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Journal of Photochemistry and Photobiology A: Chemistry 95 (1996) 245–253

Journal of PHOTOCHEMISTRY AND PHOTOBIOLOGY A:CHEMISTRY

Photophysical properties of thionine and phenosafranine dyes covalently bound to macromolecules

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Received 30 May 1995; accepted 20 October 1995

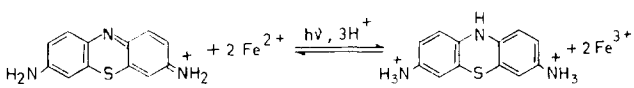
Abstract

Thionine dye was condensed with poly(acrylamidoglycolic acid) (P(AGA)) and poly(methylolacrylamide) (P(MAAM)). Phenosafranine dye was condensed with P(MAAM). The emission, absorption and fluorescence excitation spectra of thionine dye covalently linked to P(AGA) were studied. Electrochemical reduction of P(AGA)-bound thionine shows that both monomeric thionine and non-reducible thionine are bound to the macromolecule; bound non-reducible thionine is non-fluorescent in nature. The polymer-bound thionine and phenosafranine dyes in aqueous solution exhibit biexponential fluorescence decay, in contrast with the corresponding unbound dye, which is attributed to the effect of the macromolecular chain conformation and solvent environment on the photophysics of the polymer-bound dyes. The excited state which decays with a shorter lifetime is suggested to be in a more polar aqueous environment (as in the case of the unbound dyes dissolved in aqueous medium), whereas the excited state which shows a longer lifetime is situated in a hydrophobic environment inside the coil of the polymeric chain where less interaction with the solvent molecules is experienced.

Keywords: Thionine; Phenosafranine; Covalently bound dye; Macromolecule; Poly(acrylamidoglycolic acid); Poly(methylolacrylamide)

1. Introduction

The photochemistry of thiazine and phenazine dyes, covalently bound to macromolecules in homogeneous medium and as thin films coated onto electrodes, has been of interest in recent years [1]. Thiazine dyes show electron transfer reactions from the excited states when suitable reducing species are present in the medium [2] as given in Scheme 1.



Thionine Leucothionine

Accordingly on irradiation with visible light in homogeneous solutions, the thionine–iron(II) [3] and phenosafranine–EDTA [4] systems show photogalvanic potentials of 250 mV and 870 mV respectively. The efficiency of the photogalvanic cell can be improved to some extent by modifying the dye with substituents or changing the quencher [5]. Microheterogeneous reaction environments, such as micelles [6], monolayer assemblies [7], polymers [8] and chemically modified electrode systems [9], facilitate electron transfer and these systems show interesting photoelectrochemical characteristics different from the monomeric dye absorbers [10]. Recently, a number of studies have focused on the photophysical properties of amphiphilic, random, alternating and block copolymers having aromatic chromophores as pendant groups, where the polyelectrolyte is employed in order to modify the local environment of the excited chromophore by a combination of electrostatic and hydrophobic interactions. In our laboratory, we have synthesized a number of macromolecular-bound thionine and phenosafranine dyes and have investigated the photochemical and electrochemical properties in homogeneous aqueous solution [11] and at electrodes coated with the macromolecular dye [12,13]. In this paper, we describe the photophysical properties of poly(acrylamidoglycolic acid)-bound thionine (P(AGA)–TH^+), poly(methylolacrylamide)-bound thionine (P(MAAM)–TH^+) and poly(methylolacrylamide)-bound phenosafranine (P(MAAM)–PS^+); the novel heterogeneity of the excited states of the probe molecules and the nature of the polymer coil are reported.

2. Experimental details

The thionine used in this study was obtained as thionine acetate (Fluka) and was purified by the method of Clark and

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Eckert [14]. Phenosafranine dye (Fluka) was recrystallized twice from methanol. Poly(acrylamidoglycolic acid) (P(AGA)) was obtained by polymerizing acrylamidoglycolic acid (Aldrich) in water under a stream of nitrogen at 60 °C using potassium peroxydisulphate ($K_2S_2O_8$) as initiator [15]. Poly(methylolacrylamide) (P(MAAM)) was prepared by the procedure described earlier [11]. *N*-Methylolacrylamide (MAAM) was prepared by the reaction of paraformaldehyde with acrylamide. MAAM was polymerized in aqueous solution using $K_2S_2O_8$ as initiator to obtain P(MAAM).

The polymer-bound thionine was prepared by condensing thionine with the polymer according to the procedure of Kamogawa et al. [16]. Purified thionine was added to an aqueous solution of the polymer (P(MAAM) or P(AGA)) in the desired molar ratio and the mixture was kept at 90 °C for 5 h. The number of monomer units per thionine molecule in the macromolecule was varied by taking different molar ratios of thionine and the monomer. The resulting polymer-bound thionine was precipitated in large amounts of methanol and purified by repeated precipitation. The uncondensed thionine dye was removed by dialysing the sample (cellulose tubing (Sigma); molecular weight cut-off, 12 000) for several days against distilled water. Dialysis was stopped when the solvent outside the membrane showed no absorption at 600 nm, where thionine has a molar absorptivity of 5.29×10^4 $dm^3\ mol^{-1}\ cm^{-1}$.

The amount of thionine bound to the macromolecule was estimated by a titrimetric method [17]. A solution containing a known amount of ferrous ammonium sulphate in orthophosphoric acid was titrated against a known volume of polymer-bound thionine solution. The colour of the polymer-bound thionine disappeared owing to the reduction of thionine to leucothionine by ferrous ions in the presence of orthophosphoric acid. The end point was the appearance of a rose-red colour, the colour of polymer-bound leucothionine in orthophosphoric acid. From the titration data the amount of thionine present in the solution was calculated. A known volume of original polymer–dye solution was evaporated and the weight of the residue was taken as the amount of polymer–dye complex present in the original solution. From the amounts of polymer and thionine present in a given volume, the number of monomer units per dye molecule in the polymer chain (the m/d ratio) was calculated.

