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Naphthalenesulphonyl groups as fluorescence probes for examining the conformational behaviour of polyallylamine

Soichi Otsuki *, Takahisa Taguchi

Osaka National Research Institute, AIST, 1-8-31 Midorigaoka, Ikeda, Osaka 563, Japan

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Abstract

The optical absorption and fluorescence properties of polyallylamine (PAA), modified with 1- and 2-naphthalenesulphonyl (1NS and 2NS) groups, were examined in aqueous solution. The monomer emissions of both chromophores increased on addition of ethanol or HCl to the solutions. The addition of K_2HPO_4 caused a decrease in the monomer emissions for PAA containing 1.92 mol.% of 1NS and for PAA containing 4.2 mol.% of 2NS. This probably results from the contraction of the polymer conformation due to electrostatic crosslinking by phosphate ions. A similar result was obtained on addition of KCl to the same polymers and was also attributed to polymer contraction due to enhanced intramolecular interactions. However, the monomer emissions remained unchanged or increased for PAA polymers with higher chromophore contents. It is suggested that these highly modified polymers possess a compact conformation without salt, so that little conformational change is exhibited on addition of these salts. The fluorescence behaviour of the 1NS and 2NS groups was compared with that of the 5-dimethylamino-1-naphthalenesulphonyl (DNS) group. The sensitivity of 1NS and 2NS as optical probes towards the above species was higher or as high as that of DNS. In addition, the 1NS group was sensitive to the polarity of the microenvironment around the chromophore in a similar manner to the DNS group. The maximum wavelength of the monomer emission decreased as the polarity decreased.

Keywords: Absorption spectrum; Electrostatic crosslinking; Fluorescence; Intramolecular interaction; Naphthalenesulphonyl; Polyallylamine; Polymer conformation; Stacking

1. Introduction

Synthetic polymers containing hydrophilic and hydrophobic moieties have interesting physical properties in aqueous solutions. Some form organized assemblies similar to natural systems, such as micelles, vesicles or membranes [1–3]. The conformation of amphiphilic polymers in water reflects a delicate balance of forces. Coulombic forces, ion pair formation, hydrogen bonding and hydrophobic interactions all contribute to the stability of self-assembling polymer molecules [4].

Chromophores bound to polymers have been used as optical probes to study the conformational transitions in various synthetic and natural polymers [5–7]. The 5-dimethylamino-1-naphthalenesulphonyl (DNS) group has been widely used [8–11]. However, the use of non-substituted naphthalenesulphonyl groups, such as 1- and 2-naphthalenesulphonyl (1NS and 2NS), for fluorescence labelling has been studied in only a few reports, concerned with the introduction of the 2NS group into a low-molecular-weight amine [12] and energy transfer from a 1NS-labelled enzyme to a DNS-labelled substrate [13].

The photophysics of polymers containing chromophores has also been an active research field from the point of view of providing a new class of optical functional materials. Various photodriven functions of these polymers have been exploited in applications such as artificial photosynthesis [14,15], information processing [16,17], organic photoimaging materials [18,19] and photocontrolled chemimechanical systems [20,21]. Polyallylamine (PAA) possesses a side-chain carrying a primary amino group easily convertible to various functional groups and has attracted considerable attention in polymer chemistry [22]. Long alkyl chains and benzyl groups have been introduced into PAA to provide new amphiphilic polymers [23]. However, little work has been carried out on the preparation and photophysics of PAA containing chromophores [24].

* Corresponding author. Tel.: 0081 727 51 9524; fax: 0081 727 51 9628; e-mail: otsuki@onri.go.jp

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In this study, 1NS and 2NS groups were attached to PAA and used as optical probes to monitor the changes in polymer conformation. The optical absorption and fluorescence properties of 1NS- and 2NS-modified PAA were examined in aqueous solution and compared with those of DNS-modified PAA.

2. Experimental details

2.1. Materials

Poly(allylamine hydrochloride) (PAA·HCl) (M_w = 50 000–65 000; Aldrich) was used without further purification. 1NS, 2NS and DNS chlorides were of guaranteed reagent grade (Tokyo Kasei). Dibasic potassium phosphate (K_2HPO_4) (Kishida) and potassium chloride (KCl) (Nacalai) were of guaranteed reagent grade.

