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# Fluorescence probe studies on the complexation between poly(methacrylic acid) and poly(*N*, *N*-diethylacrylamide)

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**Abstract**

The complexation between poly(methacrylic acid) (PMAA) and poly(*N*, *N*-diethylacrylamide) (PDEAM) in aqueous phase was studied by UV–vis and fluorescence probe techniques. It was demonstrated that the complexation of PMAA with PDEAM occurs within a pH range of 1–6.5 and along with the complexation, the conformation of PMAA changed from a hypercoiled to a loose coiled form. The complex ratio between the two polymers is 1:1 (PMAA:PDEAM, in monomer unit). Salt effect studies showed that the complexation occurred due to formation of hydrogen bonds between the two polymers. Based upon these conclusions and the "compact micelle-like structure" for PMAA at low pH, a "ladder" model was proposed for the structure of PMAA–PDEAM complex formed at low pH.


*Keywords:* Poly(methacrylic acid); Poly(*N*; *N*-diethylacrylamide); Complexation; Conformation; Fluorescence probe

## 1. Introduction

The unique and novel properties of intelligent polymer materials, which exhibit large property changes in response to small changes in external conditions such as, for example, temperature [1], pH [2], electric fields [3], chemicals [4], offer an unlimited amount of potential applications relevant to industry, environment and the biomedical field. These applications include drug delivery systems [5], temperature-sensitive coatings [6], smart catalysts [7], and pervious membranes [8].

Poly(*N*, *N*-diethylacrylamide) (PDEAM) is a temperature-sensitive polymer [9], that exhibits a well-defined lower critical solution temperature (LCST) in water around 30 °C. It is known that the phase transition and accompanying polymer conformation changes result from a delicate balance between the hydrophobic interaction and hydrogen bonding. Recently, there has been considerable interest in the use of materials that respond to two stimulus, either mutually or independently in specific environments, with particular emphasis on temperature and pH responsive polymers that have been prepared by copolymerizing the temperature-sensitive *N*, *N*-diethylacrylamide (DEAM) with monomers containing base or carboxylic acid groups, such as methacrylic acid (MAA), to give a pH-dependent LCST [2,10].

Poly(methacrylic acid) (PMAA) is a pH sensitive polymer. Unlike other polyacids, it adopts hypercoiled conformation at low pH because of the hydrophobic interactions introduced by the methyl groups along the polymer backbone. However, on addition of base to solution, the carboxyl groups ionize and acquire negative charges. The increase in Coulombic repulsive forces results in a non-uniform sudden conformational transition from the hypercoiled to expanded form. This conformational change is reversible [11,12]. It is expected that the smart behavior of PMAA may be introduced into a temperature sensitive polymer, such as PDEAM, forms a copolymer, and this copolymer could be rendered double sensitive properties to external temperature and pH stimulus. This copolymer may form the basis of new intelligent films and hydrogels.

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At present, we have synthesized a series of temperature and pH sensitive DEAM–MAA random copolymers by free radical copolymerization techniques, and determined their temperature and pH double sensitive properties by transmittance measurements, respectively. In order to investigate further the natures of copolymer occurring phase transition and copolymer temperature and pH double sensibilities properties, we decided to investigate the interactions between PMAA and PDEAM. Fluorescence techniques, such as non-radiative energy transfer, fluorescence lifetime, fluorescence anisotropy measurements and fluorescence probe studies have been used widely to investigate interpolymer complexation [13,14], and segmental mobility and conformational behavior of polymers. The advantage of fluorescence techniques is that information about the behavior of the polymers on the molecular level can be obtained, as opposed to the bulk properties determined by non-spectroscopic techniques.

In this paper, we describe these preliminary investigation, using UV–vis and fluorescence probe techniques to study the complexation between PMAA and PDEAM, the effects of complexation upon the hypercoiled conformation of PMAA and the nature of the complexation between the two polymers.

## 2. Experimental

### 2.1. Materials

Pyrene (Py, Aldrich-96%) was purified by recrystallization from ethanol and then extracted with ethanol in a Soxhlet's extractor. Diethylamine, dichloromethane, magnesium sulfate, sodium hydroxide, acetone, methanol, diethyl ether, and hexane were used as received (analytical grade). 2,2′-Azobisisobutyronitrile (AIBN) was purified by recrystallization from ethanol. MAA was distilled under vacuum before use. Double distilled water was used throughout. The pH of the solution was adjusted using 0.5 M NaOH solution and/or 0.5 M HCl solution.

