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Probing the association behavior of poly(ethyleneglycol) based amphiphilic comb-like polymer☆

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Abstract

Amphiphilic comb-like polymer with and without intrinsic fluorophore was synthesized by the esterification of poly(styrene–maleic anhydride) copolymer with Brij35 (polyethyleneglycol (23) lauryl ether). The solution properties of this polymer in aqueous medium were investigated by pyrene solubilization experiment, surface tension, steady-state fluorescence, viscosity and laser light scattering experiments as a function of pH. Surface tension experiments suggest that the polymer is surface active throughout the pH range investigated and surface activity is very much pH dependent. Data from fluorescence, reduced viscosity and light scattering experiments indicate the possibility of two types of aggregates, intramolecular at low pH and intermolecular at high pH. Pyrene solubilization experiments suggest that the partition of hydrophobic molecules to the intermolecular aggregates is energetically more favorable than partition to intramolecular aggregates. © 2001 Elsevier Science Ltd. All rights reserved.

Keywords: Comb-like polymer; Amphiphilic polymer; Intramolecular association

1. Introduction

Hydrophobically associating polymers show unusual rheological features and high solubilization properties in aqueous medium [1–3]. These properties arise from the inter- or intramolecular association among hydrophobic groups providing hydrophobic microdomains in an isotropic aqueous solution [4–15]. Self-assemblies of block copolymers or hydrophobically modified polymers have been investigated in the field of biotechnology and pharmaceutics [16,17]. Thermodynamic considerations suggest such self-assemblies to have core-shell morphology, with the hydrophobic segments forming the core and the hydrophilic segments the protective shell. In addition to hydrophobic interactions, which give rise mainly to phase separated superstructures in aqueous systems; the driving force for aggregation may include ionic interactions and intra- and/or intermolecular hydrogen bonding.

Comb-like polymer is a special architectural variation of conventional block copolymer. A broad family of polymers can be synthesized with minor variations in structural features, such as the structure and size of the hydrophilic/hydrophobic segment, the relative frequency of occurrence of these segments, the spacing between segments, the molecular weight of the backbone, etc.

Polymers with hydrophobic backbone and oligomeric PEG chain evenly or randomly attached to this backbone, as pendant groups are one of the earliest systems investigated. Wesslen and Wesslen [17] prepared such comb-like structures by linking PEG monomethyl ethers onto acrylic and methacrylic ester copolymers through transesterification reactions. Very recently, Derand et al. [18] have investigated aggregation and viscometric properties of comb-like polymer synthesized by grafting of PEG monomethyl ether onto copolymers of maleic anhydride and styrene, methyl methacrylate, and ethylhexyl methacrylate. Employing quasi-elastic light scattering and flow field–flow fractionation methods, they suggested that in aqueous solutions, the graft copolymers are mainly present as single chains, with minor fraction of aggregates. The size of aggregates was found to increase with increase in ionic strength of the medium.

Photophysical and solution properties of naphthalene labeled styrene/*N*,*N*-dimethyl maleimido propylammonium

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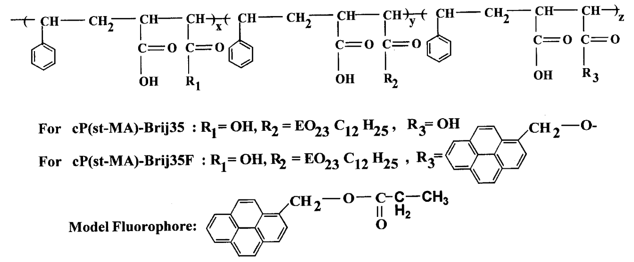

Fig. 1. Structure of CP(St–MA)–Brij35 polymer and model fluorophore.

propane sulfonate copolymer have been reported by Liaw et al. [19]. Their data show that the polymer exists as an aggregate in aqueous medium, which is disrupted by surfactants at concentrations below 10^{-4} M. Another class of associative comb-like polymers in which an oligomeric PEG chain is sandwiched between the hydrocarbon backbone and an alkyl pendant segment has been investigated by Kumacheva et al. [20] and Horiuchi et al. [21], by a combination of fluorescence probe technique and rheology experiments. The association properties of one such polymer, prepared from methacrylic acid, ethyl acrylate and $C_{20}H_{41}O–E_{35}$ macromonomer with mole ratio 49.05:50.04:0.9, respectively, suggested that at low pH (<7.5) large number of smaller micelle are formed, which aggregate to form larger structures at higher pH (>7.5).

Several different synthetic approaches have been made to design amphiphilic comb-like polymers [22–27]. Derand et al. [18] have reported the preparation PEO grafts of versatile alternating copolymer of styrene with maleic anhydride and their properties in the solution phase. Another widely studied polymer is based on alternating copolymer of maleic anhydride and *n*-alkyl vinyl ethers [22–25].

