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# Water-Soluble Copolymers. 36. Photophysical Investigations of Water-Soluble Copolymers of 2-(1-Naphthylacetamido)ethylacrylamide

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ABSTRACT: A hydrolytically stable monomer, 2-(1-naphthylacetamido)ethylacrylamide (NAEAm), has been synthesized to utilize as a label in photophysical studies of copolymers with methacrylic and acrylic acid. An increase in excimer emission relative to monomer emission ($I_E/I_M$) is observed upon increasing solution pH. The hydrophobic character of both the naphthyl chromophores and the pendent methyl groups of MAA and the effective decoupling of the naphthyl chromophores from the polymer backbone via a spacer linkage are responsible for this behavior. The effects of NaCl, urea, and Triton X-100 surfactant on solution properties indicate that hydrophobic interactions among naphthyl chromophores are responsible for formation of an intramolecular "hypercoil" at high pH. Fluorescence measurements confirm the importance of coil dimensions and ion binding on quenching by nitromethane and copper(II) ions.

## Introduction

The covalent attachment of hydrophobic chromophores such as naphthalene and pyrene to polyelectrolytes can result in microheterogeneous water-soluble systems that are in some respects micellelike. The chromophores within these copolymers may be protected from the bulk solution via hydrophobic self-association,[1-4] and, in some instances, large hydrophobic compounds may be solubilized within such microenvironments.[5,6] The photophysical behavior of pendent chromophores is dependent on whether the polymer coil is expanded or micellelike. For example, excimer formation in amphiphilic polyelectrolytes is dependent on the degree of ionization of the polymer (pH of the solution).[7] Generally, as the degree of ionization (pH) increases, segmental Coulombic repulsions cause the polymer coil to expand, increasing the separation between chromophores and decreasing the degree of excimer formation. However, we have recently reported an amphiphilic copolyelectrolyte in which expansion of the polymer coil with increasing pH causes an increase in excimer intensity.[8] We have proposed that this behavior is due, at least in part, to the "decoupling" of the chromophore from the polymer backbone by a relatively long, flexible spacer group. Unfortunately, inherent hydrolytic instability of the chromophore[9] hindered further investigations of this system.

This paper reports the synthesis of a hydrolytically stable analogue of the previously studied chromophore, copolymerization with both acrylic and methacrylic acid, and initial studies of the photophysical properties of the resulting water-soluble copolymers. The goals of this work are (1) to understand how changes in polymer conformation affect the environment of the chromophore and (2) to elucidate the effect of chromophore concentration on polymer conformation.

## Experimental Section

**Materials.** All chemicals used in the preparation of the monomers, polymers, and model compound were purchased from Aldrich Chemical Co. and were purified using previously reported methods.[10] Reagent-grade solvents were used without further purification, with the exception of *N,N*-dimethylformamide (DMF), which was dried over 4A molecular sieves and distilled under reduced pressure.

**Instrumentation.** The UV absorption spectra of the samples were measured using a Perkin-Elmer Lamda 6 spectrophotometer.

IR spectra were recorded on a Perkin-Elmer 1600 FTIR spectrophotometer.

NMR spectra were recorded with a Bruker AC-300 spectrometer.

Steady-state fluorescence spectra of the polymer solutions were measured at room temperature with a SPEX Fluorolog-2 fluorescence spectrometer equipped with a DM3000F data station.

Fluorescence decay curves were measured with a Photochemical Research Associates single-photon-counting instrument. The nonlinear iterative deconvolution technique was used to fit the decay profiles.

**Synthesis of 2-(1-Naphthylacetamido)ethylacrylamide (NAEAm).** 2-(1-Naphthylacetamido)ethylacrylamide (NAEAm) was prepared from 1-naphthylacetic acid (NAA) in a three-step process. The first step involved the formation of the activated succinimide ester of NAA (NAA–NHS) (Scheme I), the second, monoacylation of ethylenediamine with NAA–NHS (NAA–EDA) (Scheme II), and the third, the preparation of 2-(1-naphthylacetamido)ethylacrylamide (NAEAm) (Scheme III).

1-Naphthylacetic acid (NAA; 5.00 g, 27.0 mmol) and *N*-hydroxysuccinimide (NHS; 3.10 g, 27.0 mmol) were slurried in 60 mL of $CHCl_3$ at room temperature. Dicyclohexylcarbodiimide (DCC; 5.58 g, 27.0 mmol), a coupling agent, in $CHCl_3$ (20 mL) was added slowly to this suspension with rapid stirring. Addition of DCC gradually solubilized *N*-hydroxysuccinimide. Upon complete dissolution of NHS, a white precipitate (DCC–urea) was immediately formed. After addition of DCC was complete, the reaction was allowed to continue for 1 h. The product was filtered, and the filtrate, analyzed via thin-layer chromatography (silica, $CH_3CN$ eluent). Near-quantitative conversion of the acid to the succinimide ester (NAA–NHS) was observed.

