least 20% organic solvent. In the absence of P18C6, λ_{em} changes nearly linearly with dioxane content from 500 nm in water to 434 nm in 90% dioxane (ref 10 and our results).

Above about 30% dioxane or 40% ethanol the fluorescence intensity and λ_{em} are close to those found in the same solvent mixtures in the absence of P18C6. For ethanol this is nearly the same concentration where the optical spectrum of picrate does not show any binding of this anion to P18C6.[2] However, dialysis measurements would be more conclusive in determining the relationship between solute binding and ethanol or dioxane content.

Conclusions

The polymers PVBG and P18C6 dissolved in water appear to be interesting compounds for the study of binding of organic solutes to neutral and charged polysoap-type molecules. This is especially true for P18C6 since the charge on the macromolecule can be controlled by the proper choice of added cation or by varying the salt concentration. Fluorescent probes are especially convenient since very low solute concentrations and, therefore, a low degree of site binding can be used. Also, interactions between the bound fluorescent probes and quenching, crown-bound cations such as Tl^+ and Pb^{2+} can be explored. Recent studies with pyrene and auramine O have shown that phenomena similar to those found for TNS and ANS are observed for neutral and cationic probes. Auramine O is especially interesting since as a cation its binding is hindered by crown-bound cations. More information regarding the motion of bound fluorescent probes in the P18C6 or PVBG domain as well as exchange processes at the polymer–water interface can be obtained from fluorescence polarization studies and fluorescence lifetime measurements using stationary and nonstationary excitation conditions.

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