There are only limited reports on study on aggregation properties of comb-like polymers and as yet no conclusive ideas on the size and shape of the aggregates in aqueous solution [9,14,15]. The general contention has been that in aqueous solution, maleic anhydride copolymers with moderate concentrations of alkyl side chains undergo conformational transition from a compact to random coil, as the degree of ionization increases. The transition, however, shifts to a higher value of ionization with increasing alkyl chain length. Dubin et al. [23,24] found that copolymers with larger hydrophobic chains (e.g. alkyl=acetyl) are insensitive to the degree of ionization since they remain in collapsed conformation at all values of pH. It is indeed obvious that the degree of ionization of the carboxylic acid group determines the conformational status of the polymer chain. At low pH, the acid groups are protonated and the polymer is essentially neutral and nonpolar which might assume a compact conformation. As the pH is raised, acid groups ionize transforming the polymer into a polyelectrolyte.

How will the system behave if the side chain itself is amphiphilic, with hydrophilic and hydrophobic blocks? Given the structural constraints, how will the system aggregate at low and high pH? To get the answers we synthesized poly(styrene–maleic anhydride) copolymer with high content of alkylated PEG grafts (Fig. 1). This paper presents our results on the aggregation behavior of this polymer monitored by pyrene partition, surface tension, steady-state fluorescence and laser light scattering techniques in different solution environment (salt, pH).

2. Experimental

2.1. Materials

Styrene, maleic anhydride, benzoyl peroxide, xylene, methyl ethyl ketone, *p*-toluene sulfonic acid, dichloro ethane were from Merck. Brij35 (polyoxyethylene (23) lauryl ether) and pyrene were purchased from Fluka. Inhibitor from monomer was washed off by standard procedure. All solvents were distilled before use. Double distilled water was used for all measurements.

2.2. Methods

^{1}H NMR spectra were produced on a Gemini 200 MHz spectrometer. IR spectra were run on a Perkin–Elmer model 882. GPC was run on a Shimadzu unit, fitted with an RI detector using a Waters 500, 10^3, 10^4 Å micro styragel columns connected in series at a flow rate of 1 ml min^{-1} (THF as eluent, narrow dispersed polystyrene as standards). Surface tension measurements were taken at 27°C on a Du Nouy tensiometer using a platinum ring having a circumference of 4 cm. Viscosity measurements were done in Schott–Geratte automatic viscometer with 0b capillary at 25°C. For fluorescence measurements, 3 ml of each solution was placed in 10 mm rectangular quartz cell, and the spectra were run in SPEX fluorolog spectrophotometer in right angle geometry. The emission spectra were accumulated with an integration time of 1 s/0.5 nm. For fluorescence quenching experiments, nitromethane was used as quencher. Few microliters (~5 μl) of a concentrated (~0.5 M) stock solution of nitromethane was added each time directly to the fluorescence cell containing 3 ml of polymer (1 g l^{-1}) solution and spectra were recorded successively.

Dynamic light scattering (DLS) measurements were performed in a laser light scattering spectrophotometer (Model DLS-700, Otsuka Electronics, Japan, fitted with a 5 mW He–Ne laser, operating at 632.8 nm). All samples were filtered through membrane filter paper with pore size of 0.45 μm. Measurements were taken at 90° angle.

2.3. Synthesis

2.3.1. Synthesis of (styrene–maleic anhydride) copolymer (poly(St–MA))

(St–MA) copolymer was prepared by precipitation polymerization of maleic anhydride and styrene in the

Table 1
Structural characteristics of comb-like polymer CP(St–MA)–Brij35 (F^*: fluorophore)

Sample	M_w	M_w/M_n	Mole percent, Brij grafted (from ^{1}H NMR)
Poly(St–MA)	45,000	2.80	–
CP(St–MA) –Brij35	83,300	2.83	35.8
CP(St–MA) –Brij35–F^*	80,500	3.05	33.3

molar ratio 1:1 in xylene using benzoyl peroxide as initiator. The reaction was carried out at 95°C for 8 h in nitrogen atmosphere with stirring. The precipitated copolymer was filtered, washed several times with xylene and methanol. Polymer is reprecipitated from chloroform by adding methanol. The copolymer was dried under vacuum at 50°C for 24 h and characterized by IR and GPC analysis.

IR: 3027 cm^{-1} (Ar H stretching), 2915 and 2847 cm^{-1} (alkyl C–H stretching), 1845 and 1772 cm^{-1} (–C=O stretching for cyclic anhydride), 745 cm^{-1} (aromatic ring vibrations).

2.3.2. Synthesis of anionic graft copolymer with and without fluorophore (poly(St–MA)–Brij35)

Poly(St–MA) (2 g), Brij35 (20 g) and PTSA (0.1 g) were dissolved in methyl ethyl ketone (150 ml). The fluorophore labeled polymer was synthesized in an identical parallel reaction which included 1-pyrenyl methanol (0.08 g). Reaction was carried out at 85°C with stirring for 24 h. After cooling, the solvent removed under reduced pressure. Polymer was purified by repeated centrifugation in cold ethyl acetate (3°C) by taking advantage of the temperature dependent solubility of PEG in ethyl acetate [26], until there was no detectable Brij35 and pyrene compound in the supernatant. Ethyl acetate was removed under reduced pressure. Purity of the polymer was tested by GPC. The final polymer was characterized by IR, ^{1}H NMR and GPC analysis.