2.2. PAA containing chromophores

To a suspension of PAA·HCl (0.6 g; 6.9 mmol) in 20 ml of methanol, 2.5 ml of 2.9 N potassium hydroxide in methanol was added. The suspension was stirred for 1 h and then left in a refrigerator overnight. The precipitate of KCl was removed by decanting and the solution was evaporated to dryness. The resulting free PAA was dissolved in water–acetone (1 : 4, v/v). The desired amount of sulphonylchloride dissolved in acetone was added and the solution was stirred at room temperature for 24 h. The solution was acidified with hydrochloric acid (HCl) and concentrated in vacuo. The residue was dissolved in water and dialysed in a Visking tube twice with aqueous sodium hydroxide and twice with water. The dialysate was stored in a refrigerator and used as a stock solution for the measurements. The PAA derivatives examined in this study were PAA containing 1.92 mol.% of 1NS group (PAA-1NS-1.92), PAA-1NS-5.0, PAA-2NS-4.2, PAA-2NS-8.7 and PAA-DNS-1.83. A PAA derivative containing more than 5.0 mol.% of 1NS group was prepared, but was not used for measurement because it was easily precipitated from dilute aqueous solution by weak shaking.

The compositions of the PAA polymers containing chromophores were calculated from the ratio of the integral of the aromatic protons to that of the aliphatic protons in nuclear magnetic resonance (NMR) spectra. The concentration of the polymer in the stock solution was calculated from the concentration of amino groups of the polymer determined by the colloid titration method. A measured amount of stock solution was diluted with water, acidified with HCl and titrated with 0.005 N potassium poly(vinyl sulphate) using methylene blue as indicator.

2.3. Measurements

^{1}H NMR spectra were taken at 500 MHz on a JEOL ALPHA spectrometer. To prepare samples for NMR spectroscopy, the stock solutions of PAA derivatives were acidified with HCl, evaporated to dryness and dissolved in D_2O.

Absorption spectra were recorded on a Shimadzu UV-2200 spectrophotometer. Steady state fluorescence spectra were run on a Hitachi F-3010 spectrofluorometer and were corrected using rhodamine B as a quantum counter. The temperatures of the water-jacketed cell holders of both spectrometers were controlled at 25 °C with a circulating bath. Solutions were prepared by diluting the stock solutions with solvents and kept at 25 °C for at least 30 min before measurement. The concentrations of the solutions were 0.0061 wt.% for PAA-1NS-1.92, 0.0028 wt.% for PAA-1NS-5.0, 0.0027 wt.% for PAA-2NS-4.2, 0.00133 wt.% for PAA-2NS-8.7 and 0.0056 wt.% for PAA-DNS-1.83. Excitation for fluorescence was achieved at 260 nm with excitation and emission slits of 5 nm for 1NS-modified PAA, at 281 nm with excitation and emission slits of 3 nm for 2NS-modified PAA and at 304 nm with excitation and emission slits of 3 nm for PAA-DNS-1.83. The absorbance at the excitation wavelength was lower than 0.07 for 1NS-modified PAA, lower than 0.13 for 2NS-modified PAA and lower than 0.08 for PAA-DNS-1.83. To calculate the relative intensity of the monomer emission I_M and the ratio of excimer emission to monomer emission (E/M), the fluorescence intensities of the monomer emission were taken at the maximum of this emission, and the fluorescence intensities of the excimer emission were taken at 395 nm for the 2NS chromophore and at 400 nm for the 1NS chromophore.

3. Results and discussion

3.1. Spectral features of PAA derivatives

The absorption and fluorescence spectra of the PAA polymers containing 2NS and 1NS groups in water are shown in Fig. 1. The spectral features of the two 2NS-modified polymers are compared. The absorption maximum around 280 nm is larger and located at a shorter wavelength for PAA-2NS-4.2. The monomer emission appearing as a structured peak at 345 nm is also larger for this polymer. However, the broad excimer emission peaking around 400 nm is stronger for PAA-2NS-8.7. These results indicate that the chromophore stacking of PAA-2NS-8.7 is more significant than that of PAA-2NS-4.2.