Phenosafranine was condensed with P(MAAM) in the same manner, and the uncondensed phenosafranine was removed by dialysing the solution against distilled water. Dialysis was stopped when the solution outside the membrane showed no absorbance at 522 nm, where phenosafranine has a molar absorptivity of 1.8×10^4 $dm^3\ mol^{-1}\ cm^{-1}$. A titrimetric method was used to estimate the amount of phenosafranine bound to the macromolecule [18]. A solution containing a known amount of titanous chloride in 4 mol dm^{-3} hydrochloric acid was titrated against polymer-bound phenosafranine under a nitrogen atmosphere at 60 °C. The colour of the phenosafranine solution disappears owing to the reduction of phenosafranine by titanous ions in the presence of hydrochloric acid to leucophenosafranine. The end point is the appearance of a light brown colour. From the titration data, the amount of phenosafranine present in the solution was calculated. The monomer to dye ratio (m/d ratio) of P(MAAM)–PS^+ was calculated as described previously for polymer-bound thionine.

The absorption spectra were recorded using a Shimadzu UV-3101 spectrophotometer. The fluorescence spectra were recorded using a Perkin-Elmer LS-5B fluorescence spectrophotometer or Hitachi 650-40 fluorescence spectrophotometer.

Controlled potential coulometry was performed using a PAR 173 potentiostat/galvanostat and a PAR 179 digital coulometer. A PAR 377 cell system, with a platinum gauze as working electrode, a platinum gauze as counter electrode (separated from the experimental solution by a Vycor plug) and a saturated calomel electrode (SCE) as reference electrode, was used. Coulometric studies were performed with constant nitrogen gas purging through the experimental solution at 298 K.

The average molecular weights of the polymer samples were determined with a Waters 501 gel permeation chromatograph connected to a Waters 401 differential refractive index detector using water as solvent. Three columns, namely ultrahydrogel linear, ultrahydrogel-500 and ultrahydrogel-120, were connected in series. The molecular weights of the polymer-bound dye samples were based on standard poly(styrene sulphonic acid) in water.

The fluorescence lifetime measurements of the polymer-bound dye samples in aqueous solution were measured using a picosecond single-photon-counting fluorescence spectrophotometer. The details of the experimental set-up are given elsewhere [19]. The excitation source was a tunable picosecond dye laser pulse, derived from a cavity-dumped dye laser, pumped by the frequency- doubled output (532 nm) of a mode-locked continuous wave (CW) Nd-YAG laser. With rhodamine 6G dye, a typical pulse width of approximately 4 ps and a pulse energy of 10–60 nJ were obtained in the 580–640 nm region. All samples containing thionine were excited at 600 nm and the emission was collected at 620 nm, 630 nm and 640 nm for unbound thionine, P(AGA)–TH^+ and P(MAAM)–TH^+ respectively. Samples containing phenosafranine were excited at 310 nm and the emission was collected at 580 nm. All the lifetime experiments were carried out at 298 K. The analysis of the emission decay was based on the iterative reconvolution method using a non-linear least-squares technique and a Marquardt algorithm for the optimization of the parameters. When the fluorescence decay is a single exponential, then

$$I(t) = A \exp(-t/\tau_1) \quad (1)$$

where A is the pre-exponential factor and τ is the fluorescence lifetime of the chromophore. The goodness-of-fit was checked by the value of χ^2 (acceptable range is 0.8–1.2) and by a plot of the weighted residuals vs. time (this must be

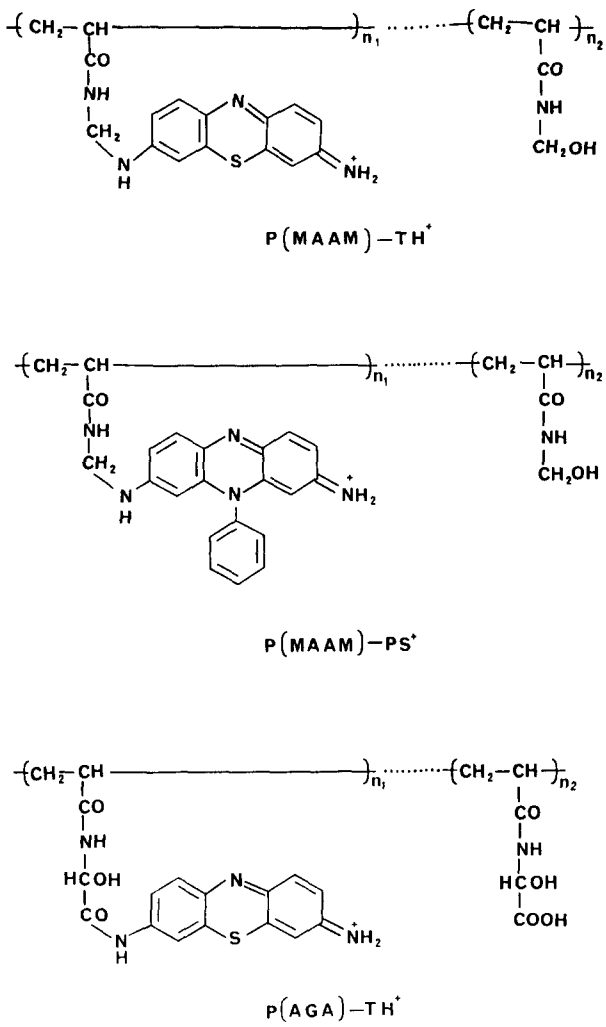

Fig. 1. Structure of polymer-bound dyes, P(MAAM)–TH^+, P(MAAM)–PS^+ and P(AGA)–TH^+.