2-(1-Naphthylacetamido)ethylenediamine (NAA–EDA) was prepared via modification of a high-dilution procedure for the monosubstitution of symmetrical diamines with acid derivatives.[11] NAA–NHS (7.64 g, 27.0 mmol) was diluted with $CHCl_3$ to a volume of 350 mL and was added dropwise with vigorous stirring to a solution of ethylenediamine (19.45 g, 324 mmol) and $CHCl_3$ (50 mL) at 0 °C. The reaction was allowed to continue at room temperature overnight. The solution was filtered, concentrated under vacuum to approximately 75 mL, washed four times with a 10% sodium chloride solution (35 mL), and dried over magnesium sulfate. Thin-layer chromatography (silica, $CH_3CN$ eluent) indicated that the primary reaction product was the monoacylated derivative, with only a trace amount of the disubstituted product. For the subsequent reaction, the amount

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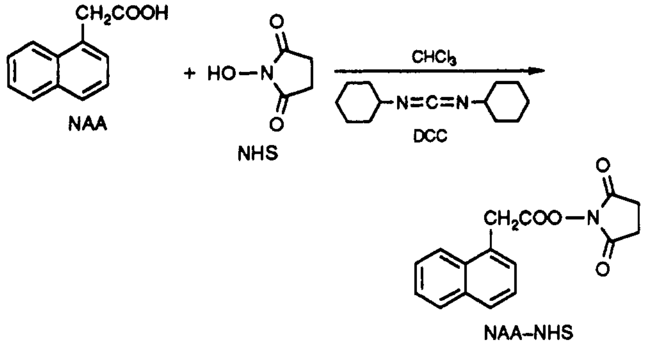
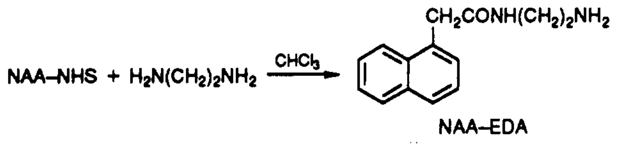
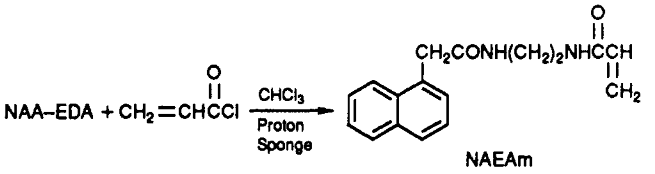
### Scheme I



### Scheme II



### Scheme III



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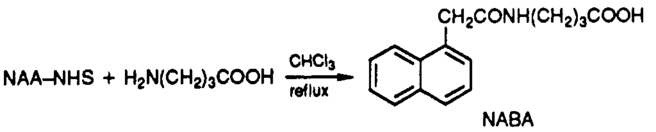


of NAA–EDA in the $CHCl_3$ solution was determined via UV absorbance measurements to be 21.5 mmol (79.6% yield).

"Proton Sponge" [1,8-bis(dimethylamino)naphthalene; 4.6 g, 21.5 mmol] was added to the NAA–EDA solution, which had been cooled to 0 °C. A solution of acryloyl chloride (1.94 g, 21.5 mmol) in $CHCl_3$ was added dropwise, keeping the temperature below 5 °C. Upon complete addition of acryloyl chloride, the reaction was allowed to proceed at room temperature overnight. The resulting solution was washed three times with 10% HCl (25 mL) and two times with DI water (25 mL). The organic layer was dried over magnesium sulfate and filtered, and the solvent, evaporated. The resulting solid was recrystallized three times from a mixture of $CH_3OH$ and $CHCl_3$ to yield 3.38 g (44.3%) of a white crystalline solid, mp 199–200 °C. FTIR (KBr): NH, 3289, aromatic CH, 3080; aliphatic CH, 2979 and 2944, C═O, 1648 and 1625 $cm^{-1}$. $^{13}C$ NMR: C═O (naphthylacetamido), 172.6; C═O (acrylamido), 166.8; aromatic and vinyl C═C 133.4–122.9; $-CH_2-$ (naphthyl), 40.2; $-CH_2-$ (ethylene spacer) 38.8 and 38.5 ppm. The purity of the compound (99.8%) was determined by HPLC.

**Synthesis of 3-(1-Naphthylacetamido)butanoic Acid (NABA).** A suspension of NAA–NHS (3.25 g, 11.5 mmol), 4-aminobutyric acid (2.37 g, 23.0 mmol), and $CHCl_3$ (50 mL) was heated to reflux for 24 h. The orange solution was washed two times with 5% HCl. After the second washing, a precipitate formed in the organic phase. This precipitate was washed with ether and dried to give 1.16 g (37.0%) of a powdery white solid, mp 120–122 °C. FTIR (KBr): OH (broad), 3448; NH, 3300; aromatic CH, 3085 and 3070; aliphatic CH, 2082 and 2051; C═O, 1702 and 1630 $cm^{-1}$. $^{13}C$ NMR: C═O (acid), 175.1; C═O (naphthylacetamido), 172.0; aromatic and vinyl C═C, 133.3–122.8; $CH_2$ (naphthyl), 39.7; $-NHCH_2CH_2CH_2COOH$, 38.2; $-NHCH_2CH_2CH_2COOH$, 23.8; $-NHCH_2CH_2CH_2COOH$, 30.4 ppm. Purity of the compound (99.9%) was determined by HPLC.

**Synthesis of Copolymers of NAEAm.** Copolymers were prepared by free-radical polymerization in purified, degassed DMF at 65 °C by using AIBN as the initiator species. After 4 h, the polymers were precipitated into ethyl acetate. Subsequent purification involved dissolving the polymers in DMF, reprecipitating into ethyl acetate, washing with ethyl ether, and drying under vacuum. Residual monomer was found to be <0.1 mol % of attached chromophore via a dialysis/HPLC technique.