The spectral features of the two 1NS-modified polymers are also compared. The absorption maximum around 290 nm is slightly smaller and is located at a longer wavelength for PAA-1NS-5.0. The monomer emission appearing as a broad peak is much smaller and is located at a shorter wavelength for this polymer. In addition, the excimer emission appears as a shoulder around 400 nm for this polymer. These observations indicate that the chromophore stacking of PAA-1NS-5.0 is more significant than that of PAA-1NS-1.92.

The spectral behaviour of sodium 1- and 2-naphthalenesulphonate (Na1NS and Na2NS), model compounds for 1NS

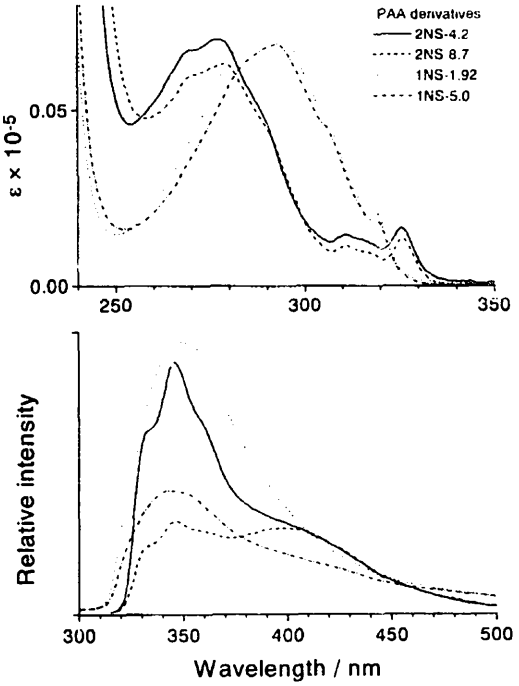

Fig. 1. Absorption and fluorescence spectra of PAA polymers in water. The fluorescence intensity is normalized to equal absorbance.

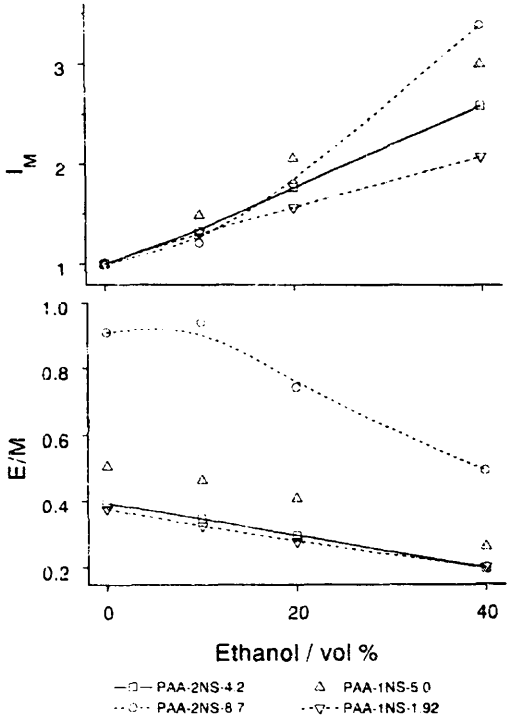

Fig. 2. I_M and E/M for PAA polymers as a function of ethanol content.

and 2NS groups bound to PAA, has been studied in the presence and absence of unmodified PAA [25]. Na2NS exhibits a stacking interaction of the naphthalene ring and predominantly forms dimers in water in the presence of PAA, whereas Na1NS exhibits little association because of the more severe steric hindrance around the sulphonate group. In contrast with Na1NS, which is only electrostatically bound to positively charged PAA, the 1NS group bonded covalently to PAA shows an excimer emission, probably because the covalent bond allows the stacking interaction of the naphthalene ring.

3.2. Effect of ethanol

The addition of ethanol to aqueous solutions of PAA derivatives is expected to affect strongly the fluorescence of the polymers. Fig. 2 shows I_M and E/M as a function of ethanol content in the solution. As ethanol is added, the I_M value increases and the E/M value decreases for all polymers. Polymer-bound hydrophobic chromophores cause the polymer chain to fold to bring non-nearest-neighbour chromophores into contact [26]. This enhances the self-quenching of excited chromophores for fluorene-labelled hydroxypropyl cellulose [27] and the quenching by the amino groups for pyrene-labelled polyethyleneimine [28]. These phenomena probably also occur in the present system. It seems that ethanol weakens the hydrophobic interaction and extends the polymer chain, which depresses both the self-quenching of the chromophores and quenching by the primary amino groups of PAA.

