Synthesis and Investigation of a New pH and Temperature Sensitive Poly(N-isopropylacrylamide)

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Abstract—A new poly(N-isopropylacrylamide) (PNIPAAm) random copolymer with a pH responsive 2-dicyanomethylene-3-cyano-4,5,5-trimethyl-2,5-dihydrofuran (TCF)-containing fluorophore was synthesized and investigated in aqueous solution. pH and temperature stimulated absorbance spectra, color, and emission spectra were studied. Acidic environment enhanced its emission intensity and blue-shifted its absorbance. pH also affected its low critical solution temperature (LCST) because of the change of the hydrophobic and hydrophilic properties of the pH sensitive fluorophore. At a certain pH, temperature induced the absorbance and emission change, however, the trend of fluorescence intensity and maximum was different at acidic and basic conditions, which was attributed to the different fluorophore arrangements in the PNIPAAm copolymer.

Keywords — LCST; pH sensor; poly(N-isopropylacrylamide); stimulated polymer; TCF.

I. INTRODUCTION

The synthesis and investigation of functional polymers as metal ion, pH, temperature, sugar, oxygen, and DNA sensors are important and challenging for not only academic interest but also for applications in disease/cancer diagnosis and treatment, environmental condition monitoring, and new biotechnological and medicinal development [1,2]. Poly(N-isopropylacrylamide) (PNIPAAm) is an interesting polymer, which has been widely studied for fundamental research and applied in drug delivery field [3] because of its typical characteristic of low critical solution temperature (LCST) around 32°C [4], which is close to body temperature and can be tuned through a copolymerization with hydrophilic and hydrophobic segments [5] and by dissolving the PNIPAAm in solutions with different ion strengths [6]. Some PNIPAAm random copolymers [7] and block copolymers [8] possessing fluorophores were reported as environmental, including temperature, solvents, metal ions, and pH stimulated probes. Along this line, we previously investigated two different PNIPAAm random copolymers as zinc, pH, temperature and DNA sensors [7g, 7h] and PNIPAAm block copolymers for solvent stimulating fluorescence [8c].

Herein, we report a new pH and temperature sensitive PNIPAAm copolymer, of which its color (absorption) and emission can be stimulated by pH and temperature. The fluorophore was constructed using 2-dicyanomethylene-3-cyano-4,5,5-trimethyl-2,5-dihydrofuran (TCF) as an electron-withdrawing group and aniline as an electron donating group (M, Scheme 1). A piperazine moiety was used as the pH sensitive group. Because the strong electron donating and withdrawing units were conjugated in the sensing moiety, M, the fluorophore emitted at red spectral window. The electron acceptor TCF is widely studied and used in nonlinear optical materials [9]. Recently, Lord et al. [10a] and Bouffard et al. [10b] used the TCF acceptor to prepare fluorophores for bioimaging. We used this acceptor as an electron withdrawing group for a new pH sensitive red emitter, M [11]. The fluorophore is also a monomer possessing a methacrylate unit, enabling it to be polymerized with N-isopropylacrylamide (NIPAAm). Because of the difference of the chemical structure of the sensing moiety (M) at basic and acidic conditions shown in P1 and P2, which enables different hydrophilic and hydrophobic properties of the fluorophore, the LCST was significantly affected by pH value. Because the fluorophore with the TCF acceptor is highly polar, its absorbance and emission are sensitive to its micro-environmental change induced by pH and temperature, which was characterized using UV-vis absorption and fluorescence spectra. Thus, a new multi-functional pH and temperature sensitive probe was successfully developed.
II. EXPERIMENTAL SECTION

Materials
The monomer M was prepared according to our previous reported procedures [11]. Azobisisobutyronitrile (AIBN), NIPAAm, and tetrahydrofuran (THF) were purchased from Aldrich and used without further purification.