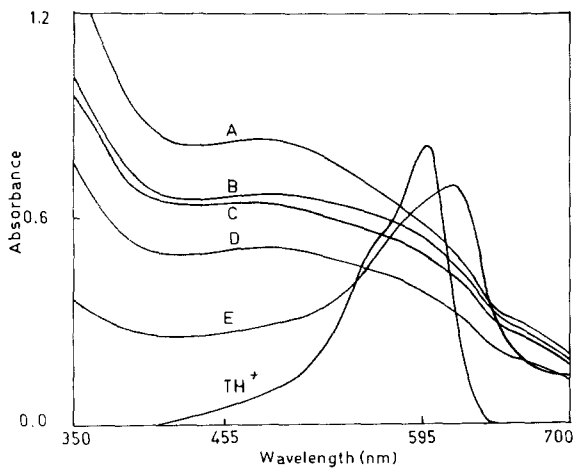

Fig. 2. Absorption spectra of thionine and polymer-bound thionine in aqueous medium. Concentration of thionine is given in parentheses. TH^+, thionine; A, P(AGA)–TH^+ (1.1×10^{-5} M), m/d = 259; B, P(AGA)–TH^+ (1.0×10^{-5} M), m/d = 310; C, P(AGA)–TH^+ (9.1×10^{-6} M), m/d = 542; D, P(AGA)–TH^+ (7.0×10^{-6} M), m/d = 865; E, P(MAAM)–TH^+ (1.35×10^{-6} M), m/d = 112.

randomly distributed around zero). If the assumed single exponential decay does not give a good fit, a double exponential decay is attempted as

$$I(t) = A_1 \exp(-t/\tau_1) + A_2 \exp(-t/\tau_2) \quad (2)$$

where A_1 and A_2 are the pre-exponential factors and τ_1 and τ_2 are the lifetimes of the fluorescing components.

3. Results and discussion

3.1. Absorption and emission spectral properties of polymer- bound dye systems

The structures of the polymer-bound dyes, i.e. P(AGA)–TH^+, P(MAAM)–TH^+ and P(MAAM)–PS^+, are given in Fig. 1. The absorption spectra of P(AGA)–TH^+ and P(MAAM)–TH^+ with different m/d ratios are shown in Fig. 2. The absorption spectrum of P(AGA)–TH^+ is broad in nature, irrespective of the chromophore loading, and shows no characteristic maxima, in contrast with that of other polymer-bound thionines [20]. The broad absorption spectral feature of P(AGA)–TH^+ in aqueous solution is not due to the aggregation of dye molecules as no change is observed in the absorption spectrum of P(AGA)–TH^+ in aqueous medium on addition of organic solvents, such as methanol, ethanol, acetonitrile and dimethylformamide; the addition of organic solvents decreases the extent of aggregation [20]. When P(AGA)–TH^+ solution in 0.5 N H_2SO_4 is coulometrically reduced at +0.1 V under a nitrogen atmosphere, the absorption spectrum of the electrolysed solution shows a decrease in absorbance at 600 nm which is due to the formation of leucothionine which does not absorb in the visible region. After complete reduction, the difference absorption spectrum between P(AGA)–TH^+ and reduced P(AGA)–TH^+ shows λ_{max} at 600 nm as illustrated in Fig. 3. This indicates that there are two components present in P(AGA)–TH^+. One component is reducible thionine, which is suggested to be monomeric thionine covalently bound to the macromolecule; the absorption spectrum of this component

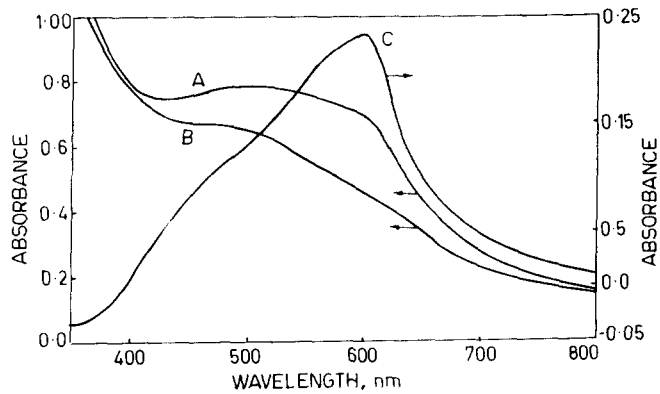

Fig. 3. Absorption spectra of polymer-bound thionine in aqueous medium: A, P(AGA)–TH^+; B, reduced P(AGA)–TH^+ (by coulometry at +0.1 V); C, difference between A and B.

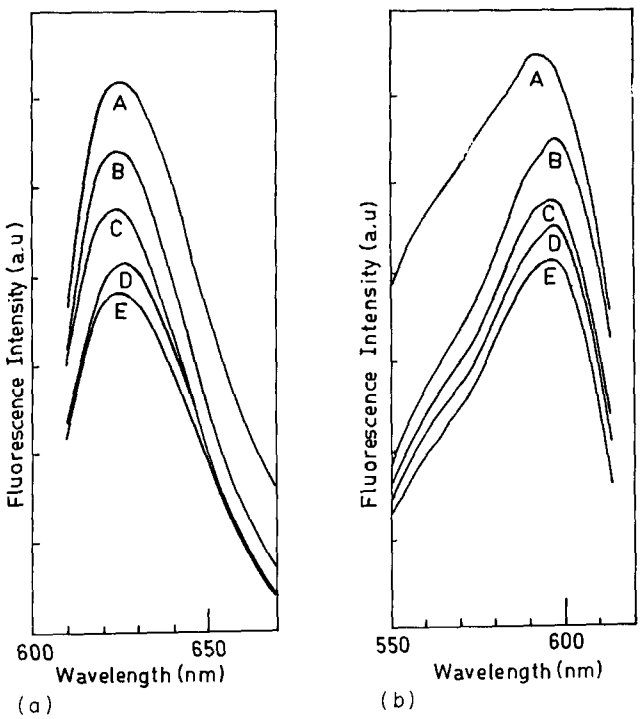

Fig. 4. (a) Emission spectra of P(AGA)–TH$^+$ in aqueous medium, $\lambda_{ex} = 600$ nm. (b) Excitation spectra of P(AGA)–TH$^+$ in aqueous medium, $\lambda_{em} = 630$ nm. The monomer to dye (m/d) ratios are 259 (A), 310 (B), 542 (C), 865 (D) and 1330 (E).

resembles that of unbound thionine with $\lambda_{max} = 600$ nm. The absorption spectrum of the non-reducible component closely resembles the absorption spectrum of polymerized thionine [21,22]. It is equally possible that some dye molecules become attached to the polymer through other bonds.

In order to understand in more detail the nature of the two components present in P(AGA)–TH$^+$, we used the gel permeation chromatography (GPC) technique. GPC of P(AGA)–TH$^+$ reveals the presence of only one polymeric species with an average molecular weight of 3.57×10^5. The results of GPC also indicate that P(AGA) is not cross-linked either before the thionine dye is condensed to the macromolecule or after the dye is attached to any appreciable extent. Therefore it is suggested that both the reducible monomeric thionine and non-reducible component present in P(AGA)–TH$^+$ are bound to the same macromolecular chain. We therefore conclude that the absorption band of P(AGA)–TH$^+$ consists of contributions from monomeric thionine and from the non-reducible dye which is photochemically inert and has very different electrochemical properties compared with the monomeric dye.