At an ethanol content of 40 vol.%, the E/M value for PAA-2NS-8.7 is 0.50, much larger than that for the other polymers. This implies that a fair amount of excimer emission remains for PAA-2NS-8.7 at this ethanol content, whereas almost no emission of the excimer appears for the other polymers. The former probably has many pairs of neighbouring chromophores on the polymer chain as expected from its large chromophore content.

Fig. 3(a) shows the fluorescence spectrum of PAA-1NS-1.92 as a function of the ethanol content. When the ethanol content is increased from 0 to 40 vol.%, the monomer emission shifts from 350 to 340 nm. This emission shifts from 345 to 340 nm for PAA-1NS-5.0, but does not shift at all for the 2NS-modified polymers. This suggests that the 1NS group can probe the polarity of the microenvironment around the chromophore. As ethanol is added to the solution, the microenvironmental polarity decreases and hence the maximum wavelength of the monomer emission decreases. As stated above, the monomer emission appears at a shorter wavelength for PAA-1NS-5.0 than for PAA-1NS-1.92, indicating that the chromophores are stacked more strongly in the former and the microenvironment around the chromophores is more hydrophobic. Ethanol probably decreases the polarity of the environment less significantly for the former.

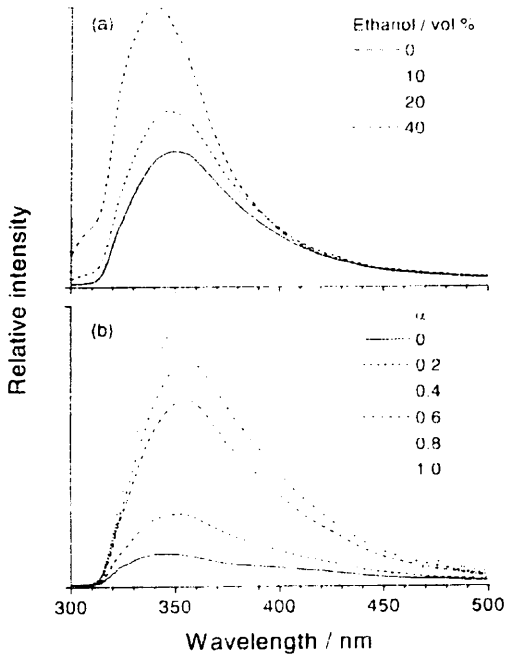

Fig. 3. Polarity dependent shift of the fluorescence maximum for 1NS-modified PAA. (a) fluorescence spectra for PAA-1NS-1.92 at various contents of ethanol; (b) fluorescence spectra for PAA-1NS-5.0 at various values of α.

3.3. Effect of protonation of PAA

The protonation of the primary amino groups of the PAA derivatives expands the polymer chain due to electrostatic repulsion and may strongly affect the spectral behaviour of the polymers. Fig. 3(b) shows the fluorescence spectrum of PAA-1NS-5.0 in water at various values of the degree of neutralization (α) adjusted by the addition of HCl to the solutions. The monomer emission increases dramatically over the whole range of α. A similar dependence on α was observed for all PAA derivatives. As the amino groups are protonated, the amine quenching continuously decreases. Together with this change, the polymer chain expands, the stacking of the chromophores becomes weaker and the self-quenching of the chromophores probably decreases.

As can be seen in Fig. 3(b), the monomer emission shifts to longer wavelengths with increasing α (345 → 356 nm). Because the maximum wavelength reflects the microenvironmental polarity, it can be concluded that the chromophore exists in the hydrophobic domain formed by the polymer and is exposed to the bulk solution as the polymer is neutralized.