Table I
Composition and Molecular Weight of NAEAm Copolymers

| | NAEAm, mol % | $M_n \times 10^{-5}$ [a] |
|---|---|---|
| NAEAm(1)AA | 0.90 | 2.49 |
| NAEAm(1)MAA | 1.02 | 2.31 |
| NAEAm(10)AA | 9.30 | 1.72 |
| NAEAm(10)MAA | 11.80 | 1.65 |

[a] Determined via membrane osmometry.

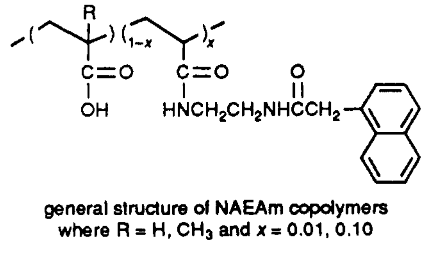
By utilization of the extinction coefficient determined for the model compound NABA in DMF ($\epsilon$ = 6687 L $mol^{-1}$ $cm^{-1}$ at 280 nm), the compositions of the copolymers were determined via UV spectroscopy. The details of the copolymers prepared are summarized in Table I.

**Methods.** Unless otherwise specified, polymer concentrations were such that the concentration of NAEAm in solution was $10^{-4}$ mol/L. Solutions were typically deaerated by $N_2$ bubbling. Samples were excited at 280 nm, and emissions at 330 nm for monomer and 420 nm for excimer were measured.

## Results and Discussion

**Synthesis and Characterization of NAEAm and Copolymers.** Recent studies in our laboratories of a novel amphiphilic polyelectrolyte[8] have indicated that, among other factors, the effective "decoupling" of the hydrophobic chromophore from the hydrophilic polymer backbone plays an important role in domain ordering and the observed photophysical properties. However, further investigations of this phenomenon required that a chromophore with long-term hydrolytic stability be prepared. Therefore, with use of a modified technique for the monosubstitution of symmetrical diamines, the hydrolytically stable 2-(1-naphthylacetamido)ethylacrylamide (NAEAm) monomer was synthesized.

An interesting feature of this synthesis is that it is possible to use diamines of varying lengths to investigate the effect of the spacer group on the photophysical properties of the polymer in solution. Furthermore, model compound studies indicate that substitution of the hydrolytically stable amide bond for the previously utilized hydrolytically unstable ester bond does not affect the photophysical properties of the chromophore (steady-state or transient), presumably due to an insulating effect provided by the methylene group between the amide bond and the naphthyl group. Finally, this synthetic technique may permit the conversion of a number of traditional probe molecules into free-radically polymerizable labels.



general structure of NAEAm copolymers
where R = H, $CH_3$ and $x$ = 0.01, 0.10

Copolymers of NAEAm with both acrylic acid (AA) and methacrylic acid (MAA) (Table I) were prepared by free-radical polymerization in *N,N*-dimethylformamide (DMF). Copolymers containing approximately 10.0 mol % NAEAm were prepared to eludicate the effect that higher degrees of incorporation of the chromophore have on solution properties. NAEAm–AA and NAEAm–MAA copolymers containing approximately 1.0 mol % NAEAm were also synthesized in order to determine the effect, if any, of comonomer on the polymer conformation and subsequently on the photophysical characteristics of the chromophore.

It has been demonstrated by a number of workers that the manner in which the chromophore (label) is incorporated into the copolymer, i.e., copolymer microstructure, is also a determining factor in the observed solution photoproperties.[7,12,13] Monomer reactivity ratios, which would provide a quantitative description of copolymer microstructures, have not as yet been determined for these comonomers; however, a qualitative assessment of monomer reactivities may be inferred through comparison of copolymerization monomer feed compositions with copolymer compositions as determined via UV absorption methods. In the synthesis of NAEAm(10)AA, an initial monomer feed composition of 15 mol % NAEAm yielded a copolymer that contained 9.8 mol % chromophore. A feed composition of 30 mol % NAEAm and 70 mol % MAA resulted in a copolymer (NAEAm(10)MAA) that contained only 11.0 mol % NAEAm, indicating a low reactivity of NAEAm with these comonomers. In solution polymerization, therefore, we expect that individual NAEAm chromophores may be separated along the polymer backbone by relatively long intervening sequences of AA or MAA mers, minimizing the number of nearest-neighbor chromophore interactions.

**Photophysical Characteristics of NAEAm Copolymers: Steady-State Emission Spectra.** Steady-state emission spectra of the copolymers of NAEAm with MAA and AA were measured both in DMF and in water at different values of pH at 25 °C (Figure 1). It is important to point out that, in each case, an increase in red-shifted emission attributable to excimer formation was observed upon changing from DMF to aqueous solutions; this is particularly noticeable for NAEAm(10)AA and NAEAm(10)MAA. (Note that, in aqueous media, a small, but measurable, emission from an excimer species is recorded.) The strong emission from an excimeric complex is believed to be due to hydrophobic interactions between naphthyl chromophores in the aqueous medium, which have been shown to occur even at low (<1.5 mol %) degrees of incorporation of chromophore in the copolymer.[14] Furthermore, the low degree of excimer formation in DMF suggests that, in aqueous solutions, non-nearest-neighbor interactions are primarily responsible for excimer formation.

Figure 2 depicts the dependence of the relative excimer (measurement taken at 420 nm) to monomer (measurement at 330 nm) emission intensity, expressed as the ratio $I_E/I_M$, on solution pH for both NAEAm(10)MAA and NAEAm(1)MAA. Interestingly, for both polymers, a decrease in $I_E/I_M$ with decreasing pH is observed.

