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A Long Wavelength Fluorescent Hydrophilic Copolymer Based on Naphthalenediimide as pH Sensor with Broad Linear Response Range

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Supporting Information

ABSTRACT: Successful operation of a microbioreactor is dependent upon the monitoring and control of pH in culture fluids. Introducing chemosensors into polymeric backbones via covalent link can be easily employed to fabricate devices and chips fixed onto microbioreactors, capable of avoiding the phase separation and concentration quenching of chromophores. With hydrophilic poly(2-hydroxyethyl methacrylate) (PHEMA, the main matrix of contact lens), a novel hydrophilic fluorescent copolymer P(NDI−HEMA) containing the pendant group of naphthalenediimide moiety (NDI) as low-cost pH chemosensor film for high-throughput microbioreactor was designed. Its film exhibits great sensitivity and stability when measured in aqueous solution, possessing an obvious fluorescent response with an excellent linearity in the pH range of 4.6−8.0 with an apparent pK_a of 6.0 corresponding to the methyl substituted piperidine nitrogen (*N*1) in NDI. The pH range is especially desirable to the fermentation monitoring of most industrially important microorganisms, such as various prokaryotes and eukaryotes. Furthermore, the long excitation wavelength at the isosbestic point of 572 nm and the emission band at about 630 nm of chromophore NDI brings several advantages such as low scattering, deep penetration, and minimal interfering absorption and fluorescence from biological system, showing a high promising application in bioprocess monitoring with a broad linear response range.

INTRODUCTION

The detection and control of pH and ions plays an important role in chemistry, biochemistry, cellular biology, and drug delivery.[1−8] Given that cell culture optimization is a labor-intensive process requiring a large number of experiments to be conducted under varying conditions, the conjunction of automated robotic systems with miniature pH and dissolved oxygen (DO) detectors has become critical in the cell cultivation via developing low-cost high-throughput microbioreactors.[9−11] Since most biological actions are carried out in aqueous system, noninvasive optical pH film sensors with hydrophilicity are preferable to biofermentation microreactors in the viewpoint of high response and low pollution to reactors.[12] Introducing chemosensors into polymeric backbones via covalent link can be easily employed to fabricate devices and chips fixed onto microbioreactors, capable of avoiding the phase separation and concentration quenching of chromophores.[13−17] Recently, water-soluble fluorescent conjugated polymers have been discussed by Wang and Zhu et al.,[18] and polydiacetylenes (PDAs) based sensors were reported by Yoon et al.[19,20] Up to date, several fluorescent copolymeric pH sensors have been exploited with using covalently immobilizable pH responsive probes.[21−23] The chemical incorporation of sensor moieties into polymeric matrix is more performable and efficient due to easy miniaturization and fabrication into devices and chips fixed onto microreactors. Generally, pH monitoring is generally performed using well-established electrochemical sensors. However, Rao et al.[24] has already pointed out that using these sensors in certain applications can be problematic. Although bioreactors with capacity of 1 L or more are equipped with pH electrodes, it is difficult to do the same for small scale fermentations such as microbioreactors (≤50 mL) or multiwell plates. Thus, monitoring high throughput bioprocessing is difficult. Moreover, attaching a pH meter to shake flasks would present multiple difficulties including expense, changes in mixing dynamics, and potential release of electrode solution into the media. Consequently, optical pH sensors[24] offer a promising alternative to the traditional glass electrodes, which require awkward wire connections. However, most reported cases are located in short emission wavelength region.[25−27] For instance, the commercially available HPTS−PEG−Dower pH film was found difficult for real-time applications in high-throughput microbioreactors due to the short excitation and emission wavelength, and high carcinogenicity from pyrene chromophore.[28] Obviously, the long wavelength emission of red or near-infrared (NIR) light could exhibit specific advantages such as low scattering, deep penetration, and minimal interfering absorption and fluorescence from biological system.