3.4. Effect of K_2HPO_4

Salts of divalent or trivalent anions, such as phosphate ion, precipitate PAA by building up crosslinks between the polymer molecules. It is expected that these salts will strongly

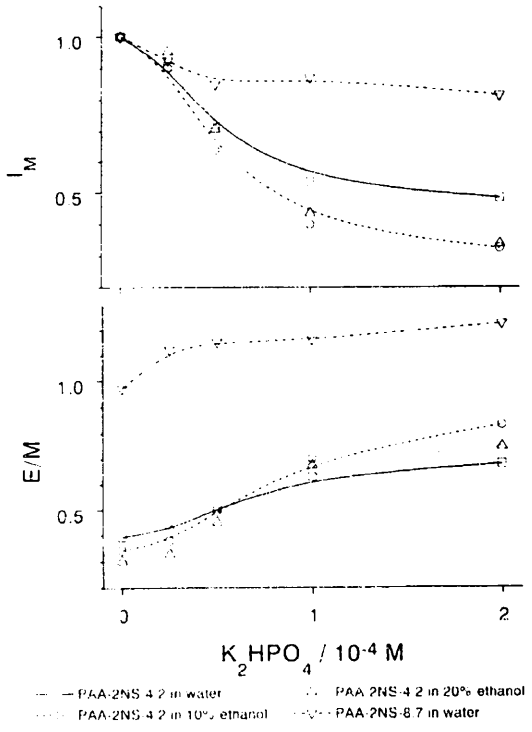

Fig. 4. I_M and E/M for 2NS-modified PAA as a function of K_2HPO_4 concentration.

affect the conformation of the polymer molecule, which will be detected via the fluorescence spectra of the chromophores bonded to the polymer.

Fig. 4 shows the effect of K_2HPO_4 on I_M and E/M for 2NS-modified polymers in water and in aqueous ethanol. As the salt is added, the I_M value decreases and the E/M value increases for both polymers. The decrease in I_M and the increase in E/M for PAA-2NS-4.2 are greater in aqueous ethanol than in water. In addition, both changes are much less for PAA-2NS-8.7 than for PAA-2NS-4.2.

Fig. 5 shows the effect of K_2HPO_4 on I_M and E/M for 1NS-modified polymers in water and in aqueous ethanol. Both I_M and E/M are almost unchanged on addition of the salt for PAA-1NS-5.0 in water. However, for this polymer, the I_M value decreases and the E/M value increases in aqueous ethanol. In contrast, the I_M value decreases for PAA-1NS-1.92 in water, although the E/M value shows little change. It is also tempting to note that the monomer emission shifts to a shorter wavelength on addition of the salt for PAA-1NS-5.0 in 10 vol.% aqueous ethanol (345 → 343 nm) and 20 vol.% aqueous ethanol (343 → 340 nm) and for PAA-1NS-1.92 in water (352 → 348 nm).

Since the addition of more than 0.0002 M of K_2HPO_4 renders the solution turbid, the polymer molecules must be combined by electrostatic crosslinking by phosphate ions leading to coagulation above this salt concentration. Presumably, crosslinking is mainly intramolecular below this salt

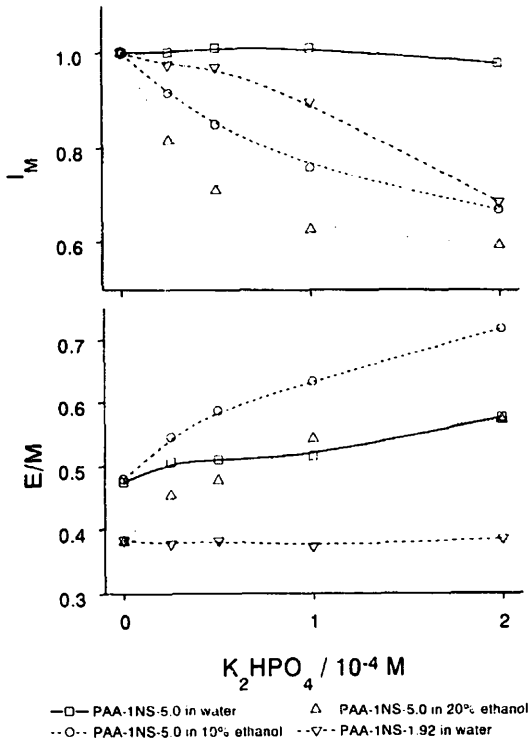

Fig. 5. I_M and E/M for 1NS-modified PAA as a function of K_2HPO_4 concentration.

concentration. In addition, the amino groups of PAA are considered to be free at this concentration, because the pH values of dilute solutions of the salt are higher than pH 10.

