

# Microstructure of Polyelectrolyte Nanoaggregates Studied by Fluorescence Probe Method

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**Abstract** The microstructure of water soluble nanoaggregates based on polyelectrolyte complex formed by the cationic comb-type copolymer poly(acrylamide-co-[3-(methacryloyl-amino)propyl] trimethylammonium chloride)-graft- polyacrylamide [P(AM-co-MAPTAC)-g-PAM] and the anionic linear polyelectrolyte sodium polyacrylate (NaPA) was investigated using the fluorescence probe technique. The fluorescence probe were 1-anilinonaphthalene-8-sulfonic acid (ANS), pyrene (Py) and 1,10-bis(1-pyrene) decane (PD). The fluorescence properties in polyelectrolyte complex solutions, which are sensitive to either micropolarity (ANS, Py) or microviscosity (PD), were related to the quantities obtained in different pure or mixed solvents. Micropolarities were quantified utilizing the polarity common index (Reichardt)  $E_T(30)$ . ANS and Py showed a variation of the micropolarity with the charge ratio of the two polymers, with the lowest polarity reached at the complex neutralization. The PD probe, by its excimer-to-monomer fluorescence intensities ratio, enabled us to evidence the effect of the composition and the comb-type copolymer grafting density on the microviscosity of the interpolyelectrolytes aggregates. It has been found that the microviscosity increased with the density of the grafting PAM chains.

**Keywords** Fluorescence probe · Polyelectrolyte complex · Micropolarity · Microviscosity

## Introduction

Polyelectrolyte complexes (PECs) are of high relevance since they offer the possibility to combine physicochemical properties of at least two polyelectrolytes. They are usually created by two polyelectrolytes carrying opposite charges, and the driving force for the PECs formation is the Coulombic interaction. This strong long-ranged interaction may leads to interpolymer ionic condensation. In other words, a phase separation occurs and the solution separates out in two immiscible liquid phases: a denser phase, which is concentrated in macromolecules and a phase poor in macromolecules [1]. Depending on the pH and ionic strength conditions, the phase separations can be alleviated and a single macroscopically isotropic phase is obtained [2–4]. The phase stability of the PECs can be also influenced by other inter-polyion interactions such as hydrogen bonding, Van der Waals forces, hydrophobic and dipole interactions [5].

Water-soluble PECs can be also obtained when both or at least one of the two polyelectrolytes has covalently bound non-ionic hydrophilic blocks such as poly(ethylene glycol) [6, 7]. It has been recently shown that the interaction between the cationic comb-type copolymer poly(acrylamide-co-[3-(methacryloyl-amino)propyl] trimethylammonium chloride)-graft- polyacrylamide [P(AM-co-MAPTAC)-g-PAM] and the anionic homopolymer sodium polyacrylate (NaPA) leads to water soluble PEC formation [8, 9]. They form core-shell nanoparticles which

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consist of hydrophobic (not water soluble) P(AM-co-MAPTAC)-PA core and hydrophilic PAM corona. The formation of PEC was studied by turbidimetry, viscosimetry, conductimetry,  $\xi$ -potential, static and quasi-elastic light-scattering [8].

The knowledge of the microstructure of such soluble PECs, in particular local polarity and local viscosity, is of great interest because of their biomedical applications [10]. Complex water soluble aggregates such as micelles, modified biopolymers, vesicles, and hydrogels show variable local properties throughout different regions of the aggregate [11]. Information on the micropolarity and microviscosity of such aggregates can be obtained by spectrophotometric methods, and in particular by the molecular probes techniques [12]. We have investigated using the absorption and fluorescence probe techniques the microstructure of polymeric and nonionic poly(oxyethylene) surfactants micelles [13–18] and have gathered information about the interactions within the surfactant-polymer and surfactant-protein complexes [18–21]. A review on the application of fluorescence techniques for characterizing of synthetic polyelectrolytes is given in ref. [22].