It is well understood that polymers of methacrylic acid undergo a nonuniform coil transition with changes in pH.[15-20] This nonuniformity has been attributed to hydrophobic associations that occur between pendent methyl groups as solution pH is lowered. Due to these interactions, poly(methacrylic acid) forms a very compact coil at low pH, the interior of which is both relatively hydrophobic and rigid. We have suggested[8] that the formation of a similar collapsed structure in NAEA/MAA copolymer is responsible for the decrease in $I_E/I_M$ with decreasing pH. Collapse of the polymer coil may alter the spatial orientation and/or mobility of the naphthalene groups (see ref 13 for a discussion of similar observations), thereby lowering the probability of forming the sandwich conformation needed for excimer formation. Alternatively, the interior of the collapsed polymer coil at low pH would not be expected to provide a driving force for the exclusive hydrophobic association of chromophores since

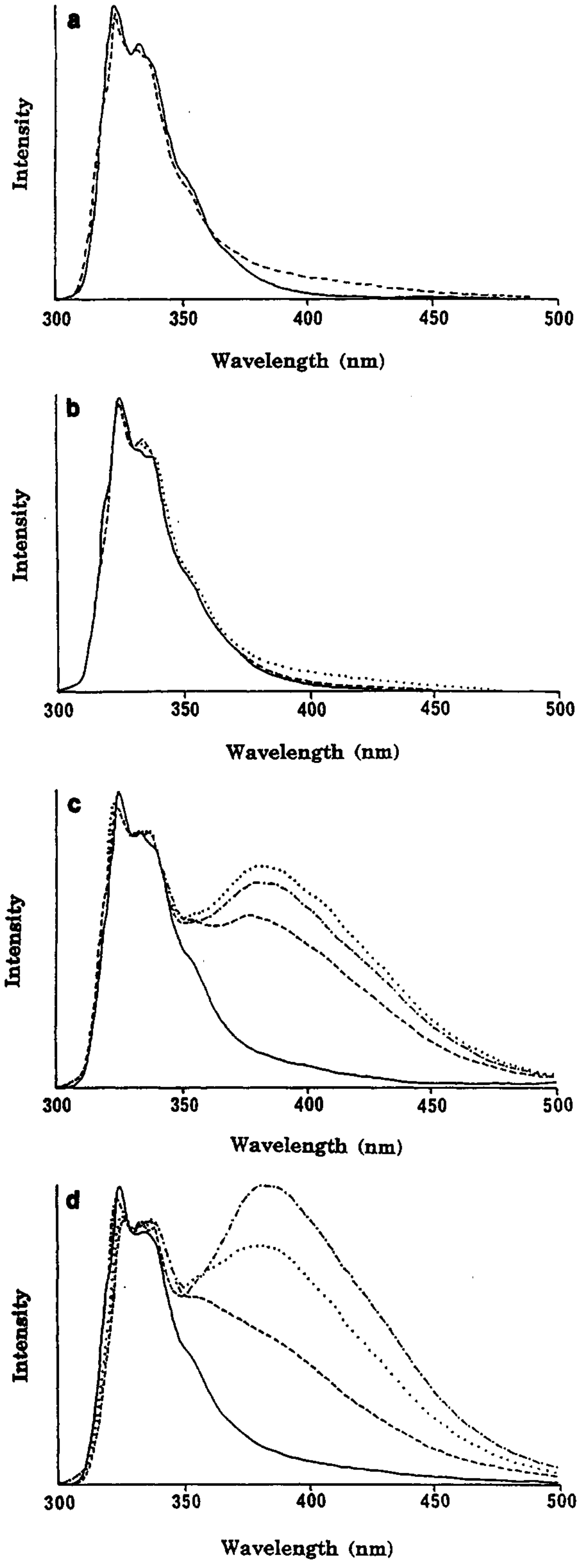


**Figure 1.** Steady-state fluorescence spectra ($\lambda_{ex}$ = 280 nm) of NAEAm copolymers in aqueous solutions at different values of pH and in *N,N*-dimethylformamide (DMF). $C_{NAEAm}$ = 0.1 mmol/L. (a) NAEAm(1)AA (- - -), pH 5.0; (—), DMF. (b) NAEAm(1)MAA (· · ·), pH 8.0; (- - -), pH. 5.0; (—), DMF. (c) NAEAm(10)AA (- - -), pH 8.0; (· · ·), pH 6.5; (- - -), pH 4.0; (—), DMF. (d) NAEAm(10)MAA (- · -), pH 8.0; (· · ·), pH. 6.5; (- - -), pH 5.0; (—), DMF.

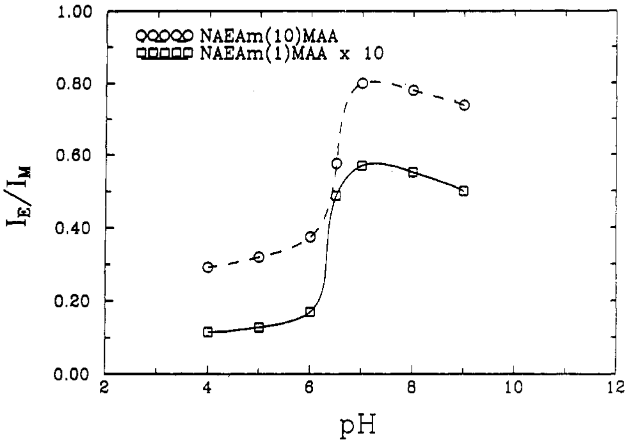


**Figure 2.** Dependence of the relative efficiency of excimer formation ($I_E/I_M$) in NAEAm(1)MAA and NAEAm(10)MAA copolymers on solution pH. $C_{\mathrm{NAEAm}}$ = 0.1 mmol/L.