### 2.2. Synthesis

#### 2.2.1. Preparation of acryloyl chloride

The preparation of acryloyl chloride is according to literature procedures [15]. A mixture of 70 g (0.97 mol) acrylic acid, 234 g (1.66 mol) benzoyl chloride, and 0.5 g (0.0045 mol) hydroquinone was distilled at a fairly rapid rate through an efficient 25 cm distilling column. The distillate was collected in a receiver containing 0.5 g (0.0045 mol) hydroquinone, immersed in ice. When the temperature at the top of the column, which remained between 60 and 70 °C for most of the distillation, had reached 85 °C, the distillation was discontinued. The crude product was redistilled through the same column and the fraction boiling at 72–74 °C at 740 mmHg was collected.

#### 2.2.2. Preparation of DEAM

A solution of 46.7 mL acryloyl chloride dissolved in 30 mL dichloromethane was gradually added to another solution of 208 mL diethylamine previously dissolved in 450 mL dichloromethane at 0 °C under $N_2$ atmosphere. The reaction mixture was stirred for 4 h at 0 °C. The precipitated salt was removed by filtration and washed with double distilled water to remove traces of the filtered solution. After drying over magnesium sulfate, the solvent was removed under reduced pressure. The crude product was distilled in the presence of hydroquinone at 85–88 °C under vacuum at 68 mmHg, to yield a colorless liquid product. $^1$H NMR ($CDCl_3$, $\delta$ ppm): 6.5 (1H, =CH–), 6.3 (1H, $CH_2$=), 5.6 (1H, $CH_2$=), 3.4 (4H, –$CH_2$–), 1.1 (6H, –$CH_3$).

#### 2.2.3. Preparation of PDEAM

PDEAM was prepared by free radical polymerization, according to literature procedures [9]. A solution of 1.27 g (0.01 mol) DEAM dissolved in 1.5 mL methanol was stirred with 8 mg (0.0488 mmol) AIBN under $N_2$ at 62 °C. Stirring was discontinued after 30 min. Heating was continued for 6 h. The polymer was cooled to room temperature and then dissolved in 5 mL acetone and precipitated from 80 mL hexane. The polymer was purified by multiple dissolutions (×3) in acetone, followed by precipitation into hexane, and then dried at room temperature under vacuum.

#### 2.2.4. Preparation of PMAA

PMAA was prepared by free radical polymerization using AIBN as initiator in benzene. Polymerization was terminated at less than 10% conversion. The polymer was purified by multiple dissolutions (×5) in methanol, followed by precipitation into diethyl ether.

### 2.3. Polymer characterization

Molecular weights were determined by laser light scattering (LLS) techniques (Brookhaven BI-2000SM, USA) using methanol as solvent. The weight-average molecular weight [$M_w$/(g mol$^{-1}$)] of PMAA and PDEAM are $7.5 \times 10^5$ and $1.24 \times 10^5$, respectively. $^1$H NMR measurements were recorded on a NMR spectrometer (BRUKER AM-400, Billerica, MA). Transmittances of the solutions were determined on a UV–vis spectrophotometer (Shimadzu UV-240, Kyoto, Japan) and pH measurements were conducted on a PHS-1 acidimeter. All fluorescence measurements were conducted on a Perkin-Elmer LS-50B luminescence spectrometer.

### 2.4. Sample preparation

Polymer solutions were prepared from their stock solutions. The concentrations of the stock solutions for PMAA and PDEAM are all 0.1 wt.%. It is to be noted that the pH of the stock solution of the polyacid was about 10, where that of PDEAM was around 7.

For the experiments involving dissolution of organic probe molecules into the water-soluble polymer solutions, the probe, Py, was initially dissolved in diethyl ether to obtain a stock solution of known concentration (ca. $10^{-3}$ mol $L^{-1}$). This solution was diluted to $10^{-5}$ mol $L^{-1}$ just before use. One milliliter of the probe solution ($10^{-5}$ mol $L^{-1}$) was injected into a 10 mL volumetric flask. The ether was evaporated at room temperature. Subsequently, a polymer solution of known pH ($10^{-3}$ wt.%) was added to the flask. To ensure solubilization and equilibration, the polymer/probe solution was sonicated for 20 min, and then left at room temperature for more than 12 h.