Characterization
Polymer molecular weight was determined using a Waters 1515 GPC coupled with UV and RI detectors, in reference to a series of polystyrene standards with tetrahydrofuran (THF) as the eluent. UV-vis absorption spectra were measured using a Perkin-Elmer Lambda 9 UV/Vis/NIR spectrophotometer. Fluorescence spectra were recorded with an Oriel InstaSpec IV spectrograph that had a charge coupled device (CCD) detector. Fluorescence quantum yields were obtained by comparing the integrated fluorescence spectra of the polymers in solutions to the fluorescence spectrum of rhodamine in ethanol (Φ = 0.65) [12] with a correction of refractive index differences. All the titrations were performed in Britton-Robinson (B-R) buffers composed of acetic acid, boric acid, phosphoric acid and sodium hydroxide.

Synthesis
Preparation of polymer P1. A mixture of 565 mg of NIPAAm (5 mmol), 10 mg of AIBN (0.06 mmol), and 24 mg of the monomer (0.05 mmol) in 5 mL of THF was degassed three times through the typical freeze-thaw approach and polymerized under nitrogen atmosphere at 65°C for 1 day. The polymer was then precipitated into ether (500 mL) to remove any potential small molecular materials. After filtration and dried under vacuum at 50 °C, 340 mg of polymer with a yield of 60% was obtained. Mn = 8500, Mw/Mn = 1.78. Fluorophore content in the polymer was determined to be 1.5 mol-% using UV-Vis spectra.

III. RESULTS AND DISCUSSION

Polymer synthesis and characterization
Polymer was prepared using random polymerization method using AIBN as the initiator. Molecular weight of the polymer was characterized using GPC.

pH response
Polymer P1 was easily dissolved in water with a solubility of at least of 5 mg/mL at room temperature. P1 has a good pH response, which was studied in B-R buffer. Typical UV-vis and fluorescence spectral changes at various pH values were given in Figures 1A and 1C using a polymer concentration of 0.1 mg/mL. 3 nm absorbance shift was observed from basic (pH 8.7) to neutral (pH 7.0) condition (Fig. 1A). Typical corresponding λmax was given in details in Table 1 of the supporting information. Much more significant blue shift was observed from the neutral to acidic condition. As the absorbance maximum shift is quite significant with 36 nm from pH 8.7 to pH 1.8, color change of the solutions at basic and acidic conditions is quite clear, which was given in Fig. 1B. Emission spectral maxima were blue-shifted slightly from pH 8.7 to pH 6.4 (Fig. 1C) and emission intensity enhanced about five to six folds depending on excitation wavelengths (Fig. 1D). The emission intensity change follows a sigmoidal (Boltzmann fitting, equation 1):
Fig. 1. A) Absorbance spectra of the PNIPAAm copolymer at different pH in B-R buffers. B) Typical colors of the solution at pH 3.3 and pH 7.4 at room temperature. C) Fluorescence intensity of the polymer at different B-R buffers excited at 496 nm. D) Integrated fluorescence intensities (from 550 – 750 nm) via pH and their Boltzmann fitting using 496 nm and 518 nm as the excitation wavelengths. F_0 represents the fluorescence intensity at pH 8.7. F is the fluorescence intensity at various pH. E) Illustration of the possible arrangements of the fluorophores in the copolymer in aqueous solution stimulated by pH and temperature, which affected the absorption and emission.
\[ \frac{F}{F_0} = \frac{m_1 - m_2}{1 + \exp\left(\frac{pH - pK_a}{p}\right)} + m_2 \]  

(1)

where, \( F \) and \( F_0 \) are integrated fluorescence intensities from 550 nm to 750 nm measured at varying pH values and at the highest pH value (pH 9.5) used during the titration, respectively. \( m_1, m_2, pK_a \), and \( p \) are empirical parameters describing the initial value \( (m_1) \), the final value \( (m_2) \), the point of inflection \( (pK_a) \), and the width \( (p) \) of the sigmoidal curve. The fluorescence intensity changes and their curve fittings are shown in Fig. 1D. The apparent \( pK_a \) value \( (pK_a) \) was 5.48 (using 496 nm as the excitation wavelength) and 5.63 (using 518 nm as the excitation wavelength) for the pH sensing film in B-R buffers.

