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Studies on the photophysical characteristics of poly(carboxylic acid)s bound protoporphyrin IX and metal complexes of protoporphyrin IX

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Abstract

The copolymers of methacrylic acid with protoporphyrin IX (PPIX) and the metal complexes, zinc protoporphyrin IX and magnesium protoporphyrin IX were synthesised and characterised. Corresponding acrylic acid copolymers were also synthesised. The steady state absorption and fluorescence spectral properties of the macromolecular bound fluorophores PPIX, Zn-PPIX and Mg-PPIX were investigated. Poly(methacrylic acid) bound protoporphyrin IX, zinc protoporphyrin IX and magnesium protoporphyrin IX show an increase in the fluorescence intensity and lifetime with increase in the pH in the range 2–8 with a marked transition around pH 6.0–7.0. The fluorophore concentration in the dilute solution of the copolymers is micromolar and the fluorophore to the carboxylic acid monomer ratios in the copolymer is around 10^{-3}. The molecular weight of the copolymers is 100 ± 10 kD. The fluorescence decay curves of all the fluorophore bound polymers follow biexponential decay fit independent of pH. Poly(MAA-co-PPIX) and poly(MAA-co-MgPPIX) undergo well marked pH induced structural transitions in the pH range of 6.0–7.0 whereas poly(MAA-co-ZnPPIX) undergoes pH induced structural transitions in the pH range of 4.0. In the case of polyacrylic acid copolymers the changes observed in the steady state and time resolved fluorescence studies are less marked. The distinct hydrophobic and hydrophilic environments experienced by the fluorophore bound to PMMA are attributed to the dynamics of the macromolecules in dilute aqueous solutions manifested by the α-methyl group present in the copolymer. The studies carried out using the fluorophores in the time windows from 2 ns to 12 ns indicate evolving trends in the dynamic coiling and reverse coiling of poly methacrylic acid chain.

Keywords: Macromolecular structural transitions; Time resolved fluorescence; Protoporphyrin IX; Zinc protoporphyrin IX; Magnesium protoporphyrin IX; Solution dynamics; Methacrylic acid copolymer

1. Introduction

Porphyrins and their metal complexes play a key role in controlling reactivities and biological functions [1–4]. Systematic studies mimicking natural systems have focussed on the electrochemical,

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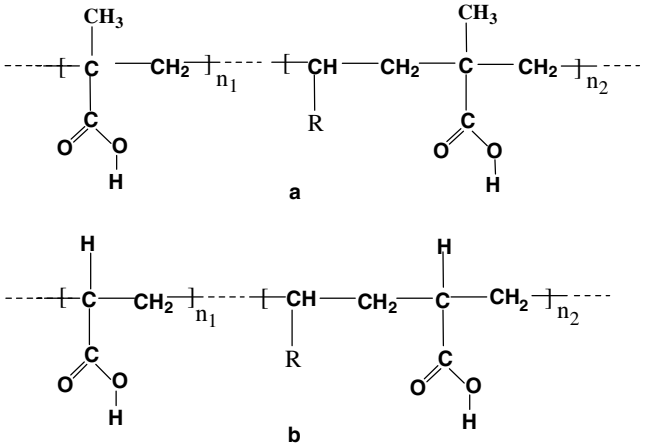
catalytic and photochemical behavior of porphyrins in various supramolecular assemblies [5] such as polymers [6], vesicles [7–10], micelles [11], monolayers [12,13] and Langmuir Blodgett films [14–16]. The microenvironment produced by the polyelectrolytes influences the photophysical and photochemical properties of the chromophores bound to the polymers [17]. The local environment of the polyelectrolytes is controlled by electrostatic forces, hydrophobic interactions and hydrogen bonding of the polymers or substitutents [18]. The fluorophores attached to the polymer indicate the characteristics of such effects manifested by the polymer backbone.

Different techniques have been employed to study the conformational transitions of polyelectrolytes induced by changes in the degree of ionisation [19]. The kinetics of expansion of poly(carboxylic acid) induced by pH jump using fluorescence technique suggest that at low pH the macromolecule adopts a hypercoiled form in order to minimise the hydrophobic interactions [20]. At high degree of ionisation and in the absence of electrolytes the chain stretches to a rod like form. The transition between these two states occurs around pH 4.5–6.5. Structural transitions of poly methacrylic acid (PMAA) and poly acrylic acid (PAA), occurring over the pH range from 2.0 to 10.0 have been investigated by means of the fluorescence probe tris(2,2′-bipyridine)ruthenium(II), $[Ru(bpy)3]^{2+}$ [21]. More recently we have used fluorescent probes with lifetimes in the range of 300 ps to 4 ns to study the dynamics of macromolecules in aqueous solutions [22].