IR: 3300–3600 cm^{-1} (broad peak for OH stretching), 3025 cm^{-1} (Ar–H stretching), 2917 and 2862 cm^{-1} (alkyl C–H stretching and ethylene oxide protons stretching), 1780 cm^{-1} (C=O, carboxylic group stretching), 1725 cm^{-1} (ester C=O stretching), 1093 cm^{-1} (C–O–C stretching), 745 cm^{-1} (aromatic ring vibrations).

^{1}H NMR: 6.8–7.6 ppm (broad peak), 3.5 ppm (ethylene oxide protons), 2.5, 2.29 and1.9 ppm (polymer backbone protons), 1.45, 1.25 and 0.8 ppm (due to alkyl protons from Brij35).

2.4. Solution preparation

The effect of acid group neutralization was studied at constant polymer concentration ($C_{\text{pol}} = 1\ \text{g l}^{-1}$) at pH values varying from 2 to 10. pH was increased by successive additions of few microliters of 0.1 M aqueous NaOH to the polymer solution, so that the change in polymer concentration at the highest pH (10) did not exceed more than 3%. For each solution, pH was checked before and after every reading. The change in pH was found to be negligible (±0.05). The polymer yields a latex at pH < 3.5, gives a hazy solution >3.5 and dissolves at pH 4.8 and above. Hence all the experiments were carried out at pH ≥ 5.

To prepare polymer solution of different concentrations at a given pH, a stock solution of the polymer ($4\ \text{g l}^{-1}$) was first made by dissolving 0.5 g of solid polymer in 500 ml 0.25 mM NaOH solution and stirred for ~18 h. The ionic strength of the solution was adjusted to 10^{-3} M by the addition of solid KCl. This stock solution was diluted with (NaOH + KCl) solution of the same pH and ionic strength.

2.5. Pyrene solubility experiments

Pyrene crystals (~2 mg) were deposited onto the walls of several volumetric flask (5 ml) by gentle evaporation of an acetone solution (1 ml) of pyrene (~10 mM) under the flow of nitrogen gas. Desired volume of polymer stock solution at a given pH was added along with a magnetic stirring bar. Volume was made up by a solution of same pH and ionic strength. The solutions were stirred at 25°C for at least 5 days in the dark. Excess pyrene microcrystals remaining in the solution were removed by centrifugation (3000 rpm, 40 min). The supernatant solution was analyzed by UV spectroscopy. The absence of microcrystals in the solution was confirmed from the fluorescence and excitation spectra of the solution [20]. An equivalent reference with the same polymer concentration but without pyrene was used in all the measurements. The absorbance at each wavelength was taken relative to the absorbance at 405 nm. The excitation coefficient ε of micellized pyrene at the major absorption peak at 338 nm ($3.58 \times 10^4\ \text{M}^{-1}\ \text{cm}^{-1}$) was taken as reported for a linear associative thickener [28].

3. Results and discussion

The essential chemistry of this polymer is shown in Fig. 1. This system could be characterized very well both with respect to backbone and the pendant groups. The molecular weight and the Brij content of the polymer are given in Table 1. We have been able to incorporate about 35 mol% of Brij compound as side chains. Earlier, Kumacheva et al. [20] and Horiuchi et al. [21] have studied a similar system with Brij component as low as 0.9 mol%.

The surface activity of such polymer is rarely studied [17]. The polymer being ionic in nature, we monitored the surface tension of the solution at fixed polymer concentration as a function of pH as well as a function of concentration at two different pH values (pH 6.26 and 9.58). Fig. 2 shows the variation of surface tension at air/water interface with pH at fixed polymer concentration of $1\ \text{g l}^{-1}$. The data indicate that the polymer is less surface active at acidic pH than at

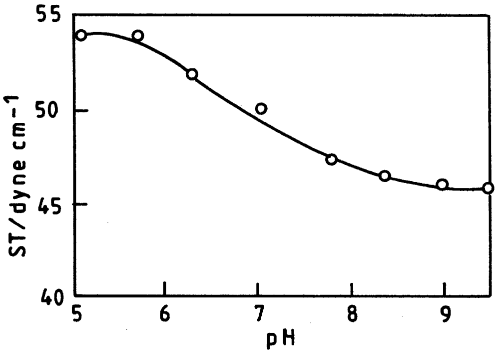

Fig. 2. Variation of surface tension at air/water interface with the pH at fixed polymer concentration (1 g l^{-1}) for CP(St–MA)–Brij35 polymer.

alkaline pH. From an initial value of 54 dyn cm^{-1}, surface tension of the solution decreases to 46 dyn cm^{-1} at pH ≥ 8. We offer the following interpretation: increase in pH would lead to greater ionization and consequent expansion of the polymer chain due to adjacent charge repulsion. This chain expansion would expose and drive the pendant dodecyl chains towards the air/water interface leading to lower surface tension.