Among fluorescence probes suitable for probing the local polarity and viscosity in microheterogeneous media, we used in the present work 8-anilinonaphthalene-1-sulphonate (ANS), pyrene (Py) and 1,10-bis(1-pyrene) decane (PD). The former two are well known polarity-sensitive probes, with different hydrophilic/hydrophobic trade-offs [11, 23–26]. In the case of ANS, the wavelength of fluorescence band maximum, yield and lifetime of fluorescence are strongly dependent on polarity of the microenvironment. ANS is practically nonfluorescent in water, while intense fluorescence is emitted when it binds in a hydrophobic site, especially on cationic charge. For this reason ANS was used as probe to investigate proteins [27–30], membranes and micelles [13, 15–17]. The more hydrophobic probe Py was also used to assess the micropolarity in micellar solutions as the ratio of the first and the third vibrational peaks of the emission spectra is sensitive to the polarity of the surrounding medium [12]. PD, the most hydrophobic probe, usually characterizes the microviscosity (or microfluidity) through examination of the intramolecular excimer formation [18].

The goal of the present study is to investigate the organization of the water soluble PECs formed by the [P(AM-co-MAPTAC)-g-PAM] and oppositely charge NaPA. The focus was on the effect of charge ratio of the two polymers and of the grafting degree of the comb-type copolymer on the core-shell conformation adopted by the PECs. The fluorescence probes mentioned above are used to assess the local micropolarity and microviscosity of the nanoaggregates.

## Experimental

### Materials and Solutions

NaPA was obtained after neutralization with NaOH of a 25 wt.% polyacrylic acid solution in water (Aldrich), of a nominal mass  $M=9.0\times 10^5$  Da. After purification, carried out with a filter membrane with a molar mass cut-off= $10^3$  Da, the polymer was obtained by freeze drying. Its molar mass was  $1.2\times 10^5$  Da, and the mass per unit charge 94 Da. Three P(AM-co-MAPTAC)-g-PAM polymers were synthesized as described in a previous work [8]. The average mass per unit charge of the polymers was conductimetrically determined with 1 mM potassium poly(vinyl-sulphate) solution and they were found equal to 1175, 2200 and 5300 Da. Henceforth, the three comb-type copolymers will be referred to as G1175, G2200 and G5300. The fluorescence probes 1-anilinonaphthalene-8-sulfonic acid, pyrene and 1,10-bis(1-pyrene) decane, all of special purity, were supplied by Molecular Probes, Eugene, OR. The chemical structures of the polymers and fluorescence probes are presented in Fig. 1.

Stock solutions of the anionic and cationic polyelectrolytes in water with molar charge concentration of  $10^{-3}$  M were prepared and thereafter different mixtures were obtained by mixing appropriate amount of the two stock solutions and additional water to reach the desired concentrations. The water used was treated with Milipore-Q water purification system.

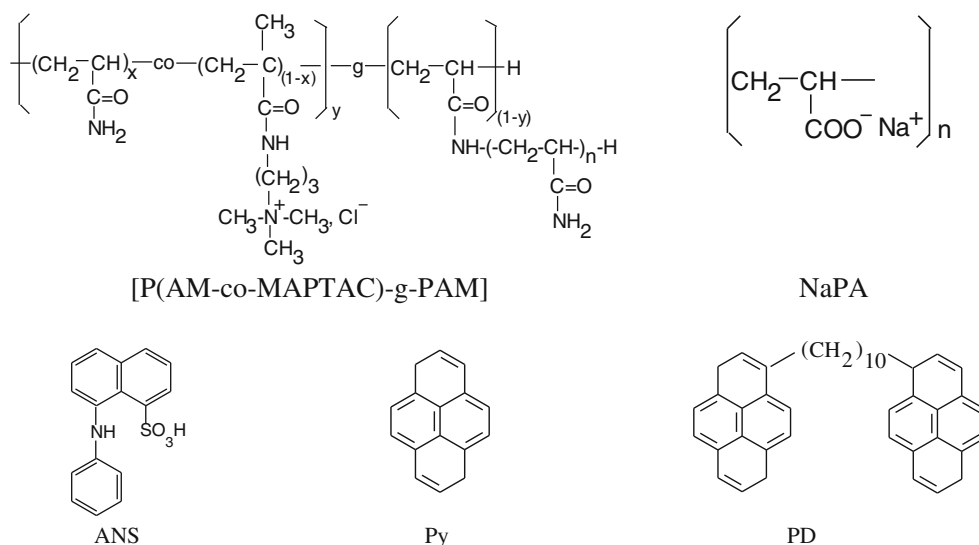
$10^{-3}$  M ANS solubilized in ethanol, and  $10^{-4}$  M Py and PD in cyclohexane represented stock solutions. Adequate amounts from these stock solutions were evaporated in a flask by means of a nitrogen stream. The polymers solutions are subsequently added, stirred and stored overnight for a good solubilization of the probe and the solution equilibration. The final probes concentrations are specified in the experimental results presentation.