Thomas and coworker [20] have used pyrene as a fluorescent probe to study the conformational transition of PMAA and PAA. The kinetics of the conformational transition of PMAA observed as a function of degree of ionisation was monitored by observing the change in the fluorescence intensity of dansyl labeled poly(carboxylic acid) [23,24]. Four stages of conformational transitions of PMAA with change in pH were proposed by Morawetz et al. Expansion of the polymer with the penetration of water molecules into the coil of PMAA takes place at pH 5.0



Scheme 1. Structures of the fluorophore used (a) poly(methacrylic acid) bound fluorophores where R = protoporphyrin IX, Mg-protoporphyrin IX or Zn-protoporphyrin IX (b) poly(acrylic acid) bound fluorophore where R = protoporphyrin.

with continuous expansion of polymer to give extended rod with increased water penetration at pH > 5.0 [25]. The fluorescence properties of thionine tagged with poly(acrylamidoglycolic acid) were studied at different pH in this laboratory earlier [26]. In the present study structural transitions occurring in nanosecond time domain are observed using covalently bound protoporphyrin and metal complexes of porphyrins in the pH range 2–14. Protoporphyrin IX is insoluble in aqueous medium and in order to prepare water-soluble PPIX and to create a heterogeneous environment, one of the vinyl groups in PPIX was copolymerised with methacrylic acid. We have used the fluorophores to monitor the nature of the conformational transition of PMMA over the pH range of 2.6–12.0, from the tight compact form at low pH extending to the larger linearly extended form at higher pH. The structural transitions at different time scales are explained in terms of solvent—polymer dynamics. The structures of the free fluorophores and polymer bound fluorophores are given in Scheme 1.

2. Experimental

Methacrylic acid and acrylic acid were obtained from Aldrich chemicals. The monomers were vacuum distilled and are used in the experiments. Poly(carboxylic acids) were prepared by the polymerisation of the corresponding monomers by free radical polymerisation as described earlier [6]. PPIX was extracted from goat blood and purified by known method [27]. This sample is identical to the commercial sample obtained from Aldrich. All other chemicals were purchased from the Qualigens and Merck Fine Chemicals. Standard methods are followed for the purification of the common organic solvents. All other chemicals were of analytical grade and are used as received unless otherwise stated. The amount of PPIX present in the polymer was estimated spectrophotometrically using the molar absorbtivity of PPIX as $1.24 \times 10^5\ M^{-1}\ cm^{-1}$ at 405 nm in dimethyl formamide (DMF). Triply distilled water was used in all the experiments. Synthesis of poly(methacrylic acid-co-protoporphyrin IX) and poly(acrylic acid-co-protoporphyrin IX) is described in a recent publication [28]. Free PPIX is eliminated by repeatedly dissolving the copolymer and reprecipitating the sample. The absorption spectrum also indicates the absence of free PPIX in the samples. NMR spectra of the sample shows that one of the vinyl group in PPIX is still present in the copolymer.

2.1. Poly(methacrylic acid-co-metallated-protoporphyrin IX)

Known weight of poly(methacrylic acid-co-protoporphyrin IX) was dissolved in a given volume of water. Excess amount (100-fold) of respective metal sulphate was added to the solution with stirring. After 12 h of stirring the free metal ions in the above clear solution of poly(MAA-co-PPIX) were removed by dialysing the sample for several days against distilled water using a cellulose bag (Sigma, cut off 12 kD). The resulting dialysed solution, poly(MAA-co-M-PPIX) where M = Zn or Mg was used for further experiments. The completion of the reaction was ascertained by monitoring the absorption spectra which shows characteristic features for the metal porphyrin complex.

2.2. Quantitative estimation of dyes present in the polymer

The amount of PPIX and metal complexes of porphyrin present in the polymer was estimated by taking known amount of PPIX bound poly(carboxylic acid) dissolved in known volume of water; the pH of the solution was adjusted to 7.0 using 0.1 M sodium hydroxide solution. The number of fluorophore units present as a ratio of the monomer present in the polymer was calculated from the known amount of the sample taken and the amount of PPIX present in the sample. For this the molar absorptivity of the PPIX present is taken to be that of PPIX in DMF: $1.24 \times 10^5\ M^{-1}\ cm^{-1}$ at 405 nm.

2.3. Absorption and fluorescence measurements

The absorption spectra of the samples were recorded using the Hewlett-Packard 8452A UV–Visible diode array spectrophotometer attached to HP 310 computer and Hitachi 320 spectrophotometer. The fluorescence spectral measurements were carried out using Perkin–Elmer LS-5B luminescence spectrometer attached to an IBM PC via RS-232C interface.

2.4. Gel permeation chromatography (GPC)

The molecular weight distribution of the homopolymers and polymer bound protoporphyrin IX in water were determined using Waters 515 by the refractive index method. Varian series RI-3 using Waters 515 HPLC pump and Waters 746 data

modules were used for the analysis of data. The samples were filtered through 0.45 μM dia. Millipore membrane filter in order to eliminate the solid particles. Ultrahydrogel 500 column for the separation was used with the flow rate of 0.6 ml/min using millipore water. Poly(vinyl alcohol) of various molecular weights dissolved in water was used as standards. The molecular weights of the copolymers are observed to be in the narrow range as the low molecular fragments pass through the dialysis membrane and the polymerisation conditions are maintained constant for all the samples.

2.5. Time correlated single photon counting

Time resolved fluorescence decays were obtained by the time correlated single photon counting (TCSPC) method as described earlier [28]. The instrument response function for this system is ~52 ps. The fluorescence decay is obtained which is further analysed using IBH (UK) software (DAS-6).