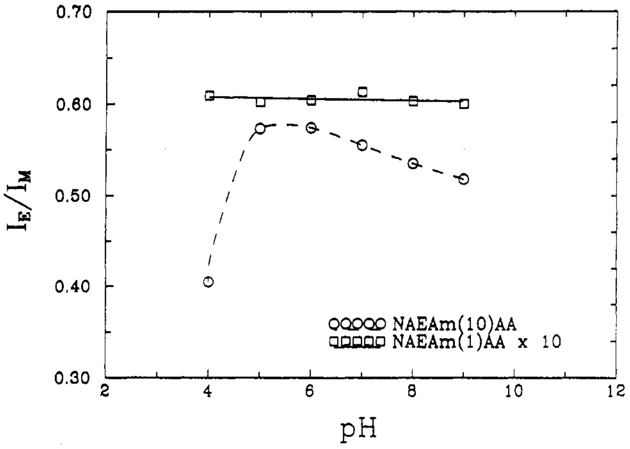


**Figure 3.** Dependence of the relative efficiency formation ($I_E/I_M$) in NAEAm(1)AA and NAEAm(10)AA copolymers on solution pH. $C_{\mathrm{NAEAm}}$ = 0.1 mmol/L.

some methyl groups and backbone elements would be present also.

$I_E/I_M$ values for two polymers of different compositions, NAEAm(10)MAA and NAEAm(1)MAA, suggest that methyl group interactions at low pH are in fact responsible for the observed decreases in excimer formation with decreasing pH.

By contrast, the pH dependence of $I_E/I_M$ for the acrylic acid copolymers, NAEAm(10)AA and NAEAm(1)AA, is depicted in Figure 3. The absence of any appreciable change in excimer intensity for the NAEAm(1)AA copolymer gives credence to the argument that hydrophobic methyl group interactions contribute significantly in the methacrylic acid copolymer at low pH.

After a slight initial increase in excimer intensity, believed to be due to the decreasing degree of ionization, NAEAm(10)AA also undergoes a decrease in $I_E/I_M$ with decreasing pH. It is important to point out that the major transition for NAEAm(10)AA occurs some 2 pH units below those observed for the methacrylic acid copolymers. We surmise that for NAEAm(10)AA the decrease in excimer intensity with decreasing pH is due to the decreased ionization associated with a pH below the p$K$ of poly(acrylic acid), approximately 4.8.[21] Once NAEAm(10)AA falls below this critical degree of ionization, the hydrophobic naphthyl groups may cause the coil to collapse in much the same fashion as do the methyl groups in methacrylic acid polymers.

At high pH in dilute solution, the associations between naphthyl chromophores are intramolecular in nature. More specifically, NAEAm(10)MAA and NAEAm(10)AA may be described by the pseudomicellar model proposed by Guillet et al.[22] in which the naphthyl groups form a hydrophobic core that is surrounded by a hydrophilic shell of carboxylate anions. Both NAEAm(10)AA and NAEAm(10)MAA (Figures 2 and 3) exhibit maxima in $I_E/I_M$ at pH values of 5 and 7, respectively, indicating closest proximity of the naphthyl chromophores. At higher pH, no further ionization of the polymer coil occurs; however, added ions screen repulsive backbone interactions, allowing some disruption of intramolecular naphthyl interactions and thus lowering slightly $I_E/I_M$ values. Apparently, NAEAm(1)AA, with no methyl and very few naphthyl hydrophobic interactions, is unaffected above pH 5 (Figure 3).

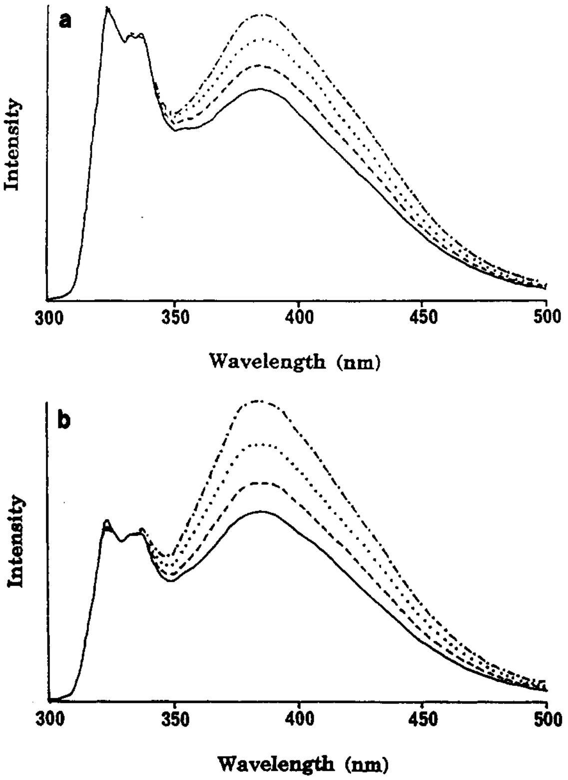


**Figure 4.** Effect of NaCl on the steady-state fluorescence spectra of copolymers containing approximately 10 mol % NAEAm: (a) NAEAm(10)AA, (b) NAEAm(10)MAA. NaCl concentrations: (—), 0%; (- - -), 2.0%; (· · ·), 4.0%; (- · -), 6.0%. $C_{\mathrm{NAEAm}}$ = 0.1 mmol/L.