With this in mind, herein we report a long wavelength fluorescent hydrophilic copolymer P(NDI−HEMA) (Scheme 1), in which a covalently immobilizable 1,4,5,8-naphthalenediimide (NDI) chromophore is used as pH-sensitive unit. As a matter of fact, the electron deficient aromatic core of naphthalenediimide has attracted much attention in a variety of areas such as semiconductor materials,[29] sensors,[30] and self-assemblies.[31]

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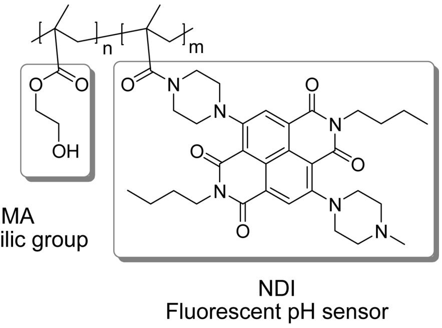
The optical properties of NDI chrompohore could be tuned over a wide spectral range by changing substituents at the core ring.[32−34] Thus, two piperidine units are incorporated as strong electron-donors to NDI-core by nucleophilic substitution for extending the push−pull electronic system with an emission band at around 630 nm, which can successfully tune the determining wavelength falling into NIR region. Furthermore, as a classical push−pull system, the fluorescence of NDI is very sensitive to pH via the photoinduced electron transfer (PET) process from methyl-substituted nitrogen to NDI fluorophore. The fluorescence intensity of P(NDI−HEMA) exhibits a linear response in a broad pH range of 4.6−8.0 with an apparent pK_a of 6.0 in aqueous solution. The present copolymeric pH sensor with excellent film-forming and hydrophilic property can be desirable to noninvasive, real-time monitoring bioprocess in high-throughput microbioreactors in most physiological environments.

RESULTS AND DISCUSSION

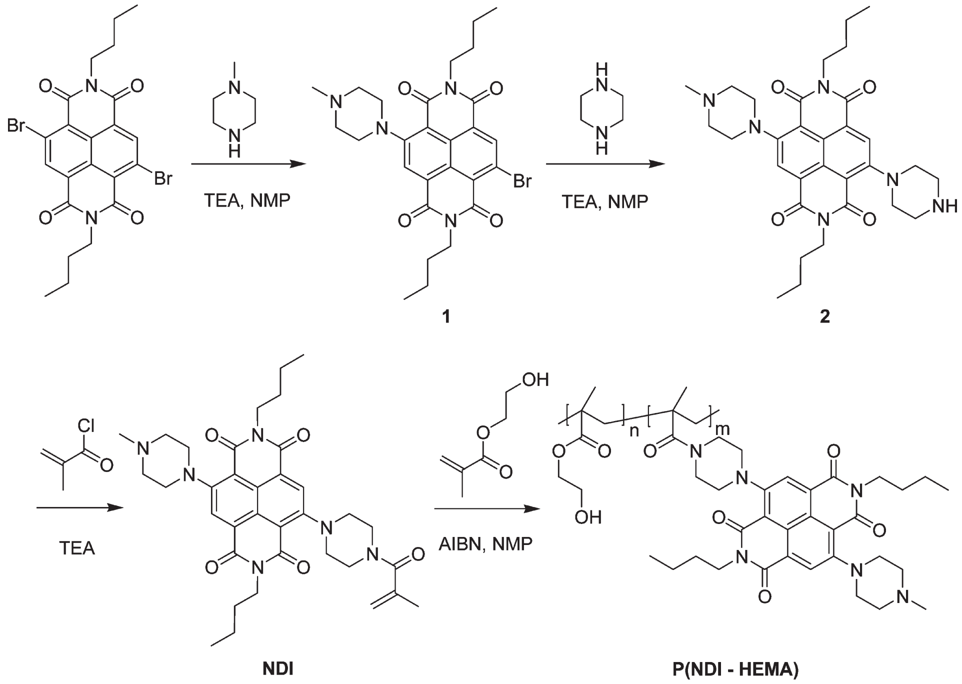
Design and Synthesis of P(NDI−HEMA). As a well-known fluorophore, NDIs are exquisite building blocks for optic-electronic functional materials owing to their long absorption band, outstanding light-fastness, chemical inertness and high fluorescence quantum yield. More importantly, their photo- and electro-chemical properties can be easily tuned with modification in chemical structures.[32−34] Generally, most functionalizations of NDI chromophore are introduced through substitutions on its dianhydride groups or core substitution at 2-, 3-, 6-, and/or 7-positions. Though core-unsubstituted NDIs are colorless and have nonfluorescent electron traps, the core substitution with electron-donating groups, especially alkylamines, provides a facile way to tune the emission wavelength from 387 nm to around 630 nm, even longer than 650 nm (falling into the NIR region).[33,35] Furthermore, functional groups could be easily incorporated into the specific NDI chromophore for developing novel NDI fluorescent systems to meet a variety of requirements in chemical and biological analyses. Recently, our group has reported two NIR fluorescent chemosensors based on core-substituted NDIs, showing excellent selectivity to zinc ions over other metal ions with a larger fluorescent enhancement centered at 650 nm.[36] In the present work, two piperidine groups were introduced into NDI-core in a stepwise manner (Scheme 2). The proton receptor of *N*-methylpiperidine was first linked to the 2-position of the NDI core, and piperidine was then incorporated into 6-position due to its convenience in introducing methylacryloyl group which could be easily copolymerized with other monomers. Here, the nucleophilic substitution of bromo groups on NDI core with piperidine could be processed under both room temperature and heating conditions. However, the treatment at room temperature with prolonged reaction time was adopted to avoid unexpected byproducts at higher temperatures.