Absorption spectra were recorded with a Perkin-Elmer Lambda 35 UV-VIS spectrophotometer, and the fluorescence measurements, both emission and excitation steady-state spectra were recorded with Jasco FP-6500 spectrofluorimeter. The absorption and fluorescence measurements were all carried out at 23 °C.

## Results and Discussion

### ANS Probe. UV-Vis Adsorption

Among the three fluorescence probe considered, ANS was the only one that had exhibited under certain circumstances a ground state interaction with G2200 or G5300 and NaPA polyelectrolyte solutions. The ANS absorption spectra at

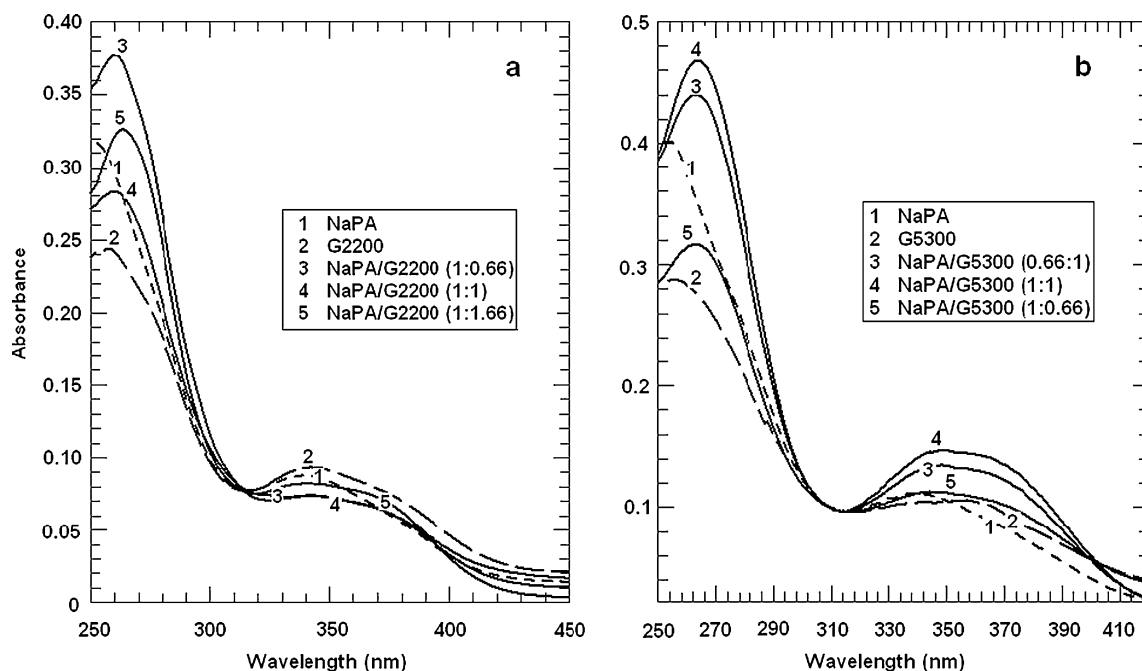
**Fig. 1** Chemical structures of the polyelectrolytes and fluorescence probes

