

# Homogeneous Fluorescent Labeling of Cationic Polyacrylamides

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**ABSTRACT:** A number of fluorescently labeled polymers have already been prepared. Since these polymers have usually been synthesized by copolymerization, the distribution of fluorescent groups will in such a case be heterogeneous because monomers always have different reactivity ratios. In this article, the preparation of fluorescently labeled cationic polyacrylamides (C-PAM) with homogeneous distributions of both fluorescent and cationic groups is described. Four fluorescently labeled C-PAM with isoindol, pyrene, anthracene, and dansyl groups were prepared in the homogeneous reaction system. These polymers were water soluble. Their wavelengths for maximum emission were in order of the above 380, 382 (smaller peak at 400), 435, and

540 nm, which thus covered a wide wavelength range. It is noteworthy that C-PAM with pyrene and anthracene groups have little fluorescence intensity at 540 nm, which is the maximum emission wavelength for C-PAM with dansyl group, making an accurate determination of the two polymers in the same solution possible. Even at the low degree of labeling, the detection limit of polymers is very low (approximately 1 ppm). © 2002 Wiley Periodicals, Inc. *J Appl Polym Sci* 86: 672–675, 2002

**Key words:** cationic polyacrylamide; fluorescent labeled polymer; PAM with isoindole; PAM with pyrene; PAM with anthracene; PAM with dansyl group

## INTRODUCTION

In recent years, fluorescent probes have been widely used not only in the field of biochemistry<sup>1</sup> but also in synthetic polymer chemistry.<sup>2,3</sup> The luminiscent techniques have been applied to conformational dynamics in solution,<sup>4–6</sup> measuring the polymer's microenvironmental pH,<sup>7,8</sup> monitoring polymerization reactions,<sup>9,10</sup> charge-transfer exciplex formations,<sup>11,12</sup> surface characterization,<sup>13</sup> polymer relaxation processes,<sup>14,15</sup> diffusion in polymer systems including gels<sup>16</sup> and polymer miscibility.<sup>17</sup>

We have carried out a series of investigation on the behavior of cationic polymers adsorbed onto anionic surfaces of monodisperse polystyrene latex,<sup>18</sup> cellulose beads, and cellulose fibers. The adsorption,<sup>19</sup> competitive adsorption,<sup>20,21</sup> reformation,<sup>22</sup> polymer exchange,<sup>23</sup> polymer transfer,<sup>24,25</sup> cleavage of polymer chains<sup>25</sup> and penetration into pores of cellu-

losic fibers have been studied. In these investigations, two different polymers have often been present, and cationic polyacrylamides with and without fluorescent labeling (dansylation)<sup>26</sup> have been utilized.

Our experiments have mostly been carried out at low electrolyte concentrations. In these cases, it was possible to determine the total amount of cationic polyelectrolyte (labeled and nonlabeled) by polyelectrolyte titration,<sup>27</sup> whereas the fluorescently labeled cationic polyelectrolyte could be determined by fluorescence intensity. The separate determination of labeled and nonlabeled polymer was thus possible. However, polyelectrolyte titration does not work satisfactorily in the presence of inorganic salts. In practical applications of cationic polymers,<sup>28,29</sup> such as wastewater treatment, papermaking, and ore separation, inorganic salts are usually present in concentrations that can lead to serious errors in the titrations. The preparation of fluorescently labeled cationic polyacrylamides (C-PAMs) with different emission wavelengths, which would avoid the need for polyelectrolyte titration, could therefore be a useful technique for investigating polymer adsorption at high electrolyte concentrations.

A number of water-soluble polymers with fluorescent groups, e.g., dansyl,<sup>30–34</sup> pyrene,<sup>35,36</sup> naphthalene,<sup>37</sup> anthracene,<sup>38</sup> and carbozol<sup>39</sup> have already been synthesized. These polymers have usually been pre-

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pared by copolymerization. The distribution of fluorescent groups between the polymer chains will in this case be heterogeneous because individual monomers always have different reactivity ratios.