The effect of ionic strength changes was further investigated (Figure 4) by studying the steady-state emission spectra of the copolymers containing approximately 10% NAEAm at pH 8.0. The spectra are normalized at the wavelength of monomer emission (330 nm). Each copolymer exhibits a discernable increase in excimer intensity with increasing NaCl concentration. For these copolyelectrolytes, an increase in sodium chloride concentration is accompanied by a decrease in the hydrodynamic size of the coil. Collapse of the hydrated coil and an increase of the polarity of the medium increase the number of favorable interactions among naphthyl chromophores, thereby producing an increase in $I_E/I_M$.

Disruption of hydrophobic association of the naphthyl groups might be expected to decrease the likelihood of excimer formation. Urea, a water-structure breaker,[23,24] was utilized to measure this effect. At pH 8.0, a significant decrease in excimer intensity is observed upon addition of urea to solutions of NAEAm(10)MAA and NAEAm(10)AA (Figure 5). Since urea was found not to quench the fluorescence of the model compound NABA, this

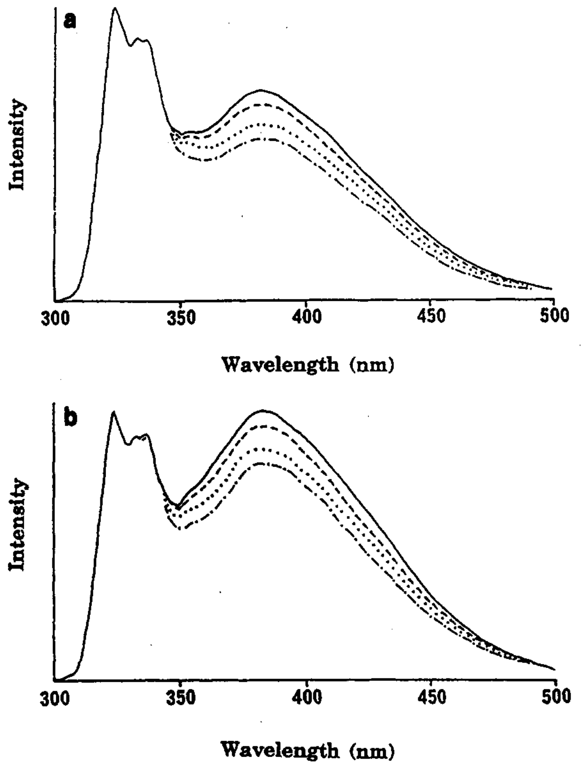


**Figure 5.** Effect of urea on the steady-state fluorescence spectra of copolymers containing approximately 10 mol % NAEAm: (a) NAEAm(10)AA, (b) NAEAm(10)MAA. Urea concentrations: (—), 0 M; (- - -), 1.5 M; (· · ·), 3.0 M; (- · -), 4.5 M. $C_{NAEAm}$ = 0.1 mmol/L.

decrease is attributed to the effective solubilization of the hydrophobic naphthyl groups by the urea. Interestingly, addition of the nonionic polyol surfactant Triton X-100 (reduced) to the polymer solutions provides a similar decrease in excimer intensity (Figure 6). However, rather than disrupting hydrophobic interactions altogether as does the urea, the surfactant provides a greater number of hydrophobic groups with which the naphthyl chromophores may associate, lessening the likelihood of chromophore interaction.

**Transient Measurements.** Fluorescence decay curves of the NAEAm copolymers and the model compound NABA for both monomer and excimer (when possible) were measured in water at different values of pH by using the single-photon-counting method (Table II). For the copolymers containing 1 mol %, the monomer decay curves could be fit satisfactorily to a double exponential by using eq 1. For NAEAm(10)MAA and NAEAm(10)AA the

$$I(t) = \sum a_i \exp(-t/\tau_i) \tag{1}$$

monomer decays were fit to a triple-exponential function. According to Chang[25] and Bai, Webber, and Chang,[3] it is possible to estimate a composite lifetime in the monomer fluorescence region by calculating the weighted average lifetimes as defined by eq 2.

$$[\tau]_M = \sum a_i\tau_i^2 / \sum a_i\tau_i \tag{2}$$

Exact interpretation of decay results in nonhomogeneous media where numerous species and environments are present is difficult; however, it is worth noting that while the average monomer lifetimes, $[\tau]_M$, of the two acrylic acid copolymers are relatively insensitive to pH, those of the two methacrylic acid copolymers seem to exhibit some dependence on the pH of the solution.