3. Results and discussion

3.1. Absorption spectral properties of polymer bound protoporphyrin IX and porphyrins complexed with metal ions

The molecular weights of the polymers investigated are found to be 100 ± 10 kD [28]. The absorption spectrum of protoporphyrin IX shows Soret band (B band) in the region 350–450 nm with the absorption maximum at 403 nm. The Q bands of the protoporphyrin IX are well resolved in DMF, which consists of four weak bands having maxima at 503, 532, 565 and 630 nm [29]. The absorption spectra of free PPIX and PPIX bound to polymer shown in Fig. 1a indicate that the absorption peaks of copolymers are not well resolved particularly in the longer wavelength region [19,21,30]. Macromolecular bound protoporphyrin IX, poly(MAA-co-PPIX) in aqueous solution (pH 4.8) shows splitting of the Soret band into two bands with absorption maximum at 375 and 409 nm as shown in Fig. 1a; the maxima of the Q bands are not altered significantly. The intensities of the B bands are an order of magnitude greater than that of the Q bands and therefore only the change in the absorption of B bands are observed well. Broadening of the absorption spectral bands of the polymeric porphyrin species in water is more likely to be due to site

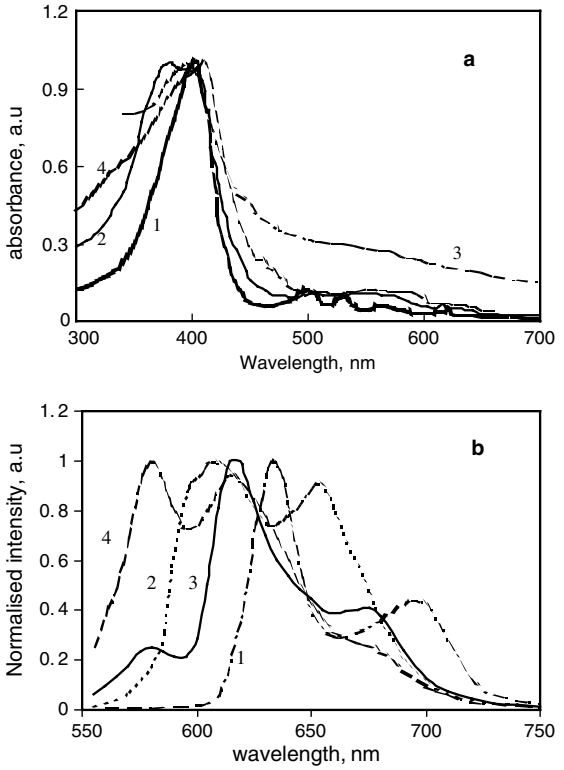


Fig. 1. (a) Normalised absorption spectra of (1) free PPIX in DMF, (2) PMMA-co-PPIX, (3) PMMA-co-MgPPIX, (4) PMMA-co-ZnPPIX. Temperature = 23.0 ± 0.5 °C. (b) Normalised emission spectra of (1) free PPIX in DMF, (2) PMMA-co-PPIX, (3) PMMA-co-MgPPIX, (4) PMMA-co-ZnPPIX; λ_{ex} at their corresponding absorption maxima of Soret band temperature = 23.0 ± 0.5 °C.

inhomogeneity in the polymer or due to interaction of PPIX from different polymer chains, since the ratio of PPIX to methacrylic acid is rather low.

At pH < 5.0 the hypercoiled PMAA exerts strong interaction with PPIX bound to the polymer resulting in the absorption spectrum different from that at higher pH. At pH > 5.0 loosening of the polymer chain due to unfolding results in the spectrum corresponding to the free porphyrin with the Soret band appearing with higher intensity. Protoporphyrin IX, insoluble in aqueous solutions has substitutents –COOH and pyrrole whose acidity constants are such that in the pH range 3.0–10.0 no significant protonation or deprotonation occurs for the fluorophore. Besides our studies using thionine and phenosafranine dyes [22], which do not, have any ionisable protons in the pH range show pH induced absorption and emission spectral changes similar to that in the present investigation. This supports our

conclusion that the observed spectral changes of the covalently bound PPIX are due to the macromolecular and solvent environment alone and not due to any significant change in the acidity of the fluorophore itself. As the monomer to PPIX ratio is as high as 10^3 in the polymer, dimerisation of PPIX is unlikely [31,32]. However, it is known that in the tightly existing super coiled polymer chains, inter polymer adducts could be formed when the fluorophores in different chains could interact [33]. It may be noted that poly(MAA-co-PPIX) in DMF shows a single peak [34] indicating the existence of monomeric protoporphyrin IX confirming the similarity of the electronic structure of free and polymer bound fluorophore particularly at pH > 5.0. Poly(AA-co-PPIX) in dilute aqueous solution shows absorption maxima at 397 nm. The free polymer PMAA or PAA does not absorb above 300 nm and hence absorption of PPIX bound macromolecule in the visible region is only due to the electronic transitions occurring in PPIX. Absorption spectra of poly(MAA-co-Zn-PPIX) and poly (MAA-co-Mg-PPIX) in aqueous solutions show absorption maxima at 402 and 400 nm, respectively for the Soret band. In addition to these two bands, Q bands are observed in the visible regions at 540 ± 3, 575 ± 3 nm and at 535 ± 3, 570 ± 3 nm for poly(MAA-co-Zn-PPIX) and poly(MAA-co-Mg-PPIX), respectively.

3.2. Fluorescence spectral properties of polymer bound PPIX and porphyrins complexed with metal ions

The emission spectra of monomeric PPIX in DMF and poly(MAA-co-PPIX) and the polymer-porphyrin systems complexed with metal ions in aqueous solutions are shown in Fig. 1b. The emission spectrum of free PPIX shows emission maxima at 636, 580 and 680 nm [6]. The emission maxima of the poly(carboxylic acid) bound PPIX are blue shifted compared to the free PPIX.