Fig. 3 indicates that polymer has a tendency to get adsorbed at the interface and that it is effective in reducing the surface tension at both pH studied. In both cases, polymer has a limiting concentration after which surface tension does not change. This is typical of conventional surfactants. The limiting concentration after which surface tension does not change is higher (1.0 g l^{-1}) at higher pH (9.6) and lower (0.5 g l^{-1}) at low pH (6.3). Two transition points are observed at higher pH. There could be multiple reasons for this. At higher pH, due to extensive ionization, the aggregates are polydispersed and many in number; or there could be a change in the conformation at air/water interface. The pre-aggregation slope indicates that at the interface, the polymer is more compact at low pH than at high pH (Fig. 3).

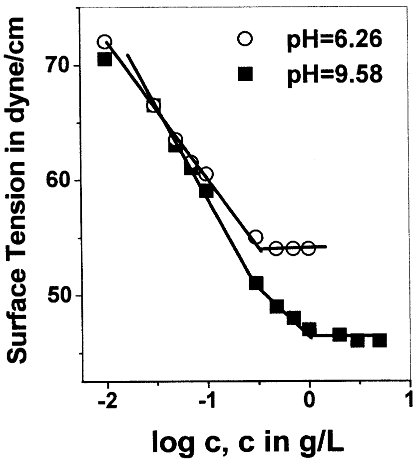

Fig. 3. Variation of surface tension at air/water interface with the polymer concentration at fixed pH for CP(St–MA)–Brij35 polymer.

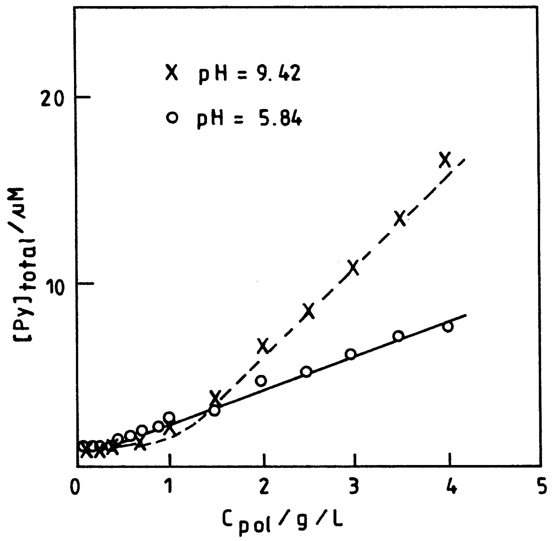

Fig. 4. Aqueous solubilization of pyrene at fixed pH (5.84 and 9.42) as a function of polymer concentration for CP(St–MA)–Brij35 polymer.

The solubility of an aromatic hydrocarbon such as pyrene in a given hydrophobic ambiance can help us to characterize the aggregate structure. The solubility of pyrene is determined exclusively by the free energy difference between Py in water and in the hydrophobic domain of the aggregates. The solubility dependence of pyrene at two different pH (5.8 and 9.4) on polymer concentration (C_{pol}) is shown in Fig. 4. At low C_{pol}, the solubility of Py increases slightly with C_{pol} and then increases more significantly in a higher C_{pol} region, giving a break point at $C_{pol} = 0.5$ g l^{-1} at pH 5.8 and 1.2 g l^{-1} at pH 9.4. The significant increase in Py solubility in the presence of polymer at different pH suggests the presence of hydrophobic microdomain at both the pH.

The equilibrium constant (k_{eq}) for the partition of pyrene between water and hydrophobic domain of the aggregate is defined as [29]

$$k_{eq} = \frac{[\mathrm{Py}]_{hd}}{[\mathrm{Py}]_{w}}(C_{pol} - \mathrm{cac}) \tag{1}$$

where $[\mathrm{Py}]_{hd}$ and $[\mathrm{Py}]_{w}$ are the concentrations of pyrene (mol l^{-1}) in hydrophobic domain and in water, respectively. C_{pol} and cac are the concentration and critical association concentration of the polymer, respectively. We have taken the limiting concentration of 0.5 g l^{-1} for pH 5.8 and 1 g l^{-1} for pH 9.4 as cac obtained from surface tension measurement, to calculate the k_{eq}. From the slope of Fig. 4, we estimate k_{eq} to be 2.63 l g^{-1} at low pH (5.84) and 13.26 l g^{-1} at high pH (9.42) using $[\mathrm{Py}]_{w} = 0.7$ μM. In terms of liquid droplet model [29] the partition coefficient (K_V) is defined as

$$\frac{[\mathrm{Py}]_{hd}}{[\mathrm{Py}]_{w}} = K_V\left(\frac{V_{hd}}{V_{w}}\right) \tag{2}$$