The intramolecular crosslinking may result in contraction of the polymer chain and enhancement of the amine quenching for PAA-2NS-4.2 and PAA-1NS-1.92. This also enhances the stacking interaction of naphthalene rings for PAA-2NS-4.2 as judged by the increase in E/M. However, the E/M value shows little change for PAA-1NS-1.92, probably because of its small chromophore content and because the nature of the 1NS chromophore is different from that of the 2NS chromophore. For polymers possessing high chromophore contents, such as PAA-2NS-8.7 and PAA-1NS-5.0, the addition of K_2HPO_4 causes little or no effect on the fluorescence of the chromophores. For these polymers, the polymer chains are sufficiently contracted without salt due to strong chromophore stacking and, hence, intramolecular crosslinking of the polymer chains is fairly limited.

Both a decrease in I_M and an increase in E/M are detected for PAA-2NS-8.7 in water, but are negligible for PAA-1NS-5.0 in water, when K_2HPO_4 is added to the solutions. Therefore it can be concluded that a chromophore content of 8.7 mol.% is not sufficient for 2NS-modified polymers to depress completely the quenching effect of the salt; however, a chromophore content of 5.0 mol.% is sufficient for 1NS-modified polymers. In other words, the chromophore content required to suppress the effect of K_2HPO_4 is lower for the 1NS group than for the 2NS group. Since the 1NS group is substituted along the short axis of the naphthalene ring, stacking of the ring may cause a more severe steric hindrance than in the case of the 2NS group.

Both the decrease in I_M and the increase in E/M, observed on addition of K_2HPO_4, are greater in aqueous ethanol than in water for PAA-2NS-4.2. The effect of the salt cannot be detected in water and can only be observed in aqueous ethanol for PAA-1NS-5.0. These observations imply that ethanol strengthens the effect of K_2HPO_4 on I_M and E/M. Because the polymer chain is extended in aqueous ethanol without salt, the addition of the salt contracts the chain conformation more significantly.

The monomer emission shifts to a shorter wavelength on addition of K_2HPO_4 for PAA-1NS-5.0 only in aqueous ethanol and for PAA-1NS-1.92 in water. The contraction of the polymer chain probably makes the microenvironment around the chromophores more hydrophobic. This phenomenon occurs only when the polymer chain is flexible due to the presence of ethanol or to low chromophore contents.

3.5. Effect of KCl

If a salt exhibits no specific interaction with a polymer, the degree to which the salt will contract or extend the chain conformation will be dependent on the hydrophilic and hydrophobic balance of the polymer [29,30]. Therefore the polymer conformation can be studied by examining the effect of a salt of a monovalent anion on the fluorescence behaviour of the covalently bound chromophores.

Fig. 6 shows the effect of KCl on I_M and E/M for 2NS-modified polymers in water and in aqueous ethanol. As the salt is added, the I_M value decreases and the E/M value increases for PAA-2NS-4.2. However, as the content of ethanol increases in the solution, the effect of KCl on I_M and E/M becomes less marked, especially at low salt concentrations. In contrast, the I_M value is enhanced and the E/M value is almost unchanged with increasing concentration of the salt for PAA-2NS-8.7 in water.

Fig. 7 shows the effect of KCl on I_M and E/M for 1NS-modified polymers in water and in aqueous ethanol. The addition of the salt increases both I_M and E/M for PAA-1NS-5.0 in water. Both increases in I_M and E/M become less marked with increasing content of ethanol. Furthermore, the monomer emission shifts to a longer wavelength at ethanol contents of 0 and 10 vol.% (345 → 348 nm), but not at an ethanol content of 20 vol.%. In contrast, the I_M value decreases and the E/M value increases slightly on addition of KCl for PAA-1NS-1.92 in water. Moreover, this emission shows a small concomitant short-wavelength shift (352 → 350 nm).