The fluorescence spectrum of P(AGA)–TH$^+$ is shown in Fig. 4(a). No fluorescence emission was observed from electrochemically reduced P(AGA)–TH$^+$. The excitation spectrum of P(AGA)–TH$^+$ is shown in Fig. 4(b). The excitation spectrum of P(AGA)–TH$^+$ differs from its absorption spectrum, but resembles the absorption spectrum of monomeric thionine in aqueous medium; this indicates that monomeric thionine in P(AGA)–TH$^+$, which absorbs around 600 nm, fluoresces, but non-reducible thionine, which absorbs around 450–500 nm, does not. It should be noted that the photophysical properties discussed in this investigation relate to the monomeric dye bound to the macromolecule only and the non-reducible dye bound to the polymer does not seem to influence the excited state properties of the former as detailed below.

Unbound phenosafranine shows an emission maximum around 585 nm when excited at 522 nm and P(MAAM)–PS$^+$ shows an emission maximum around 575 nm when excited at 530 nm, as reported earlier [12]. From the spectral studies, it is concluded that the spectral properties of the dyes bound to the macromolecules are quite similar to those of the unbound dyes, indicating that the electronic structure of the chromophore is hardly affected by the macromolecule.

3.2. Lifetime studies of polymer-bound thionine and phenosafranine excited states

The photophysics of the thionine dye is well documented [23]. In aqueous solution, thionine shows fluorescence with a lifetime of 310 ps on excitation at 600 nm. In organic solvents, the fluorescence lifetime of thionine increases (450 ps in ethanol and 760 ps in tert-butyl alcohol) with an increase in the fluorescence quantum yield [23]. The difference between the lifetimes of thionine in water and tert-butyl alcohol is quite large, demonstrating the effect of the polarity of the microenvironment on the decay of the excited state. In our experiments, thionine dye in aqueous medium shows a fluorescence decay corresponding to a single exponential with a lifetime of 306 ps, which is in close agreement with the value reported earlier [23].

In the case of P(AGA)–TH$^+$ and P(MAAM)–TH$^+$, the decay curves are fitted satisfactorily to a biexponential decay, regardless of the loading of the dye in the polymer chain, as given in Figs. 5(a) and 5(b) respectively for typical cases. The fluorescence lifetimes (τ), pre-exponential factors (A) and average lifetimes $\langle\tau\rangle$ of P(AGA)–TH$^+$ and P(MAAM)–TH$^+$ are given in Table 1. The short-lived component is the major emitting species in both systems. As the unbound dye is completely removed during dialysis in P(AGA)–TH$^+$ and P(MAAM)–TH$^+$, the multiexponential fluorescence decay is characteristic of monomeric thionine bound to the polymer, since the non-reducible component is non-fluorescent and plays no apparent role in the emission decay behaviour of P(AGA)–TH$^+$ and P(MAAM)–TH$^+$. The emission properties of both polymers are quite similar, and the electronic structure of the dye is hardly affected by the polymer chain; however, the macromolecular environment influences the photophysical properties of the excited state. The decay times of the short-lived component (τ_1) in P(MAAM)–TH$^+$ and P(AGA)–TH$^+$ are similar to that of unbound thionine in the aqueous phase (approximately 300 ps); however, the decay times of the long-lived component (τ_2) in P(MAAM)–TH$^+$ and P(AGA)–TH$^+$ are significantly longer (greater than 700 ps). The presence of a long-