For complexation measurements, all samples of different PMAA to PDEAM ratios ($x_{MAA} = n_{MAA}/(n_{MAA} + n_{DEAM})$: 0; 0.1; 0.2; 0.3; 0.4; 0.5; 0.6; 0.7; 0.8; 0.9; 1.0) were prepared in a similar manner. The method is described by using an example, that is the preparation of a solution containing 2.8 $\times$ $10^{-6}$ mol% of PMAA and 2.8 $\times$ $10^{-6}$ mol% of PDEAM ($x_{MAA}$ = 0.5, in residue unit, pH 4.0). To make this solution, 0.1 mL of PMAA stock solution (2.8 $\times$ $10^{-4}$ mol%) and 0.1 mL of PDEAM stock solution (2.8 $\times$ $10^{-4}$ mol%) were added, respectively, to a 10 mL volumetric flask with shaking. The mixture was diluted to about 9 mL and its pH was adjusted to 4.0 using 0.1 mol $L^{-1}$ HCl solution and/or NaOH solution. The solution obtained in this way was diluted to 10 mL with double distilled water before using.

### 2.5. Analytical methods

Because complexation between PMAA and PDEAM will change solution transmittance, the determination of the complexation is focused on measuring transmittances for PMAA–PDEAM complex system. Otherwise, it is reported that a significant change in conformational behavior of polymers occurs upon complexation [13,14]. Therefore, fluorescence probe measurements should find increasing use in this aspect.

## 3. Results and discussion

### 3.1. UV–vis spectrophotometric studies

Concentration of the polymer solution used for the determination of complexation was 0.1 wt.%. Because of its sensitivity to the changes in the turbidity of the solution [9], 550 nm was selected as analyzing wavelength. The sample solution was put in a sample holder and double distilled water was adopted as reference for the measurement.

The complexing behavior of the polymer solutions was determined by studying the turbidity of PMAA, PDEAM, and PDEAM/PMAA complex system solutions over a wide pH range, from pH 3.5 to 10. Fig. 1 depicts that the transmittances for PMAA, PDEAM, and PMAA–PDEAM (1:1, in residue unit) solutions, respectively, at a polymer concentration of 0.1 wt.%, were measured as a function of pH. With reference

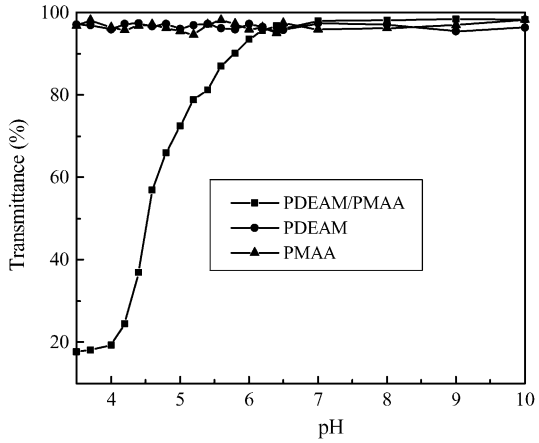


Fig. 1. Plots of transmittance (%) against pH in PMAA, PDEAM, and PMAA–PDEAM aqueous solutions (0.1 wt.%, for PMAA, PDEAM, or PMAA–PDEAM).

to Fig. 1, it reveals that the transmittance of PMAA solution is almost pH independent. This result can be understood by considering the fact that the hydrophobic interaction introduced by the methyl groups along the polymer backbone at lower pH will dominate PMAA conformational behavior and PMAA will form many hydrophobic microdomains under this hydrophobic interaction at pH lower 6.5. The hydrophobic interaction makes PMAA to adopt hypercoiled conformation at lower pH and not to form intermolecular association through hydrogen bonding. So, the transmittances of PMAA solution are pH independent. For PDEAM solution, its transmittance is also pH independent, indicating that the polymer may adopt relatively open coil conformation within the wide pH range studied. It is easy to understand that the PDEAM is a temperature sensitive polymer and has no base or carboxylic acid groups along the polymer backbone, so, the intermolecular association of PDEAM solution through hydrogen bonding at lower pH does not occur. But, compared with transmittances of PMAA, PDEAM solutions, with reference to Fig. 1, there is a transition in transmittances of PMAA–PDEAM complex system solution at pH lower than 6.5. Clearly, the decrease in transmittances of PMAA–PDEAM complex system solution can be attributed to the complexation of PMAA with PDEAM. Compared the transmittances of PMAA–PDEAM complex system with the PMAA system or the PDEAM system at pH higher 6.5, their transmittances have no much different, indicating that there is no complexation between the two polymers in this pH range. This result can be understood by considering the fact that at pH higher 7 [12], PMAA exists as polyanions, and the negatively charged structure is unfavorable for the H-bonding formation and for the complexation between the two polymers. At pH lower than 6.5, the carboxylic acid groups along the PMAA backbone exists as polyacids partially, and the lower the solution pH is, the more the content of the non-ionized carboxylic acid groups along the PMAA backbone is. For PMAA, the non-charged structure is favorable for the H-bonding formation and

complexation between PMAA and PDEAM. In order to further confirm the complexation between PMAA and PDEAM described above, some fluorescence probe solubilization experiments were conducted.