The emission spectrum of poly(MAA-co-PPIX) in aqueous solution at pH 4.5 shows maxima at 615 nm and 656 nm, which is 21 nm blue shifted as compared to free PPIX. The shift in the emission maxima of poly(MAA-co-PPIX) is attributed to the presence of the supercoiling of the polymer at that pH. In the supercoiled state, the hydrophobic microdomains are formed as suggested from the emission intensity. In general, it is well known that the fluorophore in the hydrophobic environment shows enhanced fluorescence intensity. The fluorescence intensity enhancement in the hydrophobic medium is known to be due to the slower radiationless decay. However, hydrogen bonding between carboxylic acid and fluorophores in the hydrophobic microdomain also shifts the emission maxima towards blue rather than to the red, which induces the non-radiative relaxation of the fluorophores present in the aqueous medium with the reduction in the fluorescence quantum yield [35]. In the case of fluorophores such as naphthalene and pyrene red shift is indeed observed, as these fluorophores are not affected by the interaction with the carboxylic acid groups in the polymer. Poly(AA-co-PPIX) shows emission maxima at 624 nm. The smaller blue shift observed in poly(AA-co-PPIX) is presumably due to the weak hydrophobic environment exerted by PAA polymer chain on the bound PPIX. The emission spectra of poly(MAA-co-Zn-PPIX) and poly(MAA-co-Mg-PPIX) show maxima of 580 ± 3, 630 ± 3 nm for the former and 628 ± 3 nm for the latter (Fig. 1b). Similar results have been reported for the poly(acrylamide) copolymer with Zn-PPIX in aqueous solution [40].

3.3. Effect of pH on the fluorescence spectral properties of poly(MAA-co-PPIX)

Variation in the emission intensity of the polymer bound PPIX systems as a function of pH is shown in Fig. 2. Fluorescence intensity increases gradually while increasing the pH from 2.6 to 5.0. In aqueous solution at lower pH (pH < 6.0) the polymer PMAA exists in the hypercoiled state, in which the polymer adopts more hydrophobic or tightly coiled conformation [17,36,37]. It is imperative that in the tightly coiled polymer conformation, the –COOH groups of the polymers strongly interact with the fluorophore through hydrogen bonding with the pyrrole nitrogen, which accelerates the non-radiative decay process from the emitting state. As stated earlier with the fluorophore at monomer to fluorophore ratio of 10^3, dimer formation of the fluorophore is unlikely. This behaviour is in sharp contrast to the behaviour of other fluorophores such as pyrene and naphthalene bound to PMAA where the emission intensity reaches a maximum at pH less than 5.0 [21,24,25]. In the present case fluorescence intensity increases gradually with increase in pH up to 6.0 and above this pH, a sharp increase in the fluorescence intensity was observed. The fluorescence intensity remains unchanged with further increase

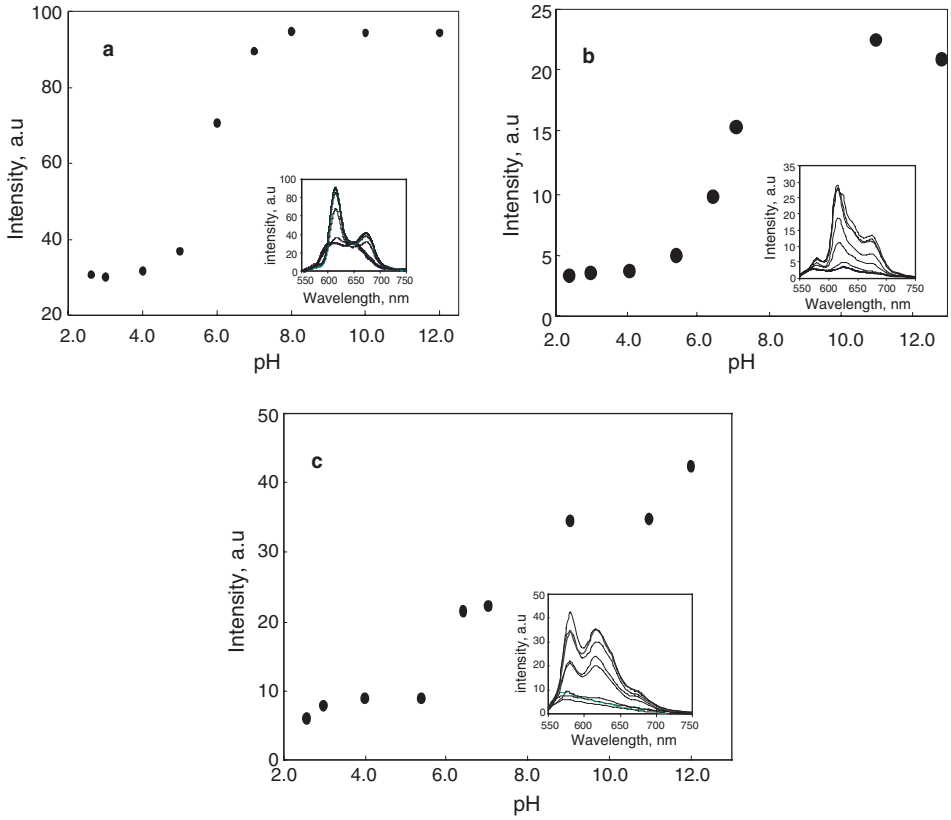


Fig. 2. Plot of emission intensity at λ_{em} max vs pH of poly(MAA-co-PPIX) (a); poly(MAA-co-MgPPIX) (b); poly(MAA-co-ZnPPIX) (c). Inset: emission spectra of poly(MAA-co-PPIX) (a); poly(MAA-co-MgPPIX) (b); poly(MAA-co-ZnPPIX) (c) at different pH; temperature = 23.0 ± 0.5 °C.

in the pH up to 12.0. Variation of fluorescence intensity maxima at 622 and 674 nm with pH shown in Fig. 2a (inset) reveals that the fluorophore is present in a strongly hydrogen bonded polar environment of the polymer in the supercoiled state up to pH 5.5. Complete ionisation of poly(methacrylic acid) is known to occur only above pH 7.0 [20]. The deprotonation of –NH in the pyrrole unit of PPIX does not occur at pH ~7.0.