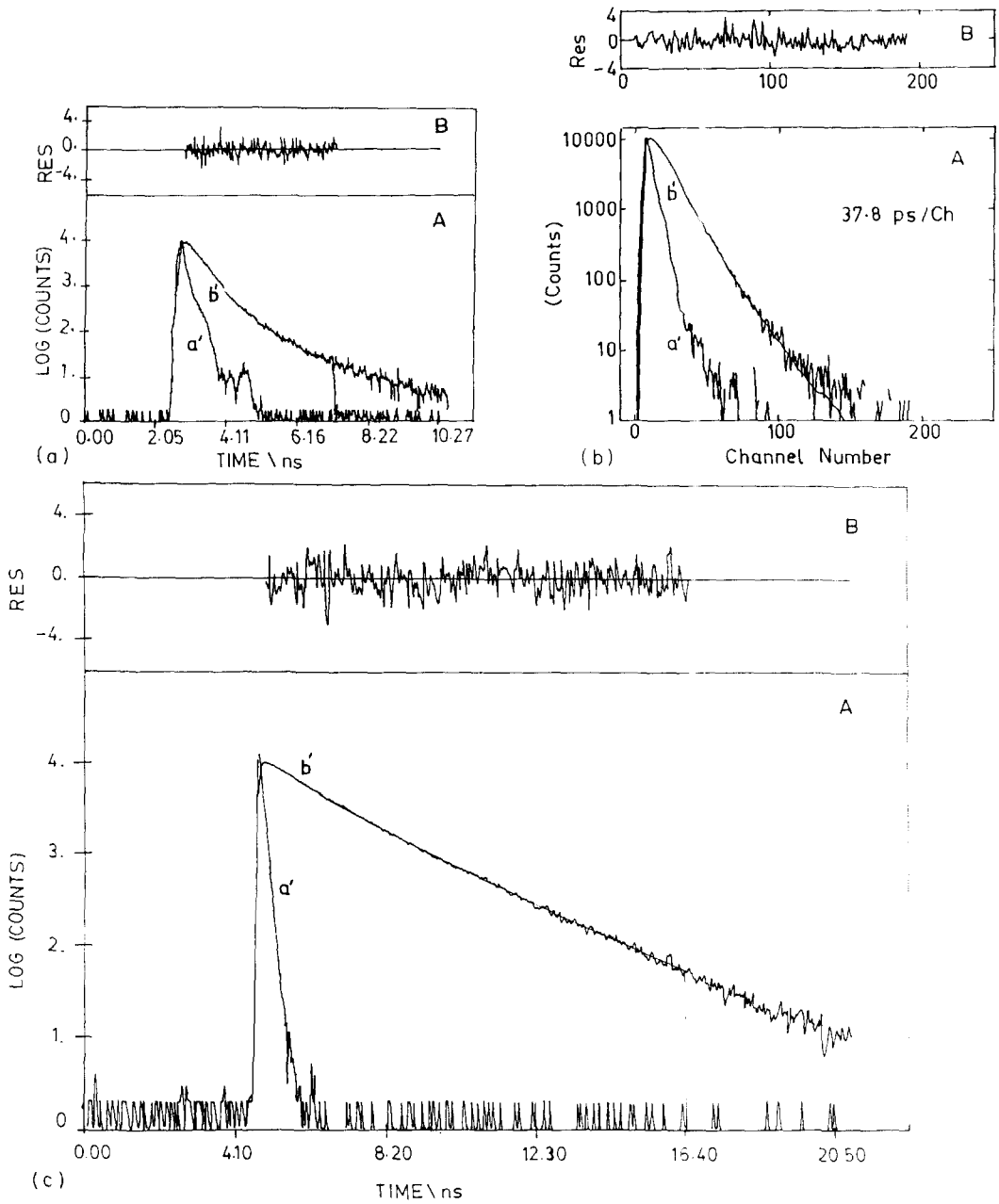

Fig. 5. (a) (A) Typical fluorescence decay profile of P(AGA)–TH^+ in aqueous medium, $\lambda_{ex} = 600$ nm and $\lambda_{em} = 630$ nm; a', lamp profile; b', sample decay profile. (B) Residuals of decay profile. (b) (A) Typical fluorescence decay profile of P(MAAM)–TH^+ in aqueous medium, $\lambda_{ex} = 600$ nm and $\lambda_{em} = 640$ nm; a', lamp profile; b', sample decay profile. (B) Residuals of decay profile. (c) (A) Typical fluorescence decay profile of P(MAAM)–PS^+ in aqueous medium, $\lambda_{ex} = 310$ nm and $\lambda_{em} = 580$ nm; a', lamp profile; b', sample decay profile. (B) Residuals of decay profile.

lived component in the emission decay of polymer-bound thionines suggests that the microenvironment around the thionine chromophore is not exactly uniform within the time frame of the excited state decay processes.

The excited state lifetimes (τ) provide information on the environment of the probe molecules attached to the macromolecules. When fluorescent probes reside heterogeneously, a multiexponential decay is observed [24–28]. In amphiphilic polymers (polyelectrolytes containing aromatic chromophores as pendant groups), the hydrophobic groups interact with each other to form hydrophobic microdomains and charged segments of the polymer surround the microdomains providing a micellar structure [29]. The chromophores reside inside the polymer chains or are exposed to the aqueous phase depending on the conformation of the macromolecule. The biexponential fluorescence decay of the polymer-bound thionines, observed in the present case, indicates that thionine dyes are located at the solvent pool or in the hydrophobic interior of the polymer coil. In both polymer-bound dyes P(MAAM)–TH^+ and P(AGA)–TH^+, the dye molecules which are located in the solvent pool decay with a fluorescence lifetime corresponding to the excited state lifetime of unbound thionine in aqueous solution (approximately 300 ps). The dye molecules residing in the interior of the

Table 1
Fluorescence lifetimes (τ) and amplitudes (A) for unbound thionine and polymer-bound thionine (P(AGA)–TH$^+$ and P(MAAM)–TH$^+$) in aqueous solution. The maximum error in the fluorescence lifetime is less than 20 ps. λ_{ex} = 600 nm and λ_{em} = 620, 630 and 640 nm for unbound thionine, P(AGA)–TH$^+$ and P(MAAM)–TH$^+$ respectively (lifetimes are in picoseconds). The amplitudes A_1 and A_2 are given as a percentage of the total amplitude

Polymer	m/d	τ_1	A_1	τ_2	A_2	$\langle\tau\rangle$ [a]	χ^2
Thionine	–	306	100	–	–	306	1.02
P(AGA)–TH$^+$	259	315	95.1	693	4.9	333	1.12
P(AGA)–TH$^+$	310	307	94.8	721	5.2	328	1.20
P(AGA)–TH$^+$	542	316	93.0	770	7.0	347	1.20
P(AGA)–TH$^+$	865	321	91.2	1010	8.8	381	1.03
P(AGA)–TH$^+$	1330	375	90.7	1380	9.3	468	0.99
P(MAAM)–TH$^+$	112	392	94.1	661	5.9	407	1.20
P(MAAM)–TH$^+$	164	368	92.8	697	7.2	391	1.00
P(MAAM)–TH$^+$	288	406	89.7	726	10.3	439	1.12
P(MAAM)–TH$^+$	440	410	87.0	756	13.0	455	1.20
P(MAAM)–TH$^+$	723	432	70.7	933	29.3	578	1.17

[a] $\langle\tau\rangle = \tau_1 A_1 + \tau_2 A_2$.

polymer coil do not experience the influence of the solvent; on excitation, the excited state of the dye finds a less polar environment, which increases the excited state lifetime by decreasing the non-radiative decay of the excited state.

In the case of polymer-bound thionines, the values of τ_1/τ_2 and A_1/A_2 decrease with increasing m/d ratio as shown in Figs. 6(a) and 6(b). When the chromophore loading is decreased, τ_2 and A_2 increase which causes an increase in the average lifetime. In other words, the contribution from the dye molecules exposed to the aqueous medium decreases when the chromophore loading is decreased. It is known that hydrophobic chromophores prefer to be located in less polar environments [27,30]. As thionine dye is hydrophobic in nature, it tends to be located in less polar environments (interior of the polymer coil) rather than in the aqueous phase. Thus when the loading is very low, the proportion of dye molecules present in the interior of the polymer coil is larger. Interestingly, the amplitude A_2 in the case of P(MAAM)–TH$^+$ is larger than that of P(AGA)–TH$^+$ (Table 1). As P(MAAM) is a non-ionic polymer, the coiling of the macromolecular chains is expected to be greater than in the ionic polymer P(AGA), where the carboxylate groups repel each other along the polymer chain making the polymer elongate. This more extensive coiled conformation of P(MAAM)–TH$^+$ leads to the observed increase in amplitude of the long-lived emitting species (A_2) in P(MAAM)–TH$^+$.