### 3.2. Probe studies

It is to be expected that the tightly coiled conformation of PMAA might favor solubilization of organic guests into its hydrophobic microdomains. Therefore, fluorescence probe studies should be useful in investigating the effects of complexation upon the conformational behavior of PMAA. The probe used in the current study is Py. Py was used because the fine structure of its fluorescence emission spectrum, especially the $I_3$ (383 nm)/$I_1$ (373 nm) value is highly sensitive to the changes in the polarity of its microenvironment [16,17]. The larger $I_3/I_1$ value indicates a more hydrophobic environment. This property has been widely used to monitor the conformational behavior of watersoluble polymers in aqueous phase [18].

Considering the effect of complexation upon conformational behavior of PMAA, the determination of the complexation between PMAA and PDEAM is focused on the effect of complexation upon the polarity of Py microenvironment, namely the $I_3/I_1$ values for Py. Fig. 2, the fluorescence emission spectra of Py ($10^{-6}$ mol $L^{-1}$) dispersed in PMAA ($2.8 \times 10^{-5}$ mol%, in reside unit) and PMAA–PDEAM (1:1, $2.8 \times 10^{-5}$ mol%, in reside unit) aqueous solution at pH 4.0 respectively, shows the effect of complexation upon the fine structures of the fluorescence emission spectra of Py and it reveals that complexation between PMAA and PDEAM was accompanied by PMAA conformational change as proved by the decrease in the hydrophobicity of the environment of the probe. The $I_3/I_1$ values for Py ($10^{-6}$ mol $L^{-1}$) solubilized in PMAA ($2.8 \times 10^{-5}$ mol%, in reside unit), PDEAM ($2.8 \times 10^{-5}$ mol%, in reside unit), and PMAA–PDEAM (1:1, $2.8 \times 10^{-5}$ mol%, in reside unit) complex system aqueous solutions, respectively, measured as a function of pH. The results

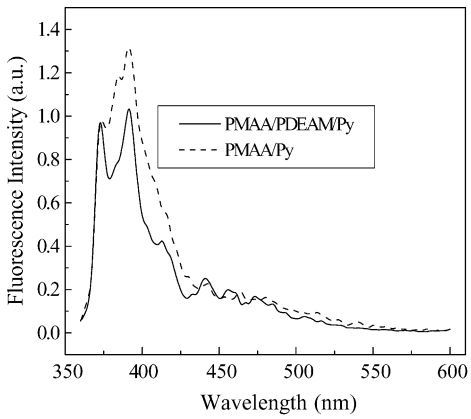


Fig. 2. Fluorescence emission spectra of Py ($10^{-6}$ mol $L^{-1}$) dispersed in PMAA ($2.8 \times 10^{-5}$ mol%, in reside unit) and PMAA–PDEAM (1:1, $2.8 \times 10^{-5}$ mol%, in reside unit) aqueous solution at pH 4.0, respectively.

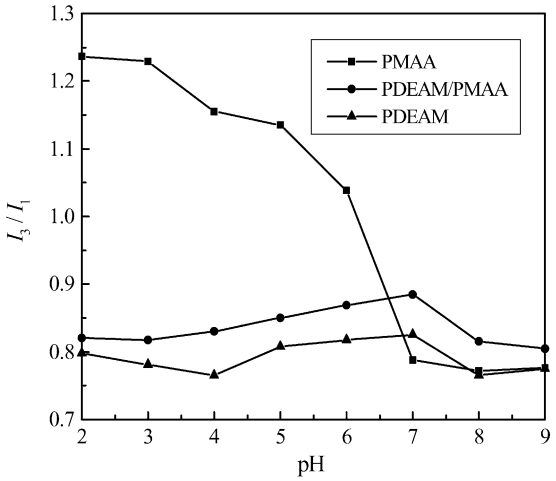


Fig. 3. Plots of $I_3/I_1$ values for Py against pH in PMAA, PDEAM, and PMAA–PDEAM aqueous solutions ($2.8 \times 10^{-5}$ mol%, for PMAA, PDEAM, or PMAA–PDEAM, and $10^{-6}$ mol $L^{-1}$, for Py).