Zana and coworker [38] have explained the formation of hydrophobic microdomains at pH greater than 7.0 where the anionic part is exposed to aqueous region while aromatic fluorophore and the hydrocarbon chains are present in the interior of the microdomain, akin to micelle formation. It appears that in both the conformational states of PMAA, the covalently bound PPIX prefers the hydrophobic environment. At high pH, as the hydrogen bonding with the –COOH of the polymer with the fluorophore is absent, emission quantum yield is enhanced. Increase in the emission intensity beyond pH 12.0 is also attributed to the deprotonation of –NH in the pyrrole unit of protoporphyrin IX. Equilibrium and kinetic behaviour of the transitions of PMAA with pH have been studied by monitoring the fluorescence properties of labeled dansyl, (5-dimethylamino-1-naphthalenesulphonate) covalently attached to the polymer chain and the present observations are in agreement with those reported earlier [23,24].

3.4. Effect of pH on the fluorescence spectral properties of poly(AA-co-PPIX)

The fluorescence intensity of the poly(AA-co-PPIX) increases with increase in pH of the solution as shown in Fig 3. As the pH increases, the fluorescence intensity of PPIX bound to PAA gradually increases up to pH 5.0 and thereafter a plateau was observed up to 12.0. The fluorescence intensity increases from 50.6% to 75% while increasing the pH from 4.0 to 6.0. There is no further increase in

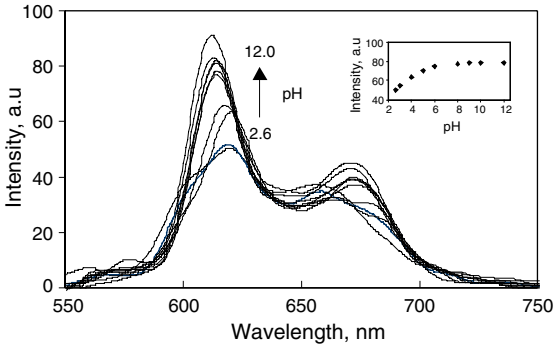


Fig. 3. Emission spectra of poly(AA-co-PPIX) at different pH; inset: plot of emission intensity maxima at 625 nm vs pH $\lambda_{ex} = 375$ nm. Temperataure = 23.0 ± 0.5 °C.

fluorescence intensity of PPIX linked PAA with increase in the pH of the solution. At low pH the polymer is in the compact state as in the case of PMAA except that the hypercoiled state is not as pronounced due to weak hydrophobicity manifested by the PAA.

Above pH > 5.0, the reverse conformation of polymer occurs due to the complete ionisation of the carboxylic acid, which leads to the formation of weak hydrophobic core. Guillet and coworker [41] have explained the intramolecular singlet energy transfer between naphthalene and anthracene covalently bound to PAA by pseudo unimolecular micellar structure formation. PAA is highly flexible and exists as rod like structure in the completely dissociated form, while in the case of poly (MAA-co-PPIX) due to the hydrophobic nature of PPIX the polymer forms more ordered micelle structure.

3.5. Emission spectral properties of the polymer bound PPIX complexed with metal ions at different pH

The emission spectra of Zn-protoporphyrin IX and Mg-protoporphyrin IX bound polymers are sensitive to the nature of the polymer chain around the fluorophore. The change in the emission spectra of poly(MAA-co-MgPPIX) by varying the pH from 2.6 to 12.0 on excitation at its absorption maxima is shown in Fig. 2b. The emission spectra of poly-(MAA-co-ZnPPIX) at various pH reveals the presence of two emission bands around 580 and 625 nm even though at low pH there is only one prominent emission band around 580 nm. Presence of another band at 625 nm is seen only after pH 5.0, while in the case of poly(MAA-co-MgPPIX) only one prominent emission band around 625 nm is observed at all pH. The characteristic peak observed at 580 nm for poly(MAA-co-ZnPPIX) is presumably due to the more stable coordination by the solvent water or carboxylate group of the polymer. The variation in emission intensity of the polymer bound Zn-PPIX with pH is shown in Fig. 2c. Fluorescence intensity increases gradually while increasing the pH from 2.6 to 6.0. Above this pH, a sharp increase in the fluorescence intensity was observed for both the systems. On further increase in the pH up to 12.0, the fluorescence intensity remains unchanged. From the above observations it is concluded that in aqueous solution at lower pH (pH < 6.0) the polymer PMAA exists in the hypercoiled state, in which the polymer adopts more hydrophobic or tightly coiled conformation. The observed spectral changes do not indicate a red shift even in the hydrophobic environment since the fluorophore with the pyrrole nitrogen interacts strongly with the carboxylic acid groups of the polymer by hydrogen bonding. At higher pH reverse coiling occurs with an increase in the fluorescence intensity in all these polymers. Coordination in the axial position by the carboxylate or hydroxide ion is indicated in the change of the emission spectra of the two systems.