The photophysics of phenosafranine dye in aqueous solution and nafion membranes has been reported previously [31]. Phenosafranine shows a fluorescence lifetime of 930 ps in aqueous medium. In organic solvents, the fluorescence lifetime of phenosafranine increases due to a reduction in the rate constant of non-radiative decay [31a]. Phenosafranine in aqueous medium in the present study exhibits a single exponential fluorescence decay with a lifetime of 980 ps, which is in agreement with the reported value [31a]. Polymer-bound phenosafranine, P(MAAM)–PS$^+$, exhibits a non-exponential fluorescence decay and, as in the case of polymer-bound thionine, the decay curves can be fitted satisfactorily to a double exponential decay. The fluorescence decay of P(MAAM)–PS$^+$ in aqueous solution is shown in Fig. 5(c) for a typical experiment; the fluorescence lifetimes τ, pre-exponential factors A and average lifetimes $\langle\tau\rangle$ of P(MAAM)–PS$^+$ are given in Table 2. The P(MAAM)–PS$^+$ samples exhibit biexponential fluorescence decay irrespective of the loading of the dye. P(MAAM)–PS$^+$ shows one lifetime very similar to that of unbound phenosafranine in the aqueous phase (approximately 980 ps) and one significantly longer (more than 2000 ps). As the fluorescence decay behaviour of P(MAAM)–PS$^+$ is similar to that of polymer-bound thionine, it is concluded that, for P(MAAM)–PS$^+$ also, the short-lived component is due to the chromophore located in the solvent pool and the long-lived component is

Table 2
Fluorescence lifetimes (τ) and amplitudes (A) of polymer-bound phenosafranine (P(MAAM)–PS$^+$) in aqueous solution. The maximum error in the fluorescence lifetime is less than 20 ps. λ_{ex} = 310 nm and λ_{em} = 580 nm (lifetimes are in nanoseconds). The amplitudes A_1 and A_2 are given as a percentage of the total amplitude

Sample	m/d	τ_1	A_1	τ_2	A_2	$\langle\tau\rangle$ [a]	χ^2
P(MAAM)–PS$^+$	393	1.2	46.0	2.56	54.0	1.9	1.10
P(MAAM)–PS$^+$	417	1.23	53.3	2.46	46.7	1.8	1.02
P(MAAM)–PS$^+$	451	1.38	56.8	2.64	43.2	1.92	0.95
P(MAAM)–PS$^+$	478	1.31	56.4	2.51	43.6	1.83	1.20

[a] $\langle\tau\rangle = \tau_1 A_1 + \tau_2 A_2$.

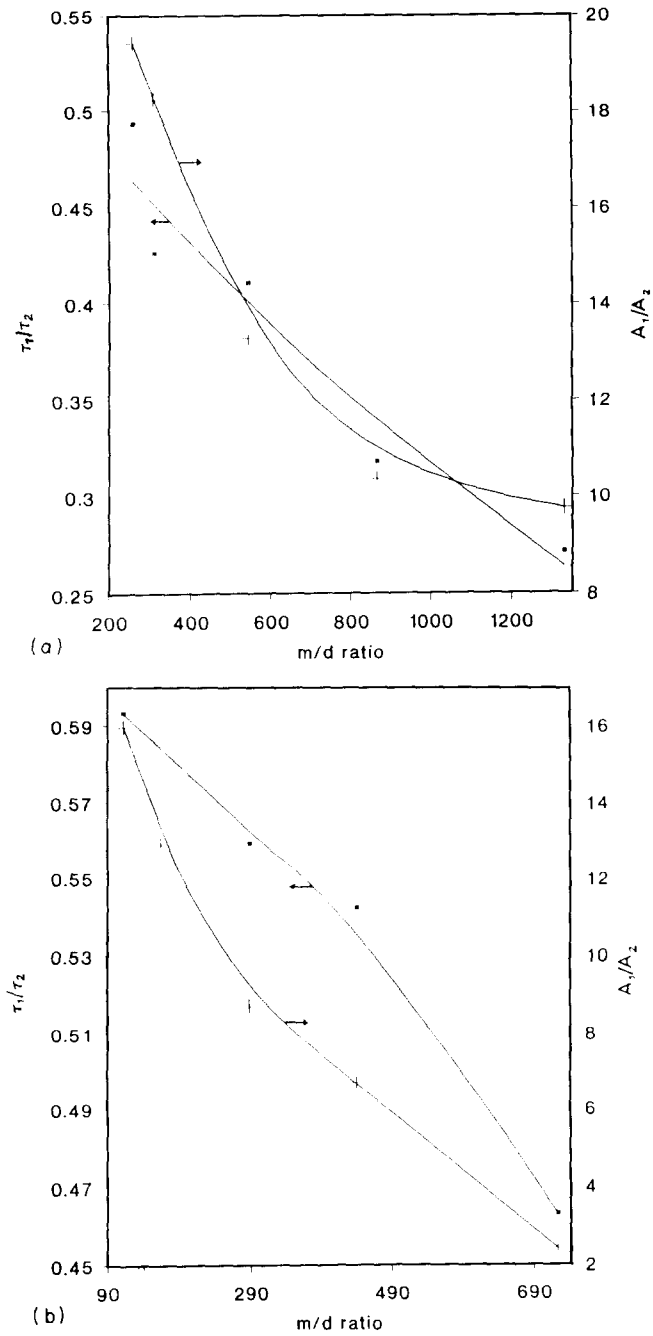

Fig. 6. (a) Plot of τ_1/τ_2 and A_1/A_2 vs. m/d ratio for P(AGA)–TH$^+$ in aqueous medium. (b) Plot of τ_1/τ_2 and A_1/A_2 vs. m/d ratio for P(MAAM)–TH$^+$ in aqueous medium.

due to the chromophore located in the interior of the polymer coil which experiences a hydrophobic environment.