are shown in Fig. 3. The Fig. 3 reveals that, for the PMAA/Py system, there is a transition in its $I_3/I_1$ values between pH 5 and 7, which corresponds to the PMAA conformational transition from hypercoiled to extended coil structure. The larger $I_3/I_1$ values for Py at lower pH are likely to be a consequence of PMAA hypercoiled structure. In contrast, the $I_3/I_1$values for PMAA/Py at higher pH should be smaller. However, for the PDEAM/Py system, the $I_3/I_1$ values for Py (with reference to Fig. 3) are significantly lower than that of the corresponding PMAA/Py system at pH lower 6.5 and is pH almost independent, indicating that the PDEAM may adopt relatively open coil conformation within the wide pH range studied. For the PMAA–PDEAM/Py complex system, the $I_3/I_1$ value at pH lower than 6.5 is also much lower than that of the corresponding PMAA/Py system. Clearly, for the PMAA–PDEAM/Py complex system, the decrease in $I_3/I_1$ value may be attributed to the complexation of PMAA with PDEAM at pH lower than 6.5. At pH higher than 6.5, there is no complexation between the two polymers, because at pH higher than 6.5, PMAA exists as polyanions and the negatively charged structure is unfavorable for the H-bonding formation between the two polymers and unfavorable for the complexation between the two polymers.

Fig. 4 presents results for the complex system at different PMAA to PDEAM ratios $x_{MAA}$ ($x_{MAA} = n_{MAA}/(n_{MAA} + n_{DEAM})$) at pH 4.0. With reference to the figure, it reveals that when the $x_{MAA}$ is lower than 0.5, the $I_3/I_1$ value for Py in the complex system basically maintains a lower constant, ca. 0.7488 ($x_{MAA} = 0$) and ca. 0.7568 ($x_{MAA} = 0.5$) and when the $x_{MAA}$ is higher than 0.5, the $I_3/I_1$ values for Py gradually increases with further increasing $x_{MAA}$. Obviously, this is a result of complexation between PMAA and PDEAM. When $x_{MAA}$ is lower than 0.5, the $I_3/I_1$ values for Py are smaller because the complexation between PMAA and PDEAM destroyed hydrophobic microdomains within PMAA. When the $x_{MAA}$ is higher than 0.5, the $I_3/I_1$ values

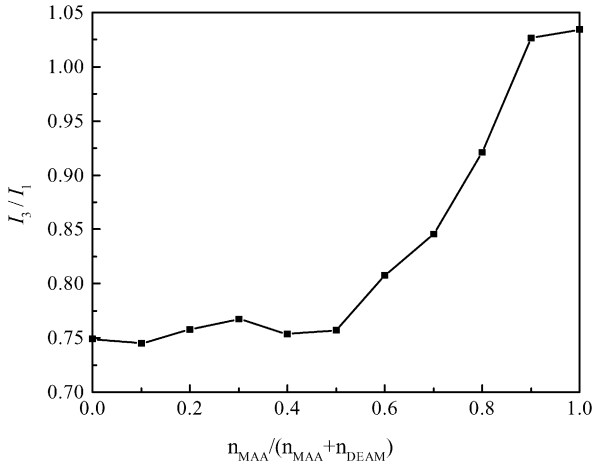


Fig. 4. Plots of $I_3/I_1$ values for Py against $x_{MAA}$ in PMAA–PDEAM (1:1, $2.8 \times 10^{-5}$ mol%, in residue unit, for either PMAA or PDEAM) aqueous solutions at pH 4.0.