3.6. Fluorescence lifetime studies of polymer bound PPIX and PPIX complexed with metal ions

Photophysics and photochemistry of protoporphyrin IX have been reported earlier [29,39]. The fluorescence lifetime of monomeric PPIX is reported to be 11.5 ± 0.5 ns in tetrahydrofuran. The fluorescence decay curve of PPIX fits to a single exponential function as given in Eq. (1)

$$I(t) = B \cdot \exp \cdot(-t/\tau) \quad (1)$$

where B is the pre-exponential factor and τ is the lifetime of the excited fluorophore. The lifetime obtained in the present study is in agreement with the reported value [39]. The fluorescence decay curves of the fluorophore in the polymers poly(MAA-co-PPIX) and the protoporphyrin complexed with the metal ions do not fit single exponential decay and the decay curves are fitted satisfactorily with the biexponential function according to the following equation:

$$I(t) = B_1 \exp(-t/\tau_1) + B_2 \exp(-t/\tau_2) \quad (2)$$

where B_1 and B_2 are the pre-exponential factors and τ_1 and τ_2 are the fluorescence lifetimes. The goodness of the fit is checked by the χ^2 values, which lie between 0.8 and 1.2, and the residuals are randomly distributed around zero. In the case of pyrene as a fluorophore, biexponential decay is observed when there is self-quenching or excimer formation where the fluorophore is influenced by a macromolecule [20]. In the present investigation the monomer concentrations in all the systems are three orders of magnitude higher than the fluorophore and hence self-quenching and excimer formation are insignificant. Furthermore, excimer formation in polymer bound PPIX is unlikely since a characteristic structureless, broad and red shifted fluorescence is not observed in the emission spectrum. The microheterogeneous hydrophobic and hydrophilic environments present around the fluorophore induced by the polymer in aqueous solution are responsible for the two lifetimes observed in these systems. PPIX is distributed randomly and the lifetimes of PPIX correspond to hydrophobic (similar to PPIX in THF) and hydrophilic (exposed to water) environments depending upon the lifetime. It is well known that in more polar environment the lifetime is shorter as compared to that in non-polar medium.

3.7. Effect of pH on the fluorescence lifetime of poly(MAA-co-PPIX) and the magnesium and zinc analogues

Fluorescence lifetimes of poly(MAA-co-PPIX) with increase in pH shown in Fig. 4. correspond to biexponential decay in the range of the pH of the solutions studied. At low pH, the fluorophore in the copolymer shows two lifetimes: 2.8 and 9.2 ns. Above pH 6.0, the lifetime increases sharply reaching a maximum of 12.2 ns above pH 9.5. The short-lived component of the lifetime is found to be independent of pH. The relative amplitude of the long lived component increases to $77 \pm 2\%$ and a plot of pH vs relative amplitudes of the corresponding lifetimes is shown in Fig. 4. The relative amplitude A_2 corresponding to the longer lifetime shows a sudden decrease around pH 6.0. The copolymers of protoporphyrin complexed with metal ions show a similar behaviour as indicated in Fig. 4.

Depending upon the pH, the polymer exists in more compact structure or reverse coiled state, in which the ionised $–COO^-$ are in the exterior and the hydrophobic groups are in the interior of the coil as suggested earlier [21] based on the luminescence studies. As the coil starts to expand, more number of PPIX are exposed to the bulk water relaxing from the supercoiled environment present at low pH. The fluorophore, being insoluble in aqueous solution, in the flexible coil of the macromolecule at high pH, prefers to be in a reverse micellar structure as indicated by the higher lifetimes at pH greater than 7.0. However, the emission studies show that at pH greater than 7.0, the quantum yield for emission is enhanced. This observation suggests that as in the case of pyrene and naphthalene as fluorophores bound to the polymers, the elongated flexible polymer chain leads to a more polar environment around the fluorophore around pH 7.0. This is clearly explained by the increase in the relative amplitudes of short lived component and decrease in the amplitude of long lived component at the intermediate state at pH 7.0.

Above pH 7.0, the polymer forms intramolecular micellar segments with the PPIX occupying the core and the ionised carboxylate in the exterior. The fluorescence lifetime increases with the increase in pH due to this type of intramolecular micellar formation. Decay associated emission spectra of PMMA-co-PPIX shown in Fig. 5c also indicates that at high pH, the spectrum corresponds to the fluorophore buried at the core of the micelle. At low pH, more polar environment due to the hydrogen bonding between the –COOH groups and the fluorophore indicates a blue shifted emission spectrum showing shorter lifetime.

One of the notable features seen for naphthalene, pyrene and dansyl as fluorophores attached to PMAA on the one hand and the fluorophores PPIX, thionine and phenosafranine [22] on the other is that in the former the environment is more hydrophilic at pH greater than six. For the latter systems more hydrophobic environment is observed. The common feature with the polymer-fluorophore in all the cases is that the supercoil to extended chain transition occurs at low pH around 6.5. The predominately aromatic hydrocarbon containing fluorophores remain surrounded by more hydrophilic environment of the polymer chain in the extended coil configuration at pH greater than 7.0. When the fluorophore is heterocyclic or ionic in nature micelle formation seems to occur forcing the coil to form micellar structure around the fluorophore pushing the water molecule to the bulk and manifesting a more hydrophobic core around the fluorophore. In the case of aromatic hydrocarbons as bound fluo-