In the target polymer, PHEMA was chosen as the main polymeric matrix owing to its high hydrophilicity. The fluorescence self-quenching induced by aggregation can also be avoided by adjusting molar proportion of the two monomers. Generally, PHEMA could be obtained by solution, suspension and UV-free-radical polymerization. In this work, solution polymerization was conducted in preparing the target copolymer P(NDI−HEMA)

Scheme 1. Chemical Structure of P(NDI−HEMA)



Scheme 2. Synthesis of Copolymer P(NDI−HEMA)



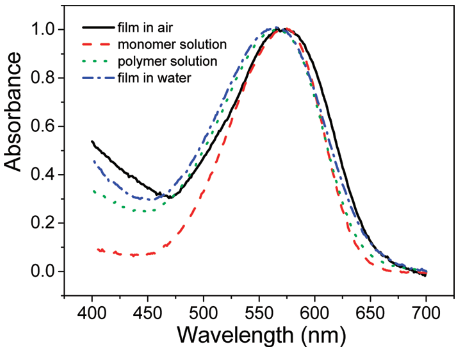


Figure 1. Normalized absorption spectra of monomer NDI and copolymer P(NDI−HEMA) in air, water, and methanol.

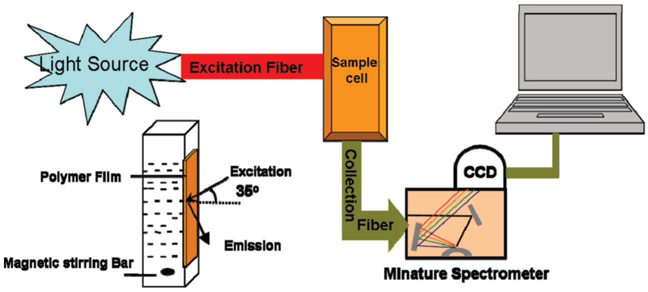


Figure 2. Schematic diagram of sensing system for fluorescent spectra: cuvette with front-face excitation and emission collection to determine the online-pH.