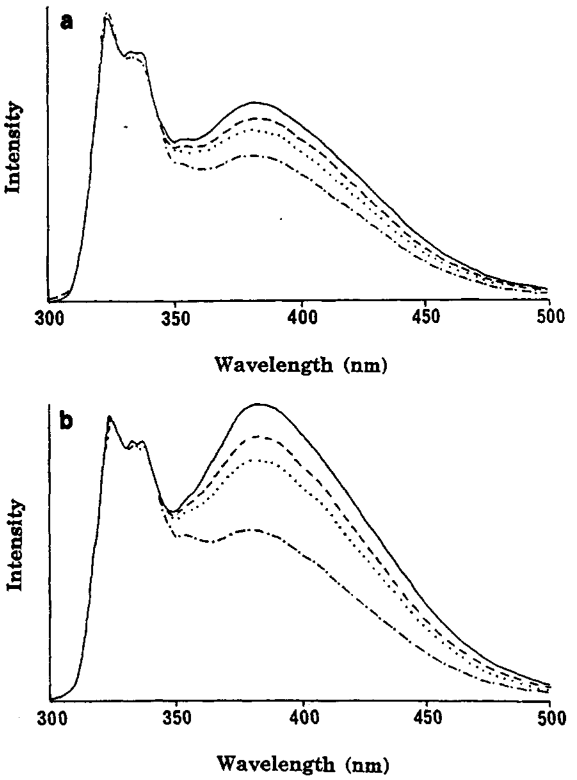


**Figure 6.** Effect of Triton X-100 on the steady-state fluorescence spectra of copolymers containing approximately 10 mol % NAEAm: (a) NAEAm(10)AA, (b) NAEAm(10)MAA. Triton X-100 concentrations: (—), 0 M; (- - -), 1.27 × $10^{-4}$ M; (· · ·), 2.66 × $10^{-4}$ M; (- · -), 5.32 × $10^{-4}$ M. $C_{NAEAm}$ = 0.1 mmol/L.

Interestingly, the lifetime of the model compound NABA in solutions of DMF, 50/50 DMF/water, and water was found to decrease from 51.1 to 43.8 to 32.6 ns, respectively. Obviously, the monomer model is extremely sensitive to water. Perhaps the change in the polarity of the microenvironment of the label associated with the transition of methacrylic acid copolymers from a collapsed to an expanded coil results in a decrease in $[\tau]_M$ with increasing pH.

Another observation concerning the decay data can be made. A distinct rise time is observed in the excimer decays for both NAEAm(10)MAA and NAEAm(10)AA reflected by the negative value for $A_2$ in the double-exponential fit at 420 nm. This suggests that rapid energy transfer to excimer-forming sites does not occur. In addition, no ground-state dimers are detected in the excitation spectrum of the excimer emission at 420 nm. The decay curves were not recorded at 420 nm for NAEAm(1)MAA and NAEAm(1)AA since the emission was too weak.

**Fluorescence Quenching Measurements.** Steady-state quenching of both monomer and excimer fluorescence of the NAEAm copolymers was studied at pH values of 5.0, 6.5, and 8.0. These values were chosen since they span the transition range associated with conformational changes of methacrylic acid copolymers. Two different types of quenchers were chosen for this study: nitromethane, a neutral noninteractive quencher, and copper(II), a cationic quencher, which has been shown to interact strongly with anionic polymers. Apparent second-order quenching rate constants $(k_q)_{app}$ calculated from Stern–Volmer plots of the steady-state fluorescence intensity versus quencher concentration in the monomer region at 330 nm for the NAEAm copolymers and the model compound NABA are summarized in Table III. It

**Table II**
**Fluorescence Decay Parameters for NAEAm Copolymers in Aqueous Solution**

| | | $I_{\mathrm{m}}$[a] | | | | $I_{\mathrm{e}}$[b] | |
|---|---|---|---|---|---|---|---|
| | pH | $\tau_1/a_1$ | $\tau_2/a_2$ | $\tau_3/a_3$ | $[\tau]$[c] | $\tau_1/A_1$ | $\tau_2/A_2$ |
| NABA | 5.0 | 33.63/0.26 | | | 33.63 | | |
| | 6.5 | 32.95/0.24 | | | 32.95 | | |
| | 8.0 | 33.43/0.26 | | | 33.43 | | |
| NAEAm(1)MAA | 5.0 | 14.14/0.13 | 54.78/0.12 | | 52.00 | | |
| | 6.5 | 13.81/0.02 | 40.20/0.11 | | 38.53 | | |
| | 8.0 | 9.92/0.03 | 34.83/0.23 | | 34.01 | | |
| NAEAm(1)AA | 5.0 | 13.05/0.04 | 38.76/0.17 | | 37.07 | | |
| | 6.5 | 14.00/0.04 | 37.12/0.23 | | 35.55 | | |
| | 8.0 | 14.40/0.04 | 35.72/0.24 | | 34.28 | | |
| NAEAm(10)MAA | 5.0 | 6.25/0.04 | 26.46/0.05 | 59.43/0.04 | 44.10 | 6.73/−0.06 | 68.44/0.12 |
| | 6.5 | 4.12/0.11 | 20.89/0.11 | 57.30/0.07 | 41.82 | 5.94/−0.08 | 66.18/0.15 |
| | 8.0 | 7.97/0.17 | 19.92/0.08 | 56.10/0.02 | 25.17 | 6.92/−0.09 | 61.58/0.14 |
| NAEAm(10)AA | 5.0 | 7.26/0.10 | 22.51/0.13 | 54.20/0.04 | 33.37 | 7.60/−0.04 | 63.26/0.09 |
| | 6.5 | 6.44/0.11 | 20.09/0.14 | 50.22/0.06 | 32.00 | 7.82/−0.08 | 60.64/0.14 |
| | 8.0 | 5.98/0.12 | 19.55/0.14 | 47.2/0.07 | 31.45 | 7.19/−0.08 | 57.63/0.14 |

[a] Monomer emission measured at 330 nm. [b] Excimer emission measured at 420 nm. [c] See eq 2 in the text.