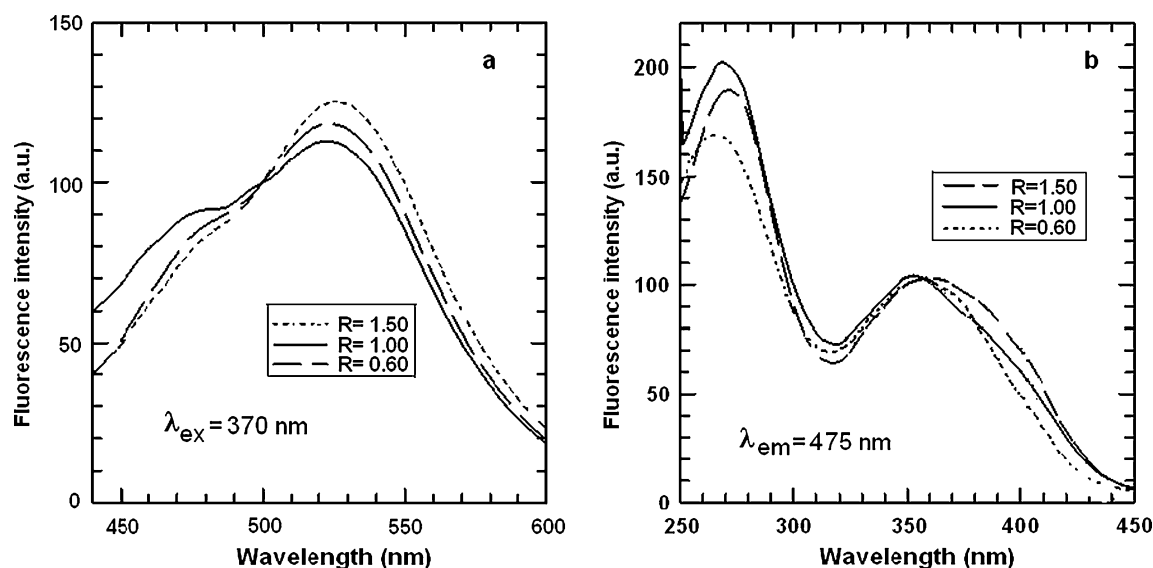
various NaPA/ G2200 and NaPA/G5300 charge ratios are shown in Fig. 2. The main features of the absorption spectra were given by two bands: a band centered at around  $\sim 265$  nm, and a second one at  $\sim 360$  nm. At closer inspection the last one consists of an overlap of two bands.

It is known that the ANS absorption spectrum does not change to a great extent with the solvent nature [24, 28, 31]. Thus, in ethanol, peaks at 219, 269, 352 (352 nm as a shoulder) and 374 nm were observed; in aqueous solution a small blue shift (around 4 nm) and lower absorbance values (integrated absorbance is about 30–40% that of ANS in ethanol for lowest two bands) compared with ethanol occur [31]. However, even these small changes of absorption

bands may be useful in highlighting the formation of the hydrophobic structures of the inter-polyelectrolyte systems in water.

In NaPA/G2200 or NaPA/ G5300 mixtures, the binding process is determined by the mutual reactivity of the reaction partners, *i.e.* ANS and polyelectrolytes. The UV band shifts bathochromic when NaPA and polycations are mixed. The shift is largest in the case of G5300, about 10 nm (Fig. 2b). Regarding the band in 300–400 nm range, it is increased in the case of 1:1 molar ratio, especially for NaPA/G5300 and the shape of the band is changed. The complex formation at 1:1 stoichiometric ratio is evidenced by the modifications of the ANS absorption spectrum.