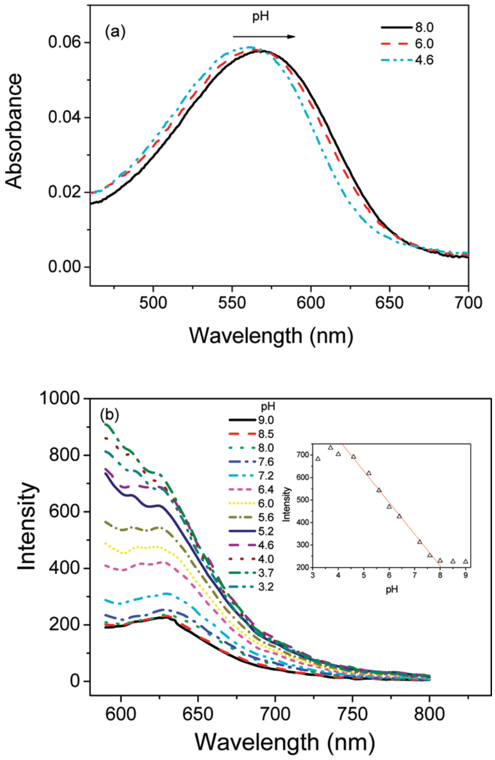


Figure 3. Spectral changes of copolymer P(NDI−HEMA) film upon various pH in aqueous system: (a) absorption and (b) fluorescence (excited at the isosbestic point of 572 nm). Inset: fitting curve of pH-dependent response to fluorescence intensity of P(NDI−HEMA) film at 628 nm, linearly with the relative coefficient of 0.998 in the pH range of 4.6−8.0. Note: for various pH values, the determination was repeated with three times, keeping the deviation below 2%.

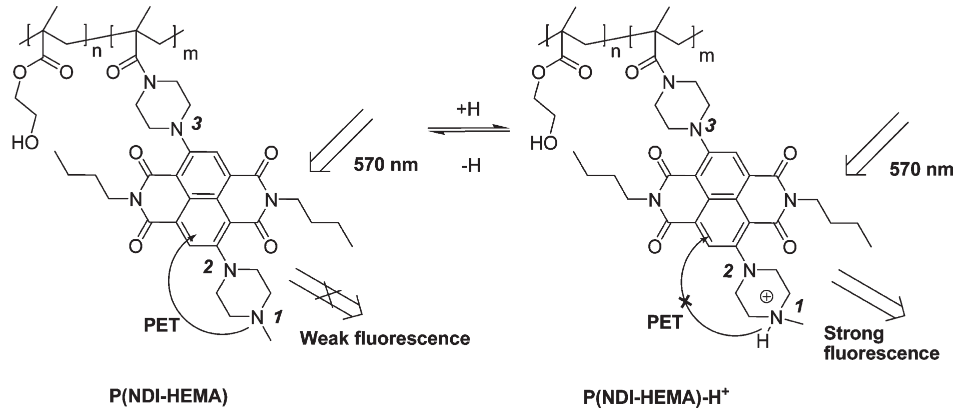
owing to its easiness in operation. The resulted copolymer was well dissolved in methanol, reprecipitated from ethyl acetate, and dried under vacuum to achieve the polymer with high purity. Since the absorption peak at about 570 nm mainly results from NDI moiety in the copolymer, the molar ratio of two monomers could be determined from the standard working curve in absorption spectra.

Absorption Properties of P(NDI−HEMA) and NDI. The absorption spectra of monomer NDI (as solution in methanol) and P(NDI−HEMA) (as solution in methanol or as thin film in air and water) are shown in Figure 1. The band shapes of the three curves of P(NDI−HEMA) are similar as that of monomer NDI in the absorption range of 470−700 nm. The absorption of copolymeric thin film in water exhibits a little blue shift by 5 nm than in air, which might be due to the solvent effect. Apparently, the main matrix PHEMA in our studied copolymer system does not have absorption in the visible region, that is, the absorption at around 570 nm is totally attributed to the fluorophore of NDI since the component PHEMA has no absorption in the region. Accordingly, the molar ratio (about 1:1100) of NDI to HEMA in this copolymer was obtained from the standard absorption working curve at 570 nm.

Optical Responses of P(NDI−HEMA) to pH. To explore the response of P(NDI−HEMA) as copolymeric pH film sensor in aqueous biosystem environment for microbioreactor, the copolymer film was obtained by spin-coating a 5 wt % methanol solution of P(NDI−HEMA) on quartz substrate. Their absorption and fluorescence properties based on the copolymer film were obtained in pure water solution by adjusting pH values with NaOH and HCl aqueous solutions (Figure 3). Obviously, the corresponding absorption peaks were red-shifted slightly from 561 to 569 nm with a clear isosbestic point at 572 nm when pH values were changed from 8.0 to 4.6. Additionally, the same absorption changes were observed under different pH values with phosphate buffers.