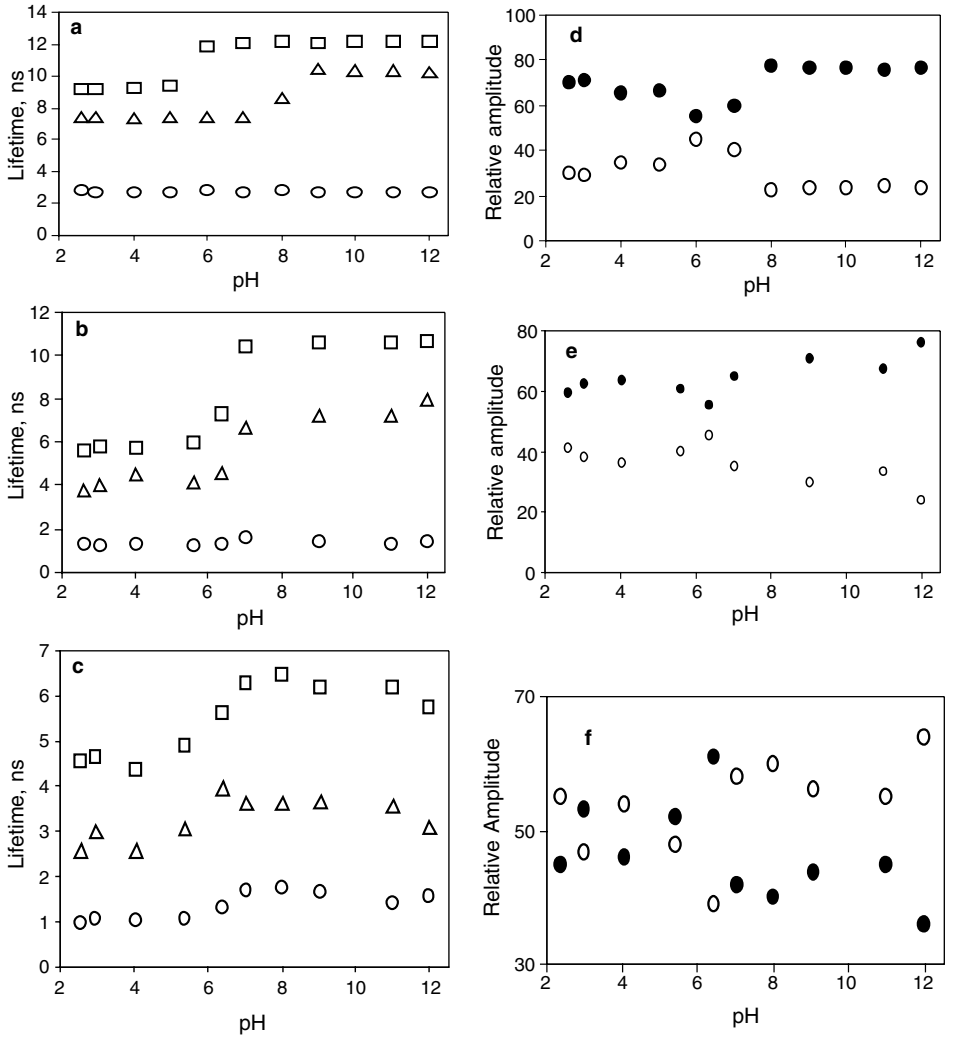


Fig. 4. Fluorescence lifetimes of poly(MAA-co-PPIX) (a); poly(MAA-co-MgPPIX) (b); poly(MAA-co-ZnPPIX) (c) vs pH; concentration 2×10^{-3} M, $\lambda_{ex} = 375$ nm; $\lambda_{em} = 622$, 626 and 580 nm for (a), (b), and (c), respectively; temperature = 23.0 ± 0.5 °C. Relative amplitudes of poly(MAA-co-PPIX) (d); poly(MAA-co-MgPPIX) (e); poly(MAA-co-ZnPPIX) (f) vs pH. (b) Relative amplitudes, ● = A_2, ○ = A_1 corresponds to τ_2 and τ_1, △ = average lifetime; □ = τ_2, ○ = τ_1.

rophores such micelle formation seems to be absent or less predominant.

The porphyrins complexed with metal ions bound to the macromolecules show somewhat shorter lifetimes. In aqueous solutions at pH < 6.5 the central metal ion, Zn(II) is coordinated to the porphyrin and the axial positions of the Zn-PPIX is presumably coordinated weakly to the counter ions in the complex. While increasing the pH the carboxylic acid gets neutralised and forms hydrogen bonding between the ionised and unionised poly(carboxylic acid)s and hence the coil becomes more compact inducing the Zn-PPIX to reside in the less polar environment at pH 6.5. As the pH of the solution increases, the coil expands and opens up with more number of Zn-PPIX exposed to aqueous medium and hence the relative amplitudes of A_1 increases and A_2 decreases till pH 9.2. In the completely neutralised state, the polymer forms reverse coil as in the case of demetalated PPIX in which the Zn-PPIX is residing inside the coil. The acetate ions in the axial position are replaced by the carboxylate anions due to the chelate effect. In the reverse coiled state, the Zn-PPIX and the coordinated carboxylate anions are located at the interior of the coil whereas all other carboxylate anions are in the exterior of the coil. Since magnesium ion forms less stable axial coordinate bond, the photophysical