3.3. Influence of the conformation of the macromolecular chain on the luminescence of polymer-bound dyes

P(AGA)–TH$^+$ in aqueous solution exhibits a marked enhancement in relative fluorescence intensity with decreasing pH (Fig. 7). Although unbound thionine itself in aqueous medium exhibits an increased relative fluorescence intensity (without an appreciable change in the emission maximum) with decreasing pH (Fig. 7), the increase for P(AGA)–TH$^+$ is greater than that of unbound thionine. This suggests that the dependence of the emission intensity of P(AGA)–TH$^+$ on the pH of the medium is due to conformational changes occurring in the macromolecule with changing pH of the solution. In polycarboxylic acids, when the pH of the solution is decreased, protonation of carboxylate groups occurs in the polymer chain, resulting in a more extensive coiled polymer conformation (hypercoil) due to the absence of electrostatic repulsion [32]. This hypercoil provides a viscous and relatively "dry" microdomain to the labelled chromophore which favours efficient emission, preventing the quenching of the excited chromophore by a quencher present in the bulk solvent. In the present situation, this is presumably the reason for the increase in the fluorescence quantum yield of P(AGA)–TH$^+$

Similarly, when the concentration of ethanol added to the aqueous solution is increased, an enhancement in the quantum yield of fluorescence occurs in P(AGA)–TH$^+$ and P(MAAM)–TH$^+$ as shown in Fig. 8. The fluorescence intensity of unbound thionine itself increases with an increase in the concentration of ethanol (quantum yield for thionine increases to $\phi_{fl}=0.23$ compared with 0.18 in water [33]). The enhancement of the relative fluorescence intensity at higher concentrations of ethanol is substantially greater in the case of polymer-bound thionine than for unbound thionine. Coiling of the polymer is expected to be greater in the presence of ethanol, which is a non-solvent for the above-mentioned polymers. As a result of the decrease in coil size, more dye molecules experience a less polar environment

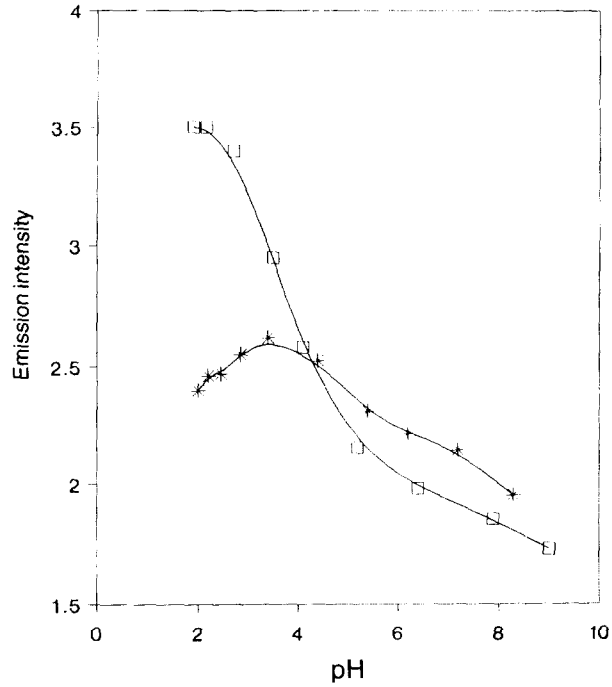

Fig. 7. pH dependence of the relative fluorescence intensity for thionine (*) and P(AGA)–TH$^+$ (□). $\lambda_{ex}=600$ nm.

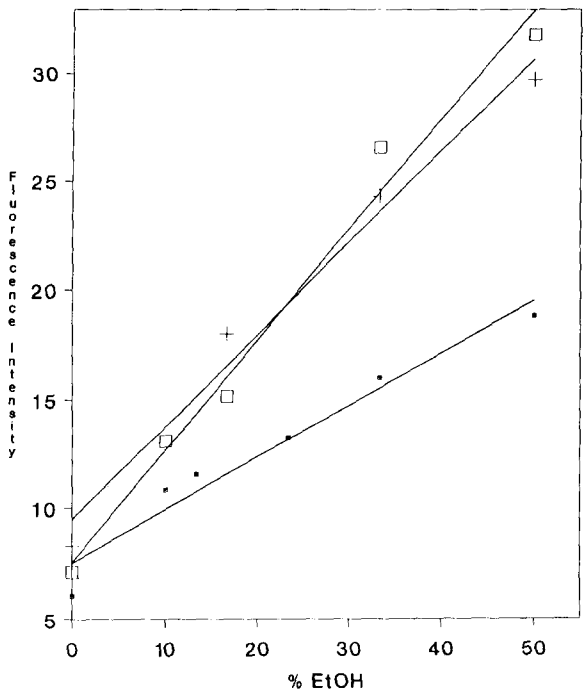

Fig. 8. Plot of the relative fluorescence intensity as a function of the percentage of ethanol for thionine (□), P(MAAM)–TH$^+$ (+) and P(AGA)–TH$^+$ (□).

Table 3
Effect of ethanol on the fluorescence lifetimes (τ) and amplitudes (A) for polymer-bound thionine (P(MAAM)–TH$^+$) (m/d = 164) in aqueous solution. The maximum error in the fluorescence lifetime is less than 20 ps. λ_{ex} = 600 nm and λ_{em} = 640 nm (lifetimes are in picoseconds)

Percentage of ethanol	τ_1	A_1	τ_2	A_2	χ^2
0	368	92.8	697	7.2	1.0
3.23	361	90.3	685	9.7	0.99
6.25	352	86.4	625	13.6	1.08
9.10	321	75.7	562	24.3	0.99

which results in an increase in the quantum yield of fluorescence. The lifetimes and amplitudes of P(AGA)–TH$^+$ in aqueous solution at different ethanol concentrations are shown in Table 3. When the ethanol concentration is increased, the amplitude of the long-lived emitting component increases. Both τ_1 and τ_2 show a decrease on addition of ethanol (Table 3). As the nature of the polymer coil changes due to the addition of ethanol, self-quenching between two thionine chromophores implies a shorter monomer lifetime. This study clearly indicates that the number of probe molecules experiencing a hydrophobic environment is greater when the polymer conformation changes from an extended conformation to a "hypercoiled" conformation.