In this investigation, the preparation of fluorescently labeled C-PAMs with homogeneous distributions of both fluorescent and cationic groups is described. Fluorescence can be detected with very high sensitivity even when the fluorescent probes are incorporated at a very low concentration. This means that the labeling has little effect on the characteristics of the polymer.<sup>19</sup> The method described in this report can be used to provide fluorescently labeled and non-labeled C-PAMs with a wide range of molecular weights and charge densities.

## EXPERIMENTAL

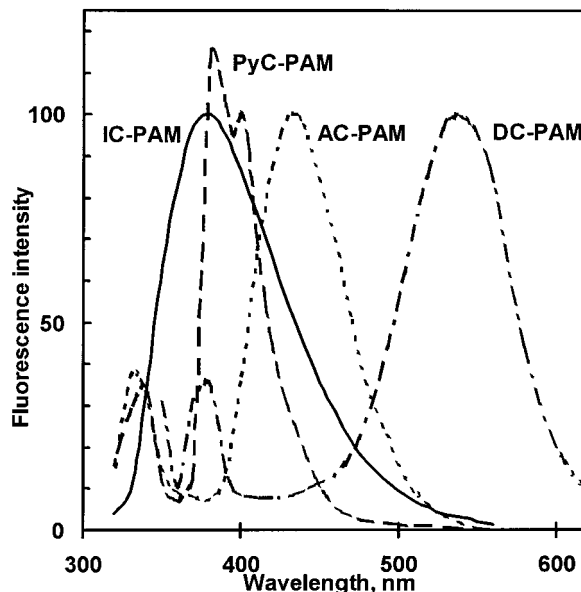
### Materials

C-PAMs with primary and quaternary amines were prepared by the modified Hofmann reaction<sup>26</sup> by adding alkaline sodium hypochlorite to an aqueous solution of polyacrylamide containing choline chloride. The polyacrylamide was synthesized as described elsewhere.<sup>26</sup> The weight average molecular weight estimated from aqueous size-exclusion chromatography.<sup>40</sup> was  $3 \times 10^5$ . The total content of amines was determined by polyelectrolyte titration<sup>27</sup> at pH 2.5 and that of quaternary amines by titration at pH 10.5.<sup>26</sup> The content of primary amines was calculated as the difference. The concentrations of primary and quaternary amines in the C-PAM used were 0.6 and 1.3 meq/g, respectively.

The fluorescent labeling reagents were 4-(5,6-dimethoxy-1,3-dihydro-1-oxo-2H-isoindol-2-yl)benzenesulfonyl chloride (isoindol chloride), 5-dimethylamino-1-naphthalenesulfonyl chloride (dansyl chloride), 1-pyrenesulfonyl chloride, and 2-anthracenesulfonyl chloride. The former two were purchased from Wako Pure Chemicals (Osaka, Japan) and the latter two from Molecular Probe, Inc. (Eugene, OR, USA). They were used without purification. Other chemicals used were of reagent grade.

### Procedure (fluorescent labeling of C-PAM)

Five grams of C-PAM were dissolved in 65 mL of deionized water. To this solution, 35 mL of dimethylformamide (DMF) was added dropwise under stirring. After cooling to 5–10°C, 1M Na<sub>2</sub>CO<sub>3</sub> was slowly added dropwise to the solution until the added phenolphthalein indicator showed a light red color and five more drops were then added. The amount of 0.4 millimole fluorescent labeling reagent (110–150 mg) was dissolved in 10 mL of DMF and was added dropwise during 15 min to the solution under vigorous



**Figure 1** Fluorescence emission spectra of fluorescently labeled cationic polyacrylamides, isoindol (IC-PAM; —), pyrene (PyC-PAM; ---), anthracene (AC-PAM; ···), and dansyl (DC-PAM; -·-·-).