Fluorescence intensity change was ascribed to photo-induced electron transfer (PET) in the pH sensor, which was suppressed by the protonation of amino groups. When a fluorophore is attached to an electron quencher (usually one or more nitrogen-containing functional groups which are non-conjugated to the fluorophore), PET occurs between them (S-Figure 1 in supporting information) [13]. In the piperazinyl group of M, the nitrogen atom in NCH\(_2\)CH\(_2\) is not directly connected to the TCF-conjugated fluorophore, of which the NCH\(_2\)CH\(_2\) moiety is a strong electron donor. PET occurs from the lone electron pair of the amine group to the acceptor TCF-containing fluorophore, making the sensor weakly fluorescent. At lower pH, however, the protonation of the amino group (structure of the sensing moiety was shown in P2) diminishes the PET effect and, in turn, leads to restoration of the fluorescence originating from the fluorophore. Hence, a remarked increase in emission intensity was observed at low pH. Because the fluorophore is polar, its fluorescence and absorbance are quite sensitive to the environment at basic, neutral, or acidic conditions. At basic and neutral conditions, the chromophore is neutral and water insoluble, when the polymer (P1) is dissolved in water, the chromophore may have some aggregations (Fig. 1E), which results in its absorbance and emission at long wavelengths. At acidic condition, the neutral amine group is protonized, resulting in an improved solubility of the sensing moieties in water. At the acidic condition, polymer structure changed to P2. The chromophore aggregation was diminished, resulting in its much blue-shifted emission and absorbance. Quantum yield at basic condition is extremely low as 0.0055 at pH 8.7. At acidic condition (pH 3.3), it was enhanced to be 0.026, which is 5 folds higher than that at pH 8.7. The polymer exhibited good pH response reproducibility (S-Figure 2 in supporting information). The high repeatability of the pH sensing indicated the highly chemical stability of the pH sensing moiety and the change is solely induced by pH variation.

**LCST properties**

LCST was characterized by measuring the transmission at 750 nm (Fig. 2A). Sharp LCST was observed at either basic or...
Fig. 3. Fluorescence change of P1 at 25 °C at pH 7.4 stimulated by temperature. B) Integrated fluorescence intensity corresponding to A at different temperature. The insert figure in B shows the emission wavelength depended by temperature. F₀ represents the fluorescence intensity at 25°C. F is the fluorescence intensity at various temperature. C) Fluorescence change of P2 at 25 °C at pH 3.3 stimulated by temperature. D) Integrated fluorescence intensity corresponding to C at different temperature. F₀ represents the fluorescence intensity at 25°C. F is the fluorescence intensity at various temperature. The insert figure in D shows the emission wavelength at different temperature.

acidic conditions. It was found at pH 7.4 the LCST was 28.2 °C, while at pH 3.3 the LCST was 31.6°C. The LCST (31.6 °C) of the polymer at acidic condition was almost the same as that of pure PNIPAAm (32 °C). The LCST (28.2 °C) of the polymer at basic condition was much lower than pure PNIPAAm. These results may be attributed to the solubility difference of the pH sensing moiety in the polymer. At acidic condition, the polymer structure is P2. The sensing moiety in P2 is more hydrophilic than that in P1, where the sensing moiety is hydrophobic. It has been known that hydrophilic segment in PNIPAAm can increase its LCST [5]. On the contrary, hydrophobic segment will decrease its LCST.

Temperature effect on color and fluorescence

Fig. 2C shows the color change induced by temperature. At room temperature (~ 22°C) of pH of 3, orange color was observed, while at 40°C purple color was observed. At room temperature of pH 7.4, slightly purple was observed, while at high temperature blue color was observed. These changes suggested the absorbance changed to long wavelength from room temperature to 40°C. Although there is strong light scattering after the LCST, the absorption spectra still showed the shifts to longer wavelength at 40°C.