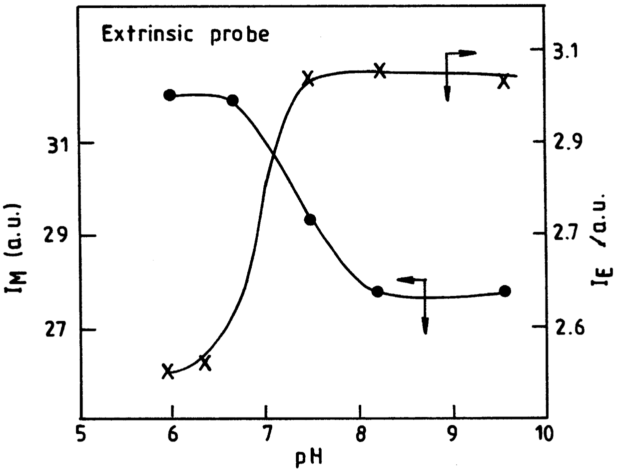

Fig. 5. Effect of pH on intensity of excimer and monomer emissions $C_{pol} = 1\ g\ l^{-1}$.

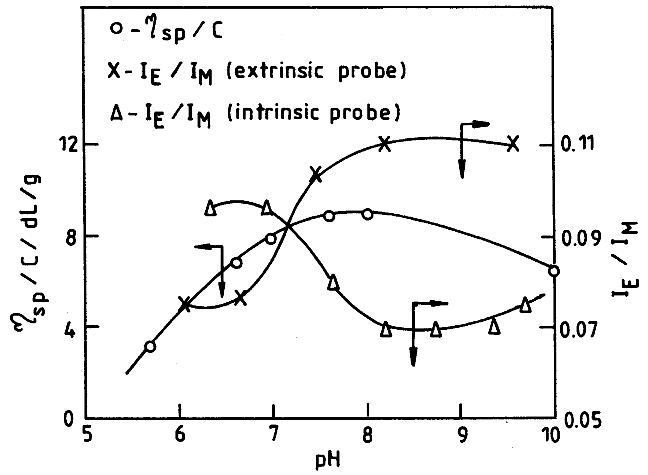

Fig. 6. Effect of pH on I_E/I_M for intrinsic and extrinsic probe and on η_{sp}/C of the CP(St–MA)–Brij35 polymer solution ($C_{pol} = 1\ g\ l^{-1}$).

and K_V is related with k_{eq} by the equation

$$K_V = \frac{k_{eq}}{h\bar{V}} \tag{3}$$

where h is the alkyl chain content of the polymer (moles of the alkyl group per gram of polymer) and $\bar{V}$ the molar volume of the hydrophobe. Taking $\bar{V} = 0.2282\ l\ mol^{-1}$ for $C_{12}H_{25}$ group[4] and $h = 0.145\ mmol\ g^{-1}$ of polymer, we find at lower pH, $K_V = 7.95 \times 10^4$ and at higher pH, $K_V = 40.09 \times 10^4$. Now K_V is simply related to the free energy (ΔG) for transfer of pyrene from water to the hydrophobic environment [30]

$$K_V = \exp\left(\frac{-\Delta G}{kT}\right) \tag{4}$$

where k is the Boltzmann constant and T the temperature. The lower value of K_V, i.e. less $-\Delta G$ (according to Eq. (4)) at pH 5.8 than that at pH 9.4 indicates that partition of pyrene molecules in the microdomain formed by the polymer at lower pH energetically is less favorable than the partition in the microdomain at higher pH.

The emission spectra of pyrene, used as a extrinsic probe, in the presence of polymer or surfactants shows a broad rather featureless emission centered at 480 nm, which is due to excimer formation [31]. Here, we monitored Py emission (6 μM) keeping polymer concentration constant at $1\ g\ l^{-1}$ at different pH. We have observed that upon increase in the pH of the solution, the monomer band decreases significantly, accompanied by an increase in excimer emission giving an isoemissive point at 448 nm. The presence of the isoemissive point suggests the presence of two types of pyrene as determined by an earlier study [20]. The plot of I_E and I_M against pH shows the decrease in monomer intensity accompanied by an increase in excimer intensity (Fig. 5) over the same pH range (pH 7–8). This indicates that the hydrophobic domain of the polymer changes at that pH range. The explanation consistent with this observation would be that at low pH that there are a large number of small aggregates in the system as reported by Kumacheva et al. [20]. When Py molecules distributed among these smaller aggregates, there is a lower probability of having two or more Py molecules in the same aggregate decreasing the I_E value. As the pH increases, larger aggregates are formed with more number of polymer chains resulting in fewer number of aggregates. Under such conditions, the occupation probability for more than one Py molecule in the same domain increases and I_E increases.