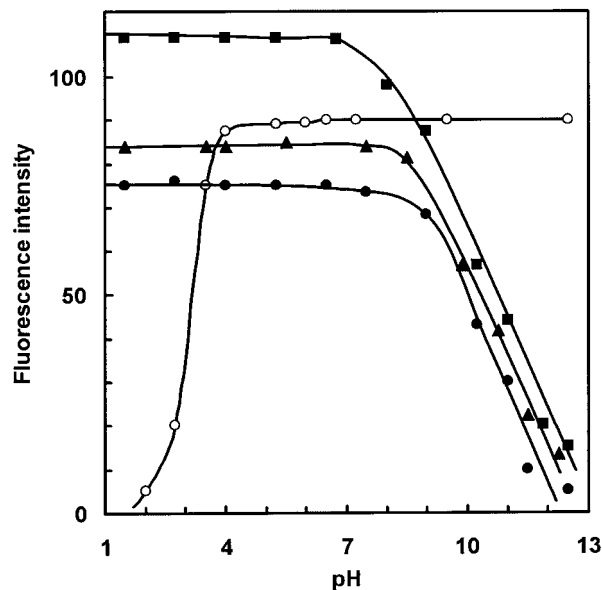
stirring and the solution was then allowed to stand for 30 min. The solution was then neutralized with cooled 1M HCl until the added methyl red indicator changed to red. The resulting polymer was purified by reprecipitation three times from water-isopropanol and dried in a vacuum desiccator.

Fluorescence measurements were made using a Perkin-Elmer LS-5 Luminescence spectrophotometer at room temperature. Bandwidth slits for both excitation and emission wavelengths were 10 nm.

## RESULTS AND DISCUSSION

The optimum excitation wavelengths for C-PAMs with isoindol (IC-PAM), pyrene (PyC-PAM), anthracene (AC-PAM), and dansyl (DC-PAM) groups were found to be respectively 310, 300, 300, and 333 nm using the pre-scan facility of the spectrophotometer. The emission spectra obtained using these excitation wavelengths are shown in Figure 1. The wavelengths for maximum emission for IC-PAM, PyC-PAM, AC-PAM, and DC-PAM were respectively 380, 382 (smaller peak at 400), 435, and 540 nm, which thus covered a wide wavelength range. It is worth noting that AC-PAM and PyC-PAM have little fluorescence intensity at 540 nm, which is the maximum emission wavelength for DC-PAM, making an accurate determination of the two polymers in the same solution possible.

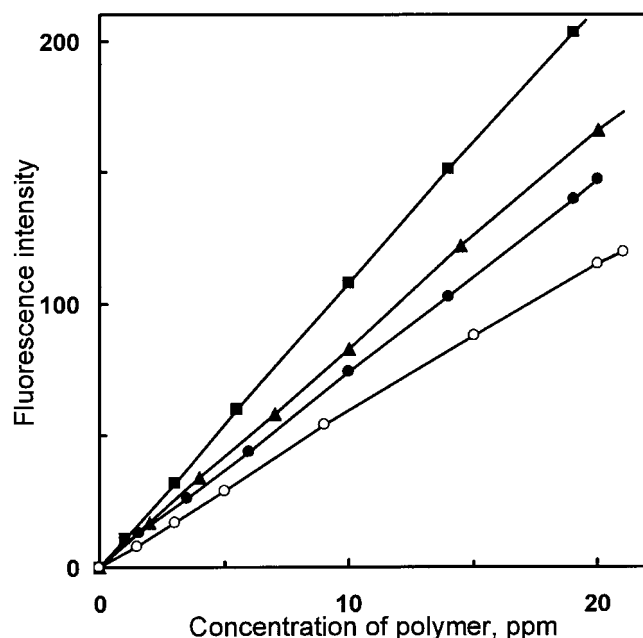
Figure 2 shows the effect of pH on the fluorescence intensity of the four labeled C-PAMs. The fluorescence intensities of PyC-PAM, AC-PAM, and IC-PAM are