Emission spectra were significantly affected by temperature (Fig. 3). For the pH 7.4 solution, from 25 to 30 °C, 6 nm emission maxima shift and slightly intensity enhancement were observed. From 30 to 39 °C, fluorescence intensity keeps on increasing without spectral shift. At temperature higher than 39 °C, the emission has no further change. The emission intensity change ratios and peak shifts were given in Fig. 3B. For pH 3.3 solution, from 25 to 31 °C, no emission maximum shift was observed. While, the emission maxima started to shift from 31 to 50 °C with continuous intensity decrease. (Figures 3C and 3D). These emission behaviors are related with both the LCST and hydrophilicity/hydrophobicity difference of the sensing moiety at various pH.
At neutral or basic condition, the sensing moiety in P1 is water insoluble. LCST affected the fluorophore aggregations (Fig. 1E). When temperature is higher than 30 °C, the environment becomes more hydrophobic, which matches the hydrophobic nature of the sensing moiety to red shift the emission maxima. The polymer may aggregate at the temperature higher than LCST, providing a viscous microdomain with the polymer to suppress the rotation of the fluorophores, which results in the fluorescence enhancement [7e]. The fluorophore re-arrangement and aggregations by temperature didn’t follow the LCST trend, however with a delay. The rearrangement seems finished when the temperature is higher than 37°C (7°C higher than the LCST). This phenomenon is different from other LCST polymers with fluorophores, of which emission intensity changes are in accordance with LCST properties [7a – 7f]. This different phenomenon might be related with the fluorophore difference, a further study is still necessary. At acidic condition, the fluorophore in P2 is water-soluble. With the temperature increases, the interaction of the fluorophore with water becomes much stronger, resulting in its red-shifted emission and the decrease of fluorophore intensities. Increasing temperature usually decreases fluorophore’s emission intensity [13c] as non-radiative decay becomes more significant at higher temperature.

IV. CONCLUSIONS

A new TCF-containing pH sensor was synthesized and polymerized with NIPAAm to generate a TCF-fluorophore-containing PNIPAAm copolymer. The polymer exhibited weak fluorescence at neutral and basic aqueous solution, but strong emission at acidic condition with a pKa of 5.63. Color change was also observed due to the pH change. LCST properties were affected by pH, which were 28.2°C and 31.6°C at pH 7.4 and 3.3 respectively. Temperature not only induced the absorption change but also emission intensity difference. At pH 7.4, the polymer exhibited weak emission, but the intensity was increased by temperature. At pH 3.0, the polymer exhibited strong emission at room temperature, but the intensity was decreased by an increase of temperature. Therefore, a new temperature and pH stimulated functional PNIPAAm functional polymer was successfully developed and investigated.

REFERENCES

Supporting information

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S-Table 1. Typical photophysical properties of the polymer in B-R buffer at different pH at room temperature.

<table>
<thead>
<tr>
<th>pH</th>
<th>$\lambda_{\text{max}}^\text{absorbance}$</th>
<th>$\lambda_{\text{max}}^\text{emission}$</th>
<th>Quantum yield$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.8</td>
<td>495 nm</td>
<td>625 nm</td>
<td>---$^b$</td>
</tr>
<tr>
<td>3.3</td>
<td>495 nm</td>
<td>625 nm</td>
<td>0.026</td>
</tr>
<tr>
<td>5.3</td>
<td>507 nm</td>
<td>625 nm</td>
<td>---$^b$</td>
</tr>
<tr>
<td>7.0</td>
<td>526 nm</td>
<td>638 nm</td>
<td>---$^b$</td>
</tr>
<tr>
<td>8.7</td>
<td>529 nm</td>
<td>642 nm</td>
<td>0.0055</td>
</tr>
</tbody>
</table>

a) quantum yield was determined using Rhodamine as standard; b) not measured.
S-Figure 1. A drawing of PET, which affected the emission intensity.

S-Figure 2. A) Reversibility of the fluorescence spectra at pH 3.3 and 8.7. B) Corresponding fluorescence intensity of Figure A. $F_0$: Integrated fluorescence intensity from 550 to 750 nm at pH 8.7 before the reversibility experiment started.