It is expected that KCl enhances intramolecular interactions, such as the hydrophobic interaction of the chromophores and the hydrogen bonding of the amino groups, inside the polymer. When the chromophore content is low, i.e. in the case of PAA-2NS-4.2 and PAA-1NS-1.92, this may lead to contraction of the polymer conformation and, hence, the

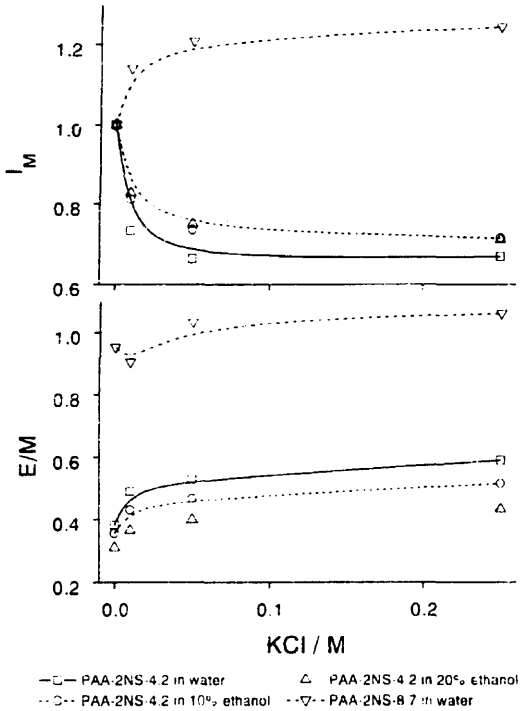

Fig. 6. I_M and E/M for 2NS-modified PAA as a function of KCl concentration.

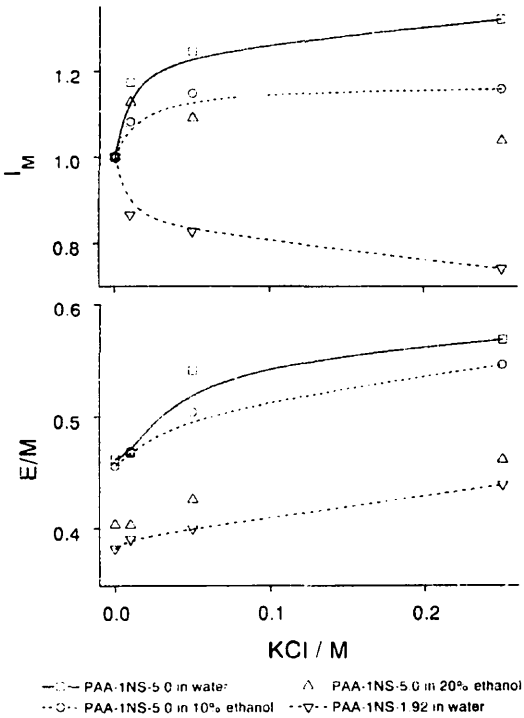

Fig. 7. I_M and E/M for 1NS-modified PAA as a function of KCl concentration.

enhancement of amine quenching. The increase in E/M indicates that the stacking interaction of the naphthalene rings becomes stronger in the presence of the salt. The short-wavelength shift of the monomer emission for PAA-1NS-1.92 indicates a decrease in the microenvironmental polarity around the chromophores, suggesting that the chromophores are transferred to the hydrophobic domain. However, the situation is quite different when the chromophore content is high, i.e. in the case of PAA-2NS-8.7 and PAA-1NS-5.0. The monomer emission value increases with increasing KCl concentration. The polymer chain is probably already compact so that the addition of the salt causes little further conformational change, although it magnifies the intramolecular interactions. This may lead to the separation of the chromophores and the amino groups into different domains and, hence, to the depression of amine quenching and enhancement of the stacking of the chromophores. This is supported by the fact that an increase in E/M, instead of a decrease, is observed for PAA-1NS-5.0, in addition to an increase in I_M. In addition, the long-wavelength shift of the monomer emission for PAA-1NS-5.0 suggests that some of the chromophores are exposed to the bulk solution in this process.