It is logical to assume that the fluorescence behavior of the intrinsic probe would reflect the dynamics of the polymer backbone, whereas the extrinsic probe would reveal the size and behavior of the hydrophobic domain formed by the polymer [31]. The emission spectrum of Py covalently attached to the polymer backbone (an intrinsic probe) also shows excimer band along with its monomer band. But the spectrum does not show any isoemissive point. The dependence of I_E/I_M on pH for both extrinsic and intrinsic probes is plotted in Fig. 6. Instead of an increase as for extrinsic Py probe, the I_E/I_M ratio decreases in the same pH range for the intrinsic probe. As pH increases, the polymer coil expands and the covalently attached pyrene molecules are separated, leading to decreased I_E/I_M. We notice another feature in this figure, a slight increment of I_E/I_M at higher pH, which could not be observed for the extrinsic probe, which implies a contraction of the polymer chain, bringing the pyrene molecules closer. The reduced viscosity plotted as a function of pH in Fig. 6, which registers a slight decrease at higher pH, confirms this possibility. Here, the reduced viscosity is plotted as a function of pH at polymer concentration $1\ g\ l^{-1}$. The reduced viscosity increases as pH increases, reaches a maximum and then suffers a slight decrease. This could be due to two events.

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[4] Molar volume of dodecyl chain was calculated from the density of dodecane.

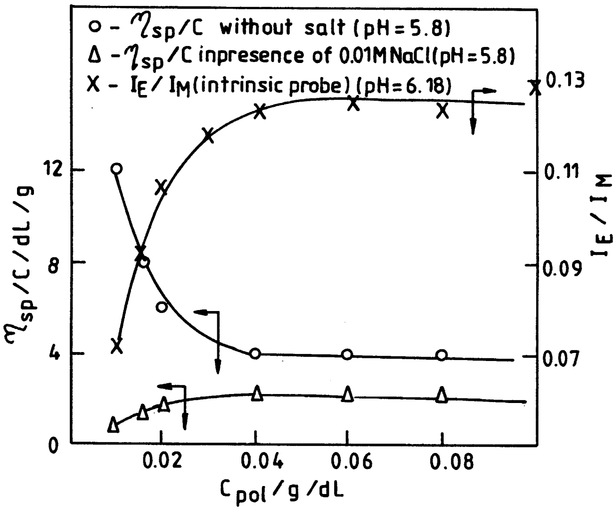

Fig. 7. Dependence of I_E/I_M (intrinsic probe) and η_{sp}/C on polymer concentration (in the presence and absence of 0.01 M NaCl).

With increase in pH, the degree of ionization of the polymer increases. As a result, the electrostatic repulsion between adjacent carboxylate anions increases and the polymer chain expands resulting in an increased reduced viscosity. The ionization of acid groups could force the hydrophobic domains into a structural reorganization. The smaller intrachain hydrophobic domains could now make way for large interchain hydrophobic domains leading to increment in the viscosity [20]. Viscosity reaches a maximum when all acid groups are ionized and all intrachain hydrophobic domains have been replaced by interchain domains. Further increase in pH could cause electrostatic shielding of the carboxyl groups resulting in slight shrinkage of the polymer chains and hence, the lowering in viscosity [32,33].

In order to determine whether the associations among hydrophobic groups at lower pH (6.18) are predominantly intra- or interchain, the effect of polymer concentration on I_E/I_M was investigated. At this pH, while both monomer and excimer intensities increase with increasing polymer concentration, there is no change in I_E/I_M above polymer concentration 0.4 g l^{-1} (Fig. 7). Insensitivity of I_E/I_M on polymer concentration at higher region strongly suggests the microdomains formed at this pH are due to the intrachain association. In the lower concentration region, increment in I_E/I_M with increase in polymer concentration (<0.4 g l^{-1}) is may be due to polyelectrolyte effect. To confirm the polyelectrolyte effect, the reduced viscosity of the polymer was measured at pH 5.8 with and without NaCl (0.01 M) as a function of the polymer concentration (Fig. 7). In the absence of NaCl, the reduced viscosity decreases as the concentration of polymer increases. This is a typical behavior of polyelectrolytes [32,33]. In dilute region, polyelectrolyte chains expand due to coulombic repulsion leading to higher viscosity. The presence of an electrolyte like NaCl screens the charge on the polymer backbone suppressing its tendency to expand.

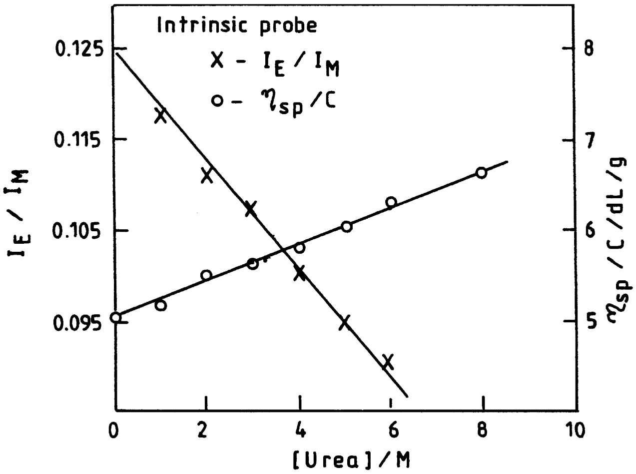

Fig. 8. Effect of urea on I_E/I_M (intrinsic probe) and η_{sp}/C of the CP(St–MA)–Brij35 of polymer solution (pH 6.18, $C_{pol} = 1$ g l^{-1}).