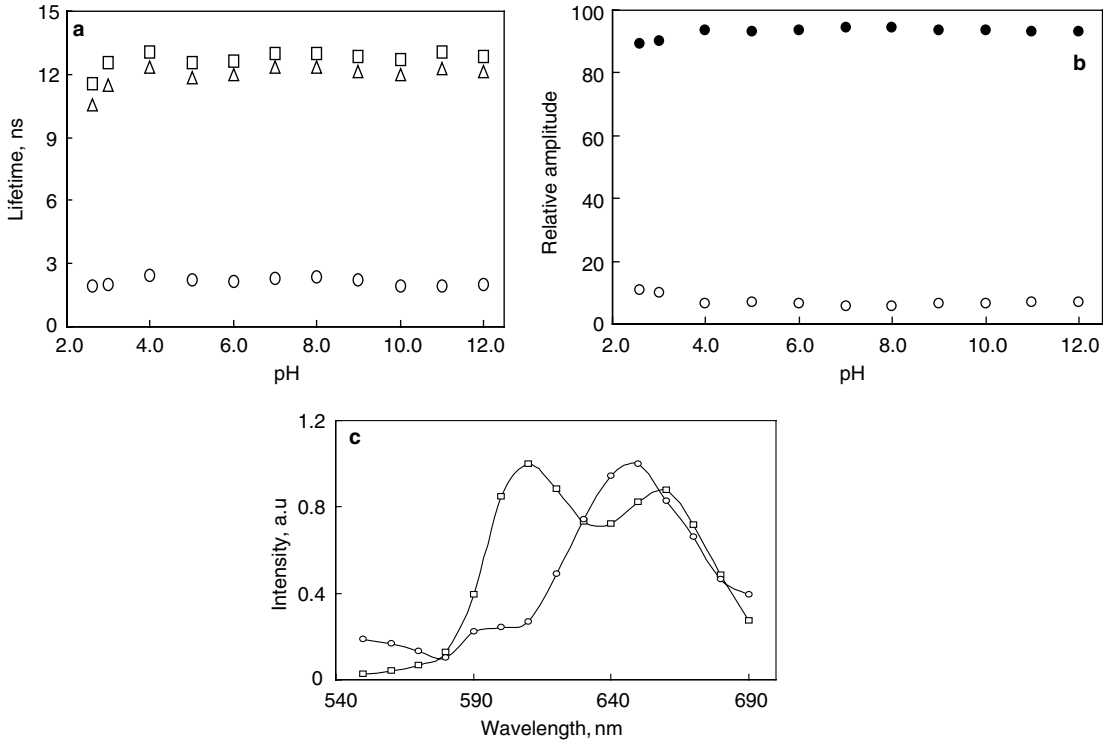


Fig. 5. (a) Fluorescence lifetime of poly(AA-co-PPIX) (□ = τ_2; △ = $\langle\tau\rangle$; ○ = τ_1). Concentration = 2×10^{-3} M; $\lambda_{ex} = 375$ nm; $\lambda_{em} = 622$ nm; temperature = 23.0 ± 0.5 °C; (b) relative amplitude of poly(AA-co-PPIX) (● = A_2, ○ = A_1) vs pH; (c) decay associated spectra of poly(MAA-co-PPIX) in aqueous solution (–□–, long lived component); (–○–, short lived component); $\lambda_{ex} = 375$ nm.

behaviour observed for magnesium porphyrin copolymer system with PMAA lies between PMAA-co-PPIX and PMAA-co-ZnPPIX systems.

3.8. Effect of pH on the fluorescence lifetime of poly(AA-co-PPIX)

Fluorescence lifetimes of poly(AA-co-PPIX) increases with increase in the pH as shown in Fig. 5a. The decay curves are fitted with the biexponential function with the lifetimes ~2.0 (15 ± 5%) and ~11.0 (85 ± 5%) ns at pH 2.6. As the pH increases, the lifetime of long lived component and their relative amplitude increases (93.0%) as shown in Fig. 5b, but there is no change in the lifetime of short lived component. The average lifetime increases gradually from 11.3 ns, which reaches a maximum of 13.5 ns in the pH range of 5.0–8.0; there is no much variation in the fluorescence lifetime above this pH. This contrasting behaviour of PMAA bound fluorophore as compared to the PAA system is due to the presence of α-methyl group, which favors reverse micelle formation in this case while PAA bound fluorophore, does not experience the effect of any reverse coiling.

4. Conclusion

The well-known biological porphyrin PPIX and metallated PPIX were covalently attached to the polyelectrolytes, poly(methacrylic acid) and poly (acrylic acid). The absorption and emission properties of the fluorophores PPIX, Zn-PPIX and Mg-PPIX bound to macromolecules were studied in the pH range 2–14. The observations suggest that the electronic properties of the fluorophores are not altered after copolymerisation with monomers. The average molecular weight distribution and the electronic spectral properties of the macromolecular bound fluorophores suggest random distribution of fluorophores in the macromolecules. The fluorescence decay curves of polymer bound fluorophores show biexponential fluorescence decay indicating the presence of fluorophores in different microheterogeneous media. Poly(MAA-co-PPIX) undergoes pH induced structural transitions in the pH range

of 6.0–7.0. Poly(methacrylic acid) exists in the coiled and reverse coiled state in the two extreme pH. In the case of PMAA bound hydrophobic fluorophores, the probe molecules are inside the hydrophobic core of the coiled and reverse coiled state. The PPIX complexed with zinc and magnesium in the +2 oxidation state shows similar behaviour and the presence of coordination site makes these metallated analogs of the copolymer more hydrophilic as indicated by the photophysical parameters. The contrasting behaviour of the PPIX and magnesium and zinc protoporphyrin as compared to the aromatic hydrocarbon fluorophores, pyrene and naphthalene in the high and low pH regions indicates that in the alkaline pH, the PPIX bound PMAA form reverse micelles and at acidic pH, extended hydrogen bonding in the supercoiled structure shows more hydrophilic character.

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