**Table III**
**Fluorescence Quenching Rate Constants for NAEAm Copolymers**

| | | $(k_{\mathrm{q}})_{\mathrm{app}}$ | |
|---|---|---|---|
| | pH | $Cu^{2+}$ [a,b] | $CH_3NO_2$ |
| NABA | 5.0 | $1.54 \times 10^{10}$ | $7.31 \times 10^{9}$ |
| | 6.5 | $2.20 \times 10^{10}$ | |
| | 8.0 | $2.62 \times 10^{10}$ | $7.31 \times 10^{9}$ |
| NAEAm(1)AA | 5.0 | $9.84 \times 10^{9}$ | $1.16 \times 10^{9}$ |
| | 6.5 | $9.81 \times 10^{9}$ | |
| | 8.0 | $9.83 \times 10^{9}$ | $1.41 \times 10^{9}$ |
| NAEAm(1)MAA | 5.0 | $1.21 \times 10^{10}$ | $4.58 \times 10^{8}$ |
| | 6.5 | $1.14 \times 10^{10}$ | |
| | 8.0 | $9.21 \times 10^{9}$ | $1.15 \times 10^{9}$ |
| NAEAm(10)AA | 5.0 | $8.48 \times 10^{11}$ | $9.05 \times 10^{8}$ |
| | 6.5 | $5.13 \times 10^{11}$ | |
| | 8.0 | $4.32 \times 10^{11}$ | $1.15 \times 10^{9}$ |
| NAEAm(10)MAA | 5.0 | $2.52 \times 10^{12}$ | $6.58 \times 10^{8}$ |
| | 6.5 | $1.50 \times 10^{12}$ | |
| | 8.0 | $6.67 \times 10^{11}$ | $1.24 \times 10^{9}$ |

[a] Monomer quenching measured at 330 nm. [b] Apparent quenching rate constants as determined from the initial slope of the Stern–Volmer plot.

should be noted that, for the $Cu^{2+}$-quenching studies, the Stern–Volmer plots deviated positively from linearity at higher quencher concentrations. The apparent Stern–Volmer constants and subsequent quenching rate constants were determined from the initial slopes of the plots of $I_0/I$ vs quencher concentration. All of the quenching constants in Table III are subject to the assumption that the individual components are quenched by the same basic kinetic interaction process; this may not be true. However, for purposes of comparison, we proceed with the assumption that the $(k_{\mathrm{q}})_{\mathrm{app}}$ values in Table III are a reasonable estimation of the overlapping quenching efficiency in the monomer region.

For the quenching of the naphthyl group fluorescence by an uncharged quencher (nitromethane), the values of $(k_{\mathrm{q}})_{\mathrm{app}}$ measured for each of the polymer solutions were reduced in comparison to those of the model compound NABA. This effect can be attributed to a decrease in diffusion of $CH_3NO_2$ to the naphthyl chromophore attached to the polymer. Only slight changes in $(k_{\mathrm{q}})_{\mathrm{app}}$ with pH for the acrylic acid copolymers NAEAm(1)AA and NAEAm(10)AA suggest that the respective polymer coils remain expanded to some degree over the pH range investigated. However, the methacrylic acid copolymers exhibit a measurable decrease in $(k_{\mathrm{q}})_{\mathrm{app}}$ as the solution pH is lowered. This indicates that, as the pH decreases, the polymer coil collapses, further hindering the diffusion of the quencher molecule into the polymer coil.

For the quenching of NAEAm with $Cu^{2+}$, one might expect that the quenching efficiency would be affected by the charge density on the polymer coil as well as by conformation. The association of $Cu^{2+}$ with ionized carboxylic acid groups is evidenced by the values for $(k_{\mathrm{q}})_{\mathrm{app}}$, which are higher than those for quenching by nitromethane, thus suggesting that, in each case, $Cu^{2+}$ is bound close to the fluorescing chromophore. The very high quenching efficiency observed for NAEAm(10)AA and NAEAm(10)MAA indicates significant enhancement in quenching due to binding of $Cu^{2+}$ in close proximity to the naphthyl chromophores. Indeed, it has been postulated[7,8] that the efficiency of a quencher capable of interaction with a labeled polymer (e.g., cationic quencher and anionic polymer) may be explained in terms of an effective concentration of quencher within the polymer coil. The effect of pH on quenching of NAEAm(10)AA and NAEAm(10)MAA by $Cu^{2+}$ may be related to a change in the accessibility of naphthyl chromophores to the bound $Cu^{2+}$ cations.

## Conclusions

Photophysical data presented above indicate that the conformations adopted by the NAEAm copolymers in aqueous solution are dependent on the pH of the solution as well as hydrophobic interactions inherent within the polymer. At high pH, NAEAm(10)MAA and NAEAm(10)AA copolymers apparently assume a pseudomicellar conformation, with the naphthyl groups forming a hydrophobic core that is surrounded by a charged shell of methacrylic acid units. The observed decreases in excimer intensity upon the addition of the water-structure breaker urea and the nonionic surfactant Triton X-100 indicate that hydrophobic interactions between naphthyl groups are indeed important in the formation of excimers at high pH. As the pH is lowered, the concomitant decreases in $I_E/I_M$ for NAEAm(10)MAA and NAEAm(1)MAA, the similar decrease in $I_E/I_M$ for NAEAm(10)AA, and the absence of a change in $I_E/I_M$ for NAEAm(10)AA indicate that the presence of hydrophobic interactions, methyl or naphthyl, is responsible for the observed phenomena. We suggest that a "dilution" of NAEAm units within the hydrophobic portion of the coil and/or nonfavorable chromophore orientation due to decreased relative mobility of the chromophores within the collapsed polymer coil are responsible for the observed decreases in excimer formation. Furthermore, the presence of a rise time in the excimer fluorescence decays indicates that rapid energy transfer to excimer-forming sites is not predominant. Finally, fluorescence quenching data indicate that the efficiency of an electrostatically bound quencher such as $Cu^{2+}$ can be related to an effective binding process to the polymer coil.

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