In order to eliminate the effect of total-reflected light in fluorescence determination of P(NDI−HEMA) film, an angle of incident light about 35 degree between the incident light (front-face excitation) and layer normal (emission collection), was chosen to determine the fluorescent response to pH for the online pH determination (Figure 2). It was successfully processed with a miniature fiber optic spectrometer and accessories from Ocean Optics to realize the convenient and noninvasive monitoring. The adopted front-face excitation and emission collection can greatly minimize the fluorescence and scatter contribution from the cell mass. At the same time, the isosbestic point of 572 nm was selected as excitation wavelength to keep the same excitation optical density from the tedious calibration, and the emission spectra of such polymer film were achieved in aqueous environment. As expected, the fluorescence intensity originating from NDI fluorophore in the system of P(NDI−HEMA) was varied upon different pH values (Figure 3b), resulting in an enhancement by about 3 times in fluorescence intensity when adjusting pH from 8.0 to 4.6. Moreover, the long-wavelength emission at about 630 nm with the long excitation at the isosbestic point of 572 nm can bring several advantages including minimal interfering absorption and fluorescence from biological samples, and low scattering, showing high promising

Scheme 3. Fluorescence Response (*OFF−ON*) to pH through PET Mechanism



applications in bioprocess monitoring with a broad linear response range.

To understand the interaction between P(NDI−HEMA) and H^+, the fluorescence under titration with H^+ was also monitored. When excited at the isosbestic point of 572 nm, there is a negligible shift in the emission peak wavelength for P(NDI−HEMA), but shows a turn-on fluorescent response with a sequential change of pH (Figure 3b). The maximum fluorescence emission shows up at pH 4.6 is reached. As reported by O'Shea, the pH sensing of arylamine-N is at strong acidic range (at pH 1.5 and 3.4 M HCl).[37a] Compared with the alkyl quaternary amine (*N*1) in the chromophore NDI, the protonation of *N*2 and *N*3 becomes much more difficult due to the strong electron-withdrawing properties of imide groups, which does not occur under weak acidic conditions. Therefore, we ascribe the pH dependent NDI fluorescence to the protonation/deprotonation of *N*1 in the pH range of 4.6−8.0. Obviously, the fluorescence enhancement of P(NDI−HEMA) upon decreasing pH values is a typical PET (photoinduced electron transfer) characteristic, that is, with little shift at emission peak wavelength, the fluorescence enhancement (*Fluorescence-ON*) in acidic environment could be attributed to the characteristic PET block from the protonation of the methyl-substituted piperidine nitrogen (*N*1) to NDI fluorophore (Scheme 3).[37b,38] There exists a PET from *N*1 to NDI chromophore under neutral condition, resulting in weak fluorescence (*Fluorescence-OFF*, Scheme 3). Considering the fact that the basicity of aliphatic amino group is higher than aromatic one, the methyl-substituted piperidine nitrogen *N*1 should be protonated preferentially. Therefore, *N*1 can act as proton recognizing group in the pH range of 4.6−8.0. According to the pH effect on spectral changes, the protonation of aromatic nitrogen of *N*2 and *N*3 (Scheme 3) does not take place at weak acidity.

Additionally, the acidity constant (pK_a) for copolymer P(NDI−HEMA) in aqueous solution was calculated using Henderson−Hasselbalch treatment (eq 1) according to the fluorimetric titration.[38]

$$log[(I_{Fmax} - I_F)/(I_F - I_{Fmin})] = pH - pK_a \qquad (1)$$

Here I_F corresponds to the fluorescent intensity of copolymeric film P(NDI−HEMA). The resulting pK_a is obtained as ca. 6.0 corresponding to the methyl substituted piperidine nitrogen *N*1 (Scheme 3), which lies in the range of near neutral conditions. As shown in Figure 3b, exactly, the titration curve exhibits a Z-like characteristic, which is quite consistent with the Henderson−Hasselbalch treatment curve. Moreover, the excellent pH-dependent fitting of P(NDI−HEMA) in aqueous solution at 628 nm is linearly responsive with the relative coefficient of 0.998 in the pH range of 4.6−8.0 (the Inset in Figure 3b), meeting the physiological range for most live activities such as pH microenvironment of Fusarium's fermentation with almost an identical range.