The decrease in I_M and increase in E/M on addition of KCl become less marked in the presence of ethanol for PAA-2NS-4.2. The increases in both values become less marked in the presence of ethanol for PAA-1NS-5.0. In addition, the I_M value begins to decrease after the initial increase in 20% ethanol for this polymer. These results indicate that ethanol weakens the ability of KCl to magnify the intramolecular interactions. As a result, ethanol depresses mainly the contraction of the polymer conformation for PAA-2NS-4.2, but mainly the separation of groups possessing different hydrophilicity for PAA-1NS-5.0. It is possible that PAA-1NS-5.0 has an intermediate nature in 20% ethanol, so that the I_M value exhibits an increase or decrease depending on the KCl concentration. It appears that ethanol weakens the effect of KCl. This is contrary to the results for K_2HPO_4, where the effect was magnified by the addition of ethanol. This is probably because KCl only changes the property of the solution, whereas K_2HPO_4 has a specific interaction with the polymer molecules.

3.6. DNS-modified polymer

PAA possessing only a covalently bound DNS group has already been synthesized [23], but its characterization is incomplete. The effect of ethanol on the fluorescence was examined for PAA-DNS-1.83. An increase in ethanol content from 0 to 20 vol.% causes a slight short-wavelength shift of the fluorescence maximum (λ_f) (545→543 nm) and an increase in the relative intensity (I_f) (1.0→1.28). This is attributed to a decrease in the solvent polarity and, at least in part, to decreased self-quenching by extension of the polymer.

In contrast, neutralization of the polymer, i.e. a change in α from zero to unity, shifts λ_I to a longer wavelength (545 → 570 nm) and decreases I_I (1.0 → 0.43). It has been reported that PAA derivatives containing long alkyl or benzyl groups and DNS groups form hydrophobic domains which incorporate the DNS groups [23]. These observations imply that PAA containing only DNS groups also forms such domains and that the chromophores which exist in these domains in water are exposed to the bulk solution when the polymer is neutralized. The polymer chain of PAA itself is probably rather hydrophobic and is somewhat contracted in water. Furthermore, the decrease in I_I, instead of an increase, indicates that the quenching by amino groups of PAA hardly occurs for this chromophore.

The effect of salts on the spectral behaviour was also examined for PAA-DNS-1.83. An increase in K_2HPO_4 concentration from 0 to 0.0002 M does not affect λ_I, but leads to a slight decrease in I_I (1.0 → 0.89). An increase in KCl concentration from 0 to 0.25 M shifts λ_I slightly to a longer wavelength (545 → 550 nm) and decreases I_I (1.0 → 0.79). These are probably the result of the enhanced self-quenching of the chromophores due to contraction of the polymer molecules.

4. Conclusions

Our primary motivation in the present work was to examine the ability of 1NS and 2NS groups to act as optical probes monitoring the polymer conformation and chemical parameters in solution. Both groups can detect the conformational change caused by the addition of ethanol or HCl to the solutions regardless of the chromophore content in the polymer. The I_{VI} value increases dramatically and the E/M value decreases in these cases. The sensitivity of 1NS and 2NS as optical probes towards ethanol and HCl is higher or as high as that of the widely used probe, DNS, whose fluorescence intensity increases slightly on addition of ethanol, but decreases on addition of HCl. However, 1NS and 2NS groups cannot always detect the concentrations of the salts K_2HPO_4 and KCl. Only polymers with low chromophore contents, PAA-1NS-1.97 and PAA-2NS-4.2, show monotonic decreases in I_{VI} with increasing salt concentration. However, when an appropriate chromophore content is used, the sensitivity of both groups towards K_2HPO_4 and KCl is higher than that of DNS, whose fluorescence intensity decreases slightly on addition of these salts.

The introduction of 1NS and 2NS groups into PAA significantly affects the solution properties of the polymer, as observed for polymers with high chromophore contents which seem to possess very compact conformations. These chromophores are fairly hydrophobic and exert strong stacking interaction in the polymer. In contrast, the DNS group is less hydrophobic and only forms weak associations on addition of K_2HPO_4 and KCl. Another feature of 1NS and 2NS groups is their susceptibility to amine quenching, as demonstrated by the strong increase in the fluorescence intensity with neutralization of the polymer (in contrast with the fluorescence of the DNS group which is little affected by this quenching process). This susceptibility increases the sensitivity of 1NS and 2NS groups towards chemical parameters such as the pH, ionic strength and concentrations of third components. In addition, the 1NS group can probe the polarity of the microenvironment around the chromophore in a similar manner to the DNS group. The maximum wavelength of monomer emission decreases as the polarity decreases. This feature further increases the potential of this type of chromophore as an optical probe.

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