**Fig. 2** UV-VIS absorption spectra of ANS ( $10^{-5}$  M) at different charge ratio (a) NaPA / G2200 and (b) NaPA/G5300



**Fig. 3** (a) Normalized emission and (b) excitation spectra of ANS ( $5 \times 10^{-6}$  M) at three charge ratio NaPA/G5300

### ANS Probe. Fluorescence Spectra

Fluorescence spectrum of ANS exhibits a wide band whose maximum shifts bathochromic with increase of solvent polarity. The shape of the fluorescence band suggests that it might originate from the overlap of two bands [15, 23, 32]. Figure 3a exemplifies the emission spectra, normalized at 500 nm, of ANS in aqueous solutions of G5300 and NaPA mixtures at three molar ratios  $R = [\text{NaPA}]/[\text{G5300}]$ . The spectra have been normalized to better highlight the two bands and their changes at varying the charge ratio around the neutral complex formation. As in reference [32], the two peaks are attributed to emission from excited state of non-planar geometry (NP) and to the emission from a planar configuration through the charge transfer (CT) excited state, respectively.

The wavelength at the maximum emission,  $\lambda_{em}^1$ ,  $\lambda_{em}^2$  and the fluorescence intensities,  $I(\lambda_{em}^1)$ ,  $I(\lambda_{em}^2)$  of the two bands were determined by the deconvolution of the fluorescence spectra with two Gaussians. The recovered variables for the ANS in the systems NaPA/G2200 and NaPA/G5300 are listed in Table 1. In the case of the NP band,  $\lambda_{em}^1$ , varied between 473 and 477 nm whereas the CT band position,  $\lambda_{em}^2$ , remains practically unchanged at varying the polyelectrolyte charge ratio. Note that the lowest value of  $\lambda_{em}^1$  was obtained near the stoichiometric charge ratio ( $\lambda_{em}^1$  reached minimum at  $R = 0.80$ ). Similar behavior of the NP and CT emission bands were reported for the NaPA/G2200 and NaPA/G5300 PECs as seen in Table 1. The ratio of the intensities  $I(\lambda_{em}^1)/I(\lambda_{em}^2)$  varies in the same line as the  $\lambda_1$  variation (see Table 1). The intensities ratio reaches a maximum at  $R = 0.80$ , when the

**Table 1** The wavelength at the maximum emission,  $\lambda_{em}^1$ ,  $\lambda_{em}^2$  and the relative intensities ratio,  $I(\lambda_{em}^1)/I(\lambda_{em}^2)$  of the two emitting bands of ANS at various ratio of PECs.  $E_T(30)$  values were determined from  $\lambda_1 = 0.573 E_T(30) + 445$  equation

Charge ratio	$\lambda_{em}^1$ (nm)	$\lambda_{em}^2$ (nm)	$I(\lambda_{em}^1)/I(\lambda_{em}^2)$	$E_T(30)$ (kcal/mol)
NaPA/G2200				
3.00	477.2	531.0	0.41	55.8
1.50	475.4	530.9	0.44	52.3
1.00	474.1	530.3	0.46	50.6
0.80	473.1	530.3	0.48	48.9
0.60	474.2	530.0	0.46	50.6
0.50	476.1	530.9	0.42	54.1
NaPA/G5300				
3.00	476.0	529.4	0.41	54.1
1.50	475.6	530.5	0.43	53.4
1.00	474.0	530.1	0.46	50.6
0.80	472.5	530.3	0.48	48.0
0.60	476.0	531.0	0.42	54.1

band intensity  $I(\lambda_{em}^1)$  is highest, indicating a less polar region.

To estimate the micropolarity of the complex calibration curves were used. They were obtained from ANS fluorescence in dioxane-water mixtures and plotting  $\lambda_1$  as a function of Reichardt solvent polarity  $E_T(30)$  of dioxane-water mixture.  $E_T(30)$  values for dioxane-water mixtures were extracted from refs. [24, 33]. The following linear relationship between  $\lambda_1$  and  $E_T(30)$  was established:  $\lambda_1 = 0.573 E_T(30) + 445$ . Based on this relationship,  $E_T(30)$  values for polyelectrolytes solutions were calculated and presented in Table 1.

The lower micropolarity, equivalent to the 80% dioxane-water solution, is observed for  $R=0.80$ , although the complex has a 1:1 stoichiometry. The difference arises from the fact that the ANS molecules contribute to neutralize cationic charge.