Fig. 8 depicts the effect of urea on I_E/I_M of the labeled polymer at pH 6.18. A significant decrease is observed upon addition of urea to the polymer solution. Since urea does not quench the fluorescence of the model compound, this decrease in I_E/I_M could be due to expansion of polymer backbone. Being an effective hydrogen bond breaker [34], urea disrupts the structure of water in a way that facilitates the dissolution of hydrophobic species, thus uncoiling the polymer chain. The fluorophore molecules get separated discouraging the formation of excimer and I_E/I_M decreases. McCormick et al. [35] report similar observation for a 20:80 mol% copolymer of 2-(1-naphthylacetyl) ethylacrylate and methacrylic acid. To confirm this possibility, reduced viscosity was measured at lower pH (6.18) at constant polymer concentration (1 g l^{-1}) in the presence of urea (Fig. 8). On increasing the urea concentration, the reduced viscosity increases which is consistent with I_E/I_M data.

Quenching of fluorescence intensity by a suitable quencher molecule gives information about the environment encountered by the probe. The efficiency of a quencher can be quantified according to the Stern–Volmer equation

$$\frac{I_0}{I} = 1 + K_{SV}[Q] \tag{5}$$

where I_0 and I are the intensities of the probe in the absence and presence of quencher, respectively, and K_{SV} the Stern–Volmer constant which quantifies the quenching efficiency. For a particular probe/quencher pair, higher magnitude of K_{SV} indicates the probe is more accessible for the quencher. Here, for the present investigation, quenching studies were performed using nitromethane (CH_3NO_2), a water-soluble neutral quencher [36]. The Stern–Volmer plot has been performed to evaluate the efficiency of the quencher at different environmental conditions and has been compared with the quenching efficiency in case of model compound.

The K_{SV}, the Stern–Volmer constant, obtained from the

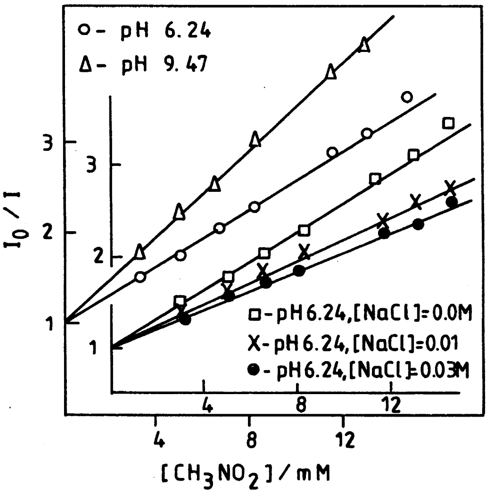

Fig. 9. Stern–Volmer plot for CP(St–MA)–Brij35 polymer ($C_{pol} = 1\ g\ l^{-1}$).

slope of the plot of I_0/I vs. [CH_3NO_2], for the model fluorophore, 1-pyrenyl methanol propionate, in aqueous medium is 1700 M^{-1} and quite independent of the pH and ionic strength (up to 0.1 M NaCl). For the pyrene labeled polymer, K_{SV} are 160 M^{-1} at pH 6.2 and 250 M^{-1} at pH 9.0 (Fig. 9). The low value of the K_{SV} for the polymer at both pH values than for the model compound indicates that the fluorophore in the polymer is less accessible for the quencher and confirms the presence of hydrophobic domains at both pH values. The shielding of predominantly hydrophobic domains by the hydrophilic part of the polymer would make the fluorophore less accessible for the quencher. The higher K_{SV} values at higher pH than the lower pH indicates the fluorophores are now more exposed. The K_{SV} values have been determined varying the salt concentration at low pH (Fig. 9). Upon increasing the ionic strength, K_{SV} decreased suggesting that the salt induces the polymer backbone to be more compact and less exposed to water and thus protects the fluorophore from quencher. At lower pH (6.24), the K_{SV} values obtained with and without urea show variation. The implication is that urea makes the fluorophore more accessible to the quencher. This is consistent with the viscosity and I_E/I_M data.

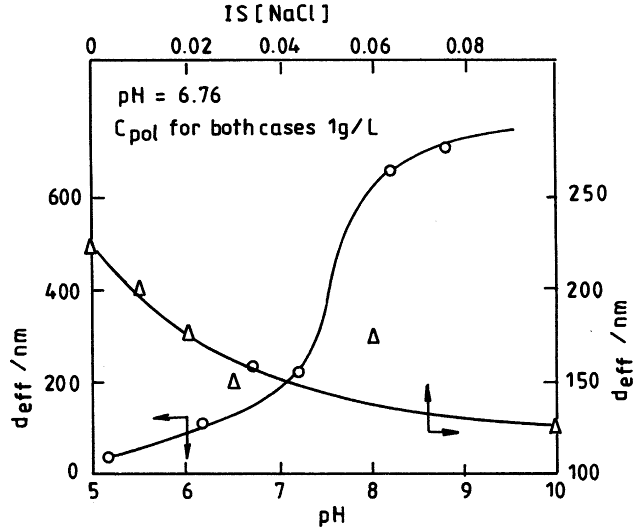

Fig. 10. Effect of pH and NaCl (at pH 6.76) on the effective diameter of the aggregate formed by CP(St–MA)–Brij35F in aqueous medium ($C_{pol} = 1\ g\ l^{-1}$).