4. Conclusions

The present investigation demonstrates that, in P(AGA)–TH$^+$, both monomeric thionine and a non-reducible component are present. From the emission and excitation spectra, it is confirmed that the non-reducible component present in P(AGA)–TH$^+$ is non-fluorescent. For the polymer-bound dyes, the fluorescence decay is biexponential irrespective of the loading of the dye on the macromolecule. The heterogeneity of the excited dye molecules is found to be due to the different locations of the binding sites which is a manifestation of the random coil nature of the macromolecule. The dye molecules which are exposed to the solvent medium show lifetimes similar to the unbound dye molecules in aqueous medium, and the dye molecules which are located inside the hydrophobic polymer coil give rise to long-lived emission as they experience a less polar environment in their excited state. In the presence of added ethanol, polymer-bound thionine shows an increase in the amplitude of the long-lived component due to a change in the conformation of the macromolecule from an extended structure to a tight coiled form ("hypercoil"). When the loading of the dye molecules is decreased in polymer-bound thionine, the proportion of the dye molecules present inside the polymer coil increases, which is consistent with the fact that the hydrophobic dye molecules prefer the macromolecular environment rather than an aqueous environment. The present work thus provides an understanding of the nature of binding of the probe molecules and the conformational properties of the polymer coil.

Acknowledgements

The investigations reported here were partly supported by a DST SERC project and by the UGC-COSIST programme. K.V was the recipient of a UGC Fellowship. The authors are grateful to Professor S. Doraiswamy, Professor N. Periasamy and Dr. G. Krishnamoorthy of TIFR, Bombay for assistance with the lifetime measurements.

References

[1] P. Natarajan, *J. Macromol. Sci., A: Chem., 25* (1988) 1285.
[2] W.J. Albery, P.N. Bartlett, J.P. Davies, A.W. Foulds, A.R. Hillman and F.S. Bachiller, *Faraday Discuss. Chem. Soc., 70* (1980) 341.
[3] E. Rabinowitch, *J. Chem. Phys., 8* (1940) 551.
[4] K.K. Rohatgi-Mukherjee, M. Bagchi and B.B. Bhowmick, *Electrochim. Acta, 28* (1983) 293.
[5] M.D. Archer, *J. Appl. Electrochem., 5* (1975) 17.
[6] J.H. Fendler and E.J. Fendler, *Catalysis in Micellar and Macromolecular Systems*, Academic Press, New York, 1975.
[7] D.G. Whitten, *Acc. Chem. Res., 13* (1980) 83.
[8] M. Kaneko and A. Yamada, *Adv. Polym. Sci., 55* (1984) 1.
[9] R.W. Murray, in A.J. Bard (ed.), *Electroanalytical Chemistry*, Marcel Dekker, New York, 1984, p. 191.
[10] R. Tamilarasan and P. Natarajan, *Nature, 292* (1981) 224.
[11] R. Tamilarasan, R. Ramaraj, R. Subramanian and P. Natarajan, *J. Chem. Soc., Faraday Trans. 1, 80* (1984) 2405.
[12] R. Ramaraj and P. Natarajan, *J. Chem. Soc., Faraday Trans. 1, 85* (1989) 813.
[13] P. Natarajan and R. Tamilarasan, in D.O. Hall and J. Morton (eds.), *Solar World Forum*, Pergamon, Oxford, 1981, p. 2204.

[14] W.D.K. Clark and J.A. Eckert, *Solar Energy, 17* (1975) 147.
[15] R. Muqbill, G. Muller, J.C. Fenyo and E. Selegny, *J. Polym. Sci., Polym. Lett. Ed., 17* (1979) 369.
[16] H. Kamogawa, M. Kato and H. Sugiyama, *J. Polym. Sci. A1, 6* (1968) 2967.
[17] R. Tamilarasan, *Ph.D. Thesis*, University of Madras, 1981.
[18] A.I. Vogel, *Quantitative Inorganic Analysis*, ELBS, London, 1975, p. 329.
[19] K.V. Bankar, V.R. Bhagat, R. Das, S. Doraiswamy, A.S. Ghangrekar, D.S. Kamat, N. Periasamy, V.J.P. Srivatsavoy and B. Venkatraman, *Indian J. Pure Appl. Phys., 27* (1989) 416.
[20] R. Tamilarasan and P. Natarajan, *Indian J. Chem., 20A* (1981) 213.
[21] T.I. Quickenden, I.R. Harrison and J.M. Austin, *J. Electrochem. Soc., Electrochem. Science Tech.*, (1985) 2176.
[22] J.M. Bauldreay and M.D. Archer, *Electrochim. Acta, 28* (1983) 1515.
[23] G.B. Dutt, S. Doraiswamy and N. Periasamy, *J. Chem. Phys., 94* (1991) 5360.
[24] E.P. Niu and K.P. Ghiggino, *J. Lumin., 46* (1990) 191.
[25] F. Buyl, A.K. Mesmaeker, A. Tossi and J.M. Kelly, *J. Photochem. Photobiol. A: Chem., 60* (1991) 27.
[26] M. Kaneko and S. Hayakawa, *J. Macromol. Sci., A: Chem., 25* (1988) 1255.
[27] N.J. Turro, J.K. Barton and D.A. Tomalia, *Acc. Chem. Res., 24* (1991) 332.
[28] B.S. Fujimoto, J.B. Clendenning, J.J. Delrow, P.J. Heath and M. Schurr, *J. Phys. Chem., 98* (1994) 6633.
[29] Y. Morishima, Y. Itoh, S. Nozakura, T. Ohno and S. Kato, *Macromolecules, 17* (1984) 2264.
[30] M.N. Szentirmay, N.E. Prieto and C.R. Martin, *J. Phys. Chem., 89* (1985) 3017.
[31] (a) K.R. Gopidas and P.V. Kamat, *J. Photochem. Photobiol. A: Chem., 48* (1989) 291. (b) H. Mohan and R.M. Iyer, *J. Chem. Soc., Faraday Trans., 88* (1992) 41.
[32] (a) D.Y. Chu and J.K. Thomas, *J. Phys. Chem., 89* (1985) 4065. (b) G. Jones II and C. Oh, *J. Phys. Chem., 98* (1994) 2367. (c) K.S. Arora and N.J. Turro, *J. Polym. Sci., Polym. Chem., 25* (1987) 259.
[33] S.S. Rathi, K. Gopalakrishnan and M.K. Machwe, *Curr. Sci., 41* (1972) 805.