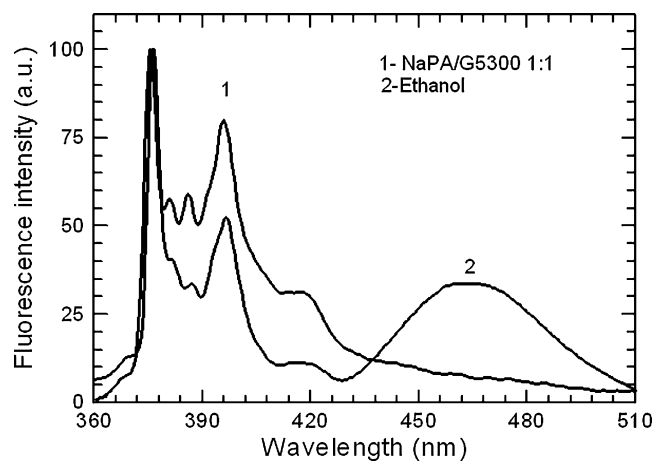
Figure 3b shows the ANS fluorescence excitation spectra in NaPA/G5300 mixed solutions obtained for NP band fluorescence emission  $\lambda_{em}=475$  nm. They resemble absorption spectra in Fig. 2b as two main excitation bands centered at  $\sim 270$  nm and  $\sim 360$  nm were found. Moreover, the main features of the two excitation bands, namely such as the position of the maxima and the broadness of the lower energy UV band depended on the charge ratio in line with the behavior of the UV-Vis absorption spectra.

#### Py Probe

This fluorescent probe is frequently used in direct micelles to emphasize the micellization process, to estimate the micropolarity with the help of standard calibration solutions, or to study the effect of additives on the micropolarity. The method is based on the extreme sensitivity of the ratio corresponding to the first (near 373 nm) and third (near 384 nm) vibronic bands fluorescence intensities on

**Table 2** The  $I_1/I_3$  ratio of  $10^{-6}$  M Py in inter-polyelectrolyte systems at different composition of NaPA-cationic polymer mixtures

Cationic polymer	Charge ratio	$I_1/I_3$
G1175	1:1	1.29
G1175	2:1	1.43
G1175	1:2	1.51
G2200	1:1	1.31
G2200	2:1	1.56
G2200	1:2	1.52
G5300	1:1	1.29
G5300	2:1	1.58
G5300	1:2	1.52
Ethanol		1.38



**Fig. 4** The normalized emission spectra of PD ( $1 \times 10^{-5}$  M) solubilized in NaPA/G5300=1:1 and in ethanol

the polarity of the medium. The ratio  $I_1/I_3$ , so-called pyrene polarity index, is often used to characterize the micropolarity of the micellar systems and to study polymer-surfactant interactions [34–36]. Pyrene is a very hydrophobic probe, its solubility in water is very low (about  $10^{-7}$  M), but it is easily solubilized in a hydrophobic medium.

The ratio of the first and third vibrational peaks of the emission spectra,  $I_1/I_3$ , for Py solubilized in polyelectrolytes solutions of different charge ratios are summarized in Table 2. We remind that  $I_1/I_3$  depends on the local polarity sensed by the probe and thus it enabled us to gain insights into PECs structure. When the complex is formed at stoichiometric charge ratio, the probe, being solubilized in a more hydrophobic zone of the aggregate senses a low polarity, lower than in ethanol. The ratio value does not seem to depend on the number of grafted chains. This is a proof of pyrene solubilization in the core of aggregate, it is the neutral PEC (consisting of cationic polymer backbone and NaPA). The  $I_1/I_3$  value in aggregate is lower than its value in ethanol for  $R=2:1$  and  $1:2$ , the probe indicates a greater polarity but lower than in water where the  $I_1/I_3$  value is 1.84.