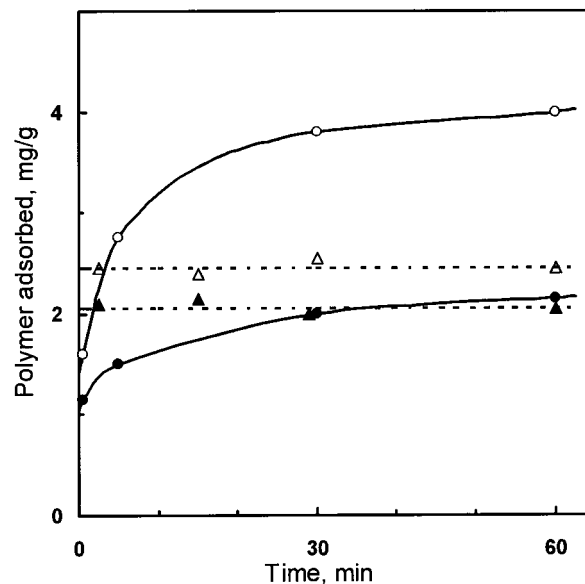
**Figure 2** Effect of pH on fluorescence intensity of IC-PAM (■), PyC-PAM (▲), AC-PAM (●), and DC-PAM (○). IC-PAM, PyC-PAM, and AC-PAM: 10 ppm, DC-PAM: 15 ppm.

practically constant at pH levels below 7, 8, and 9 respectively, but they decrease rapidly with increasing pH above these pH levels. On the other hand, the fluorescence intensity of DC-PAM is essentially constant at pH levels above 5, but decreases rapidly at pH below 4. This may be attributed to the degree of dissociation of the dimethylamino group attached to the dansyl group.

Figure 3 shows the linear relationships between the



**Figure 3** Relationship between fluorescence intensity and polymer concentration for PyC-PAM (■), IC-PAM (▲), AC-PAM (●), and DC-PAM (○). pH 5.5–6.5.



**Figure 4** Simultaneous adsorption of mixed C-PAMs with different MW but the same CD onto cellulose fibers and polystyrene latex (PSL). (○) DC-PAM on fibers, (△) DC-PAM on PSL, (●) AC-PAM on fibers, (▲) AC-PAM on PSL. Cellulose fibers (hardwood bleached kraft pulps): 1.0%; DC-PAM: 0.007%; AC-PAM: 0.007%. PSL: 1.0%; DC-PAM: 0.005%; AC-PAM: 0.005%.  $22 \pm 1^\circ\text{C}$ ,  $\text{pH } 6 \pm 0.2$ ; cellulose fibers:  $43 \mu\text{eq/g}$ ; PSL:  $5.3 \mu\text{eq/g}$ ; DC-PAM:  $1.1 \times 10^4$ , CD 1.6 meq/g; AC-PAM:  $3.0 \times 10^5$ , CD 1.5 meq/g.

concentrations of the fluorescently labeled polymers and the fluorescence intensities at their emission maxima.

From the composition, the average equivalent MW for a monomer unit of C-PAM is estimated to be 87. The amount of fluorescent labeling reagent used was 0.007 mole per average equivalent MW of C-PAM. In the previous work,<sup>26</sup> about 60% of the dansyl chloride added was incorporated into C-PAM. The fluorescent labeling reagents are all sulfonyl chlorides. If it is assumed that the reaction efficiencies of the labeling reagents are the same, the degree of substitution of fluorescent groups can be estimated to be 0.004. The characteristics of the C-PAM should not be altered by such a low level of labeling,<sup>19</sup> and Figure 3 shows that this level is sufficient for the determination of C-PAM at ppm concentrations.

As an example, the simultaneous adsorption of two cationic polyacrylamides onto cellulosic fibers in one experiment and onto polystyrene latex (PSL) particles in another experiment has been followed by using polymers with different fluorescent groups. The results are given in Figure 4. The adsorption of both polymers onto PSL particles quickly reaches a constant level with a small influence of MW. On cellulosic fibers, the adsorption increases with time due to penetration into pores with a significant difference in behavior due to the MW of the polymer.

## CONCLUSIONS

Four fluorescently labeled cationic polyacrylamides with different fluorescence emission wavelengths from 380 to 540 nm were successfully prepared in the homogeneous reaction system. Even at the low degree of labeling the detection limit of polymers is very low (approximately 1 ppm).

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