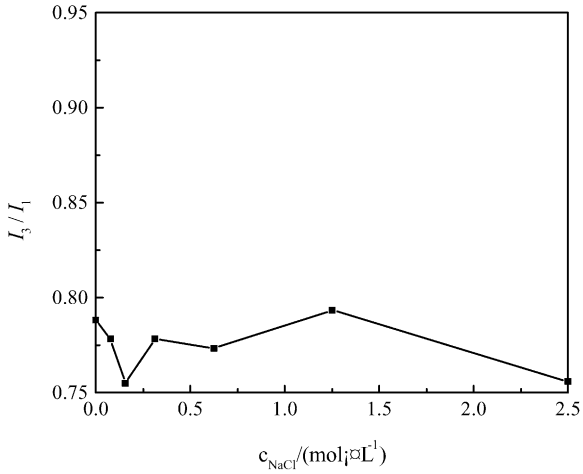


Fig. 5. Plots of $I_3/I_1$ values for Py probe against NaCl concentration in PMAA–PDEAM (1:1, $2.8 \times 10^{-5}$ mol%, in residue unit, for either PMAA or PDEAM) aqueous solutions at pH 4.0.

for PMAA–PDEAM/Py complex system begin to increase progressively from 0.7568 to 1.0345 with increasing $x_{MAA}$ from 0.5 to1.0, indicating hydrophobic microdomains of PMAA gradually form because of excessive PMAA. Above results indicates that the complexation occurs most effectively at $x_{MAA}$ about 0.5, namely at a ratio of about 1:1 (PMAA:PDEAM, in residue unit). This result can be understood by considering the fact that PDEAM is a typical H-bond acceptor and PMAA a H-bond donor because of the amide group in DEAM and carboxyl group in MAA. Each residue unit in PDEAM or in PMAA only has one functional group. Because all of the interaction sites are equally accessible and can participate in the complexation, the ideal complexation is complete at stoichiometric ratio of about 1:1 (PMAA:PDEAM, in residue unit), namely $x_{MAA}$ 0.5. When the $x_{MAA}$ exceeds 0.5, because of excessive PMAA, the hydrophobic microdomains of complex system gradually form, and the $I_3/I_1$ values for Py also progressively increase with increasing PMAA content. The higher the PMAA concentration is in complex system at pH 4.0, the more the hydrophobic microdomains are, and the greater the $I_3/I_1$ values for Py are also.

### 3.3. Nature of complexation

In order to gain further understanding of the nature of the cpmplexation between PMAA and PDEAM, an experiment was undertaken to study the effect of NaCl on the interaction. Fig. 5 depicts the $I_3/I_1$ values for PMAA–PDEAM/Py complex system at pH 4.0 as a function of NaCl concentration. Inspection of the figure reveals that the $I_3/I_1$ values for the complex system do not change very much with increasing NaCl concentration. Furthermore, the $I_3/I_1$ values for PMAA–PDEAM/Py complex system in NaCl solution at pH 4.0 are also significantly lower than that for PMAA/Py system. Therefore, the nature of the complexation between PMAA and PDEAM may be not electrostatic attraction, which was commonly found in other polyelectrolyte complex systems [14,19], but most likely hydrogen-bonds formation.

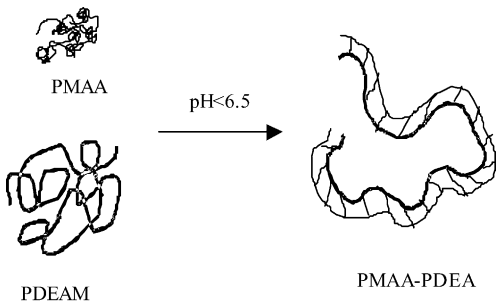


Fig. 6. Schematic diagram of the model for the structure of PMAA–PDEAM.

Considering the “compact micelle-like structure” for PMAA at low pH [20] and the experimental results described above, it might be reasonable to propose a “ladder model” for the structure of the PMAA–PDEAM complex (ref. Fig. 6). In the model, it was suppose that the two polymer chains would be connected by hydrogen bonding. With this model it should have no difficult to explain all the results described above.

## 4. Conclusion

UV–vis and fluorescence probe studies show that interpolymer complexation between PMAA and PDEAM is both pH and molar ratio dependent and a major conformational change occurs when PMAA is mixed with PDEAM in aqueous phase at pH lower than 6.5. The conformational change of PMAA from hypercoiled to loose coiled form is evidenced by the decrease in the hydrophonic microdomain size or domain number. At pH 4.0, the complexation occurs most efficiently at $x_{MAA}$ 0.5, namely at a molar ratio of about 1:1 (PMAA:PDEAM, in residue unit), suggesting that almost all

of the segments of PMAA have taken part in the complexation. Introduction of NaCl has little effect upon complexation between PMAA and PDEAM showing that the nature of the complexation is hydrogen bonding and non-Coulombic interaction. Based upon these conclusions and the "compact micelle-like structure" for PMAA at low pH, a "ladder model" was proposed for the structure of PMAA–PDEAM complex formed at low pH.

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