Film Sensitivity and Stability of Copolymer P(NDI−HEMA). Given that the copolymer is expected to be applied as pH chemosensor in low-cost microbioreactor, it is vital that such a polymer film should be hydrophilic rather than water-insoluble. As a pH sensor in water, the hydrophilicity can ensure proton to reach the chromophore, that is, assuring the protonation of the methyl-substituted piperidine nitrogen (*N*1) to NDI fluorophore to realize the response of pH. Consequently, the pH response of the sensing film is dependent upon the proton permeability into polymer matrix. Actually, the obtained copolymer exhibits excellent hydrophilicity due to the specific matrix of PHEMA. As discussed above, due to the larger molecular weight, P(NDI−HEMA) could be only well soluble in methanol and ethanol, but less soluble in DMF and DMSO, and poorly dissolves in water. Owing to the good film-forming property of PHEMA, copolymer P(NDI−HEMA) can also be easily spin-coated to form an ideal film on quartz or glass substrate for fabricating devices. Generally, the pH response of sensing film is dependent upon the proton permeability into polymer matrix and the strength of binding interaction between protons and polymer sensors.[39,40] Expectedly, the copolymeric film of P(NDI−HEMA) exhibits a high sensitivity to pH in water. To further study on the thickness influence of the film to pH, the fluorescence responses based on two different films with thicknesses of 60 and 20 μm were investigated in water at pH of 6.6 and 7.6. The fluorescence of P(NDI−HEMA) film reached the equilibrium for less than 1.5 min, showing s high sensitivity to proton with a fast response (Figure 4).

Moreover, in order to satisfy the long time process in microbioreactors, both the shape and sensing ability of the film should be stable enough. Two pH points were utilized to undergo a further experiment. The thin film of P(NDI−HEMA) on quartz could be treated repeatedly at least for a week, showing good stability in water to adapt long time online monitoring (Figure 5). Accordingly, the hydrophilic film of P(NDI−HEMA) with high sensitivity and stability could fully meet the requirements of a continuous bioreactor.

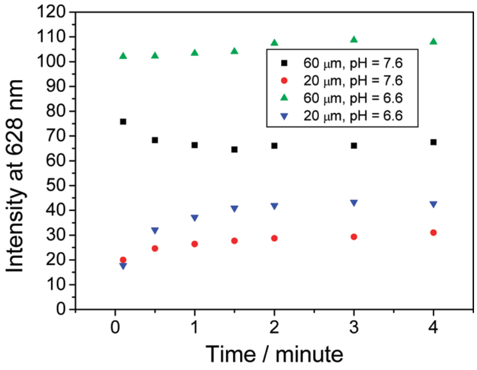


Figure 4. Changes in fluorescence intensity as a function of time for the films of P(NDI−HEMA) with different thickness (60 and 20 μm) dipped into water under different pH values.

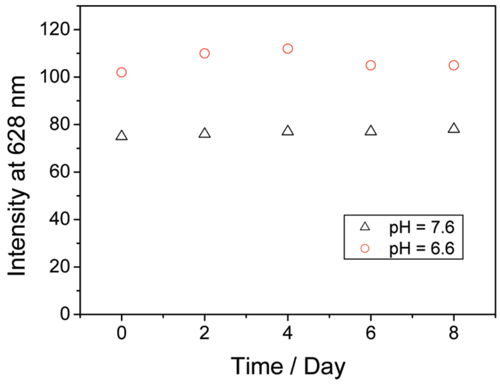


Figure 5. Repeatability of P(NDI−HEMA) film (60 μm) in aqueous solution under different pH at 6.6 and 7.6.