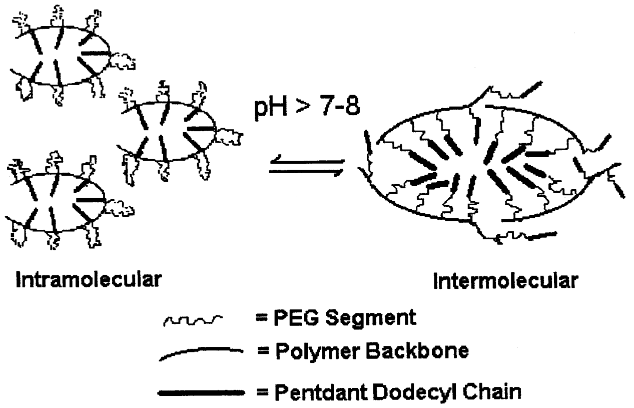

Fig. 11. Conceptual illustration of intra- and intermolecular aggregates.

One way to measure the size of aggregates in solution is by the DLS [37]. The diffusion coefficient of the mobile species is determined by a commulant analysis of the auto-correlation function decay assuming a log-normal fit to the distribution of diffusing species. Hydrodynamic radii R_H are calculated through the Stokes–Einstein equation

$$R_H = \frac{kT}{6\pi\eta D_m} \qquad (6)$$

where η is the solvent viscosity, D_m the diffusion coefficient, k the Boltzmann constant and T the temperature. The effect of pH on the effective diameter d_{ef} ($d_{ef} = 2R_H$) of the polymer aggregate, as measured by DLS are shown in Fig. 10. A certain increase in d_{ef} has been observed at pH ~ 7.2. Above pH 8, d_{ef} does not change considerably with change in pH. Fig. 10 also shows the effect of salt (NaCl) on the d_{ef} at low pH (6.76). On addition of salt, the d_{ef} reduces. The same observation was made in case of viscosity measurements also. Addition of salt suppresses the ionization of the polymer leading to more compact aggregates.

The essence of the analysis of the results is illustrated in Fig. 11. The backbone of the polymer consists of free carboxylic groups from maleic anhydride comonomer and phenyl groups from styrene comonomer. At lower pH, few of these carboxylic groups are ionized. As every alternative unit of the backbone contains a phenyl moiety, at this pH, it becomes hydrophobic. As a result, intrachain hydrophobic microdomains are formed through the association of these phenyl groups, which are also shared by the pendant dodecyl groups from the same polymer. These intrachain microdomains are protected by the hydrophilic PEG chains. Due to this intrachain association, polymer is less surface active, smaller in size and larger in number. As hydrophobic domains are smaller in size, these domains solubilize less amount of pyrene molecules and hence, system shows lower pyrene partition coefficient. Upon increment in pH of the system, acid groups are ionized and the backbone becomes completely hydrophilic in nature. At this stage, pendant dodecyl groups from different polymer chains are responsible for the polymer association. As several chains associate to form the aggregates, these are larger in size but lesser in number. These larger interchain aggregates are capable to dissolve more amounts of pyrene molecules than the smaller intrachain aggregates leading to higher pyrene partition coefficient. Reporting the aggregation behavior of a series random copolymer of sodium-(acrylamido)-2-methylpropanesulfonate and a macromonomer of nonionic surfactant ($C_{12}E_{25}$), Noda et al. [38,39] suggest the possibility of intra- or interchain aggregates depending on the concentration of the polymer. They observed that at concentration $>10\ g\ l^{-1}$, a network structure is formed resulting in significant enhancement in solution viscosity. In the present study, the concentration used (except Py partition experiment) is as low as $1\ g\ l^{-1}$ and insufficient to lead to secondary aggregation. The lower increment in viscosity due to transition from intra- to intermolecular upon increase in pH of the system confirms the absence of this secondary aggregation for present study.

4. Conclusions

Surface tension, pyrene partition, steady-state fluorescence, viscosity and laser light scattering have been used to study the aggregation behavior of a PEG based comb-like polymer as a function of pH. The data presented above clearly show that the surface activity, the nature and size of the aggregates formed by the polymer in water are very much pH dependent. At low pH, the polymer forms more compact monolayer at air/water interface and is less surface active compared to that at higher pH. Intramolecular aggregates are formed at $pH < 7.4$ and the aggregates behave as a polyelectrolyte at this pH range. As pH is raised, the intrachain aggregates are replaced by interchain aggregates with larger hydrophobic domain.

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