**Table 3** The  $I_E/I_M$  ratio of PD in various solvents and neutral PECs

Solution	$I_E/I_M$
G1175:NaPA=1:1	0.30
G5300:NaPA=1:1	0.06
Ethanol	0.34
Ethanol-glycerine 1:1 (v/v)	0.29
Hexadecane	0.27
Tetraethylene glycol	0.02
84% Tetraethylene glycol-16% water	0.05
80% Tetraethylene glycol-20% water	0.07



## PD Probe

This hydrophobic derivative of pyrene may form intramolecular excimers. The process depends on the rate of conformational change of the molecule which is sensitive to the viscosity of the probe microenvironment [36, 37]. Hence the  $I_E/I_M$  ratio, where  $I_M$  stands for the intensity of the monomer fluorescence and  $I_E$  the intensity of the excimer fluorescence, is used to estimate the microviscosity.

Because of its high hydrophobicity, the solubilization zone of PD is in the core of the aggregate, as was established in the case of polymeric micellar solutions [18]. The PD probe may also evidence (only qualitatively) the micropolarity variation in the solubilization region, by the change in the ratio of monomer vibronic bands intensities measured at 377 nm and 397 nm, but it is known that this probe is less sensitive to polarity compared with pyrene [18].

Emission spectra of PD ( $1 \times 10^{-5}$  M) solubilized at the stoichiometric charge ratio NaPA/G5300 = 1:1 is shown in Fig. 4. For the sake of comparison, Fig. 4 illustrates the PD emission spectra in ethanol. The fluorescence intensities of the excimer,  $I_E$ , and monomer,  $I_M$ , were measured at  $\lambda_E = 465$  nm and  $\lambda_M = 377$  nm, respectively. The  $I_E/I_M$  ratio in different media is presented in Table 3. One can observe a striking difference between the excimer intensity in ethanol and in G5300-NaPA aggregates (Fig. 4). The presence of a weak excimeric band in PECs implies that the PD experienced a highly viscous microenvironment that is similar to that found in TEG solvent. On the other hand, the fluidity of the NaPA/G1175=1:1 complex is higher as compared to that found for NaPA/G5300=1:1. It is therefore concluded that the conformation adopted by the P(AM-co-MAPTAC) moiety and NaPA polymer in the core of the PECs depend on the density of the grafting chains. This peculiar can be understood by the fact that the core-shell radial organization of the inter-polyelectrolyte complex is affected by the density of grafted chains. It has been recently shown by Monte Carlo simulations on a coarse grained model of the G2200:NaPA complex that the core-shell organization was enhanced by increasing the grafting chain density [38]. A better segregation of the core at increasing the chain density would explain the higher viscosity sensed by PD in neutral NaPA/G5300 complex as compared with that in NaPA/G1175 complex. For NaPA/G5300=1:1 the microviscosity is comparable with 81% tetraethylene glycol-19% water.

## Conclusions

Microstructure characterization of the water soluble nanoaggregates based on the complex formed between the

cationic comb type copolymer poly(acrylamide-co-[3-(methacryloylamino)propyl]trimethylammonium chloride) –graft–polyacrylamide, P(AM-co-MAPTAC)-g-PAM, and the anionic polyelectrolyte poly(sodium acrylate), NaPA, was provided by the fluorescence probe technique. Three cationic polyelectrolytes that differed by the number of grafted chains, denoted as G1175, G2200 and G5300 have been studied. Three fluorescent probes: ANS, Py and PD have been used. The fluorescence properties relevant for the micropolarity and microviscosity sensed by probes were all affected by the PECs formation. Micropolarity, indicated by ANS, expressed in equivalent  $E_T(30)$  polarity scale, is lower in the neutral G2200:NaPA and G530:NaPA complexes, it is equivalent to that of 80% dioxane - 20% water mixture. Pyrene, a more hydrophobic probe compared with ANS, showed also a variation of the micropolarity with the charge ratio of the two polymers, with the lowest polarity reached again at the complex neutralization. The microviscosity of the complex core experienced by PD probe increased with the density of the grafting PAM chains. For NaPA /G5300= 1:1 the microviscosity is comparable with 81% tetraethylene glycol-19% water.

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