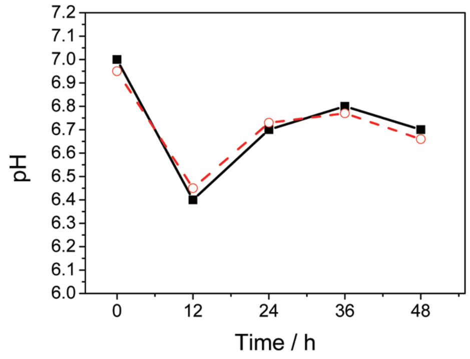


Figure 6. Variation in the pH in Fusarium fermentation system during the course of fermentation measured with common pH meter (black solid line) and with P(NDI−HEMA) film (red dashed line).

Finally, the pH sensing film of P(NDI−HEMA) was applied in a Fusarium fermentation system to perform continuous online measurement of pH. The fluorescent intensity of film in fermentation liquid was detected each 12 h with the P(NDI−HEMA) pH film sensor and the pH meter, respectively (Figure 6). The figure shows that pH tested in fermentation system fit well with the working plots and the error is within bounds, which implies that such a pH sensor film has a broad response in application for microbioreactors.

Also the commercially available HPTS−PEG−Dower pH film[28] was selected to test in parallel. Since the pH film would lose its activity once leaving solvent, HPTS−PEG−Dower must be dipped in distilled water for activation before and after use. In contrast, the copolymeric P(NDI−HEMA) can be used directly and repeatedly without specific pretreatment or storage. Moreover, the pyrene chromophore utilized as fluorescent moiety in HPTS−PEG−Dower pH film may be carcinogenic, and not suitable for biosystem. With easier synthesis, lower toxicity, longer wavelength emission and excellent linear relevance to pH, the chemosensor film of P(NDI−HEMA) could be potentially applied as a low-cost microbioreactor pH indicator for high-throughput bioprocessing.

■ CONCLUSIONS

In summary, a long wavelength fluorescent hydrophilic copolymer P(NDI−HEMA) was developed as a pH chemosensor for bioprocess monitoring, in which a covalently immobilized 1,4,5,8-naphthalenediimide (NDI) chromophore is used as pH-sensitive unit. In the target polymeric chemosensor system, PHEMA was chosen as the main polymeric matrix due to its high hydrophilicity and good film-forming ability. The fluorescence self-quenching induced by aggregation can also be avoided by adjusting molar proportion of the two monomers. The adopted front-face excitation and emission collection can greatly minimize the fluorescence and scatter contribution from the cell mass. Its film shows high sensitivity to environmental acidity with excellent linear relevance to pH values in the range of pH 4.6−8.0, which is located at the physiological pH range for satisfying most of biosystems. The isosbestic point at 572 nm was utilized as excitation wavelength to avoid the tedious calibration. The long wavelength emission approaching NIR range could ensure minimal interference from biological samples, and low scattering. In terms of the convenience in synthesis and good linearity relevance to pH, the chemosensor film of P(NDI−HEMA) could be potentially applied as a low-cost microbioreactor pH indicator for high-throughput bioprocessing.

■ EXPERIMENTAL SECTION

^{1}H NMR and ^{13}C NMR spectra were obtained by a Bruker AV 500 or 400 Spectrometer. High resolution mass spectra (HRMS) were obtained by a Waters LCT Premier XE spectrometer. Absorption spectra were recorded on a Varian Cary 100 spectrometer, and fluorescence spectra were obtained by using a Varian Cary Eclipse spectrometer. The molecular weight and molecular weight distribution (M_w/M_n) was determined with a Waters 1515 Gel Permeation Chromatography (GPC) using DMF as the mobile phase. The starting material of 1,4,5,8-naphthalenetetracarboxylic dianhydride was commercially available from TCI. The pH value was adjusted by NaOH and HCl aqueous solution, and demarcated on a Mettler Toledo FE20 pH meter.

Synthesis of Compound 1. A mixture of *N*,*N*′-dibutyl-2,6-dibromo-1,4,5,8-naphthalenediimide (108 mg, 0.2 mmol), *N*-methylpiperidine (20 mg, 0.21 mmol), triethylamine (0.5 mL), and *N*-methylpyrrolidin-2-one (NMP, 0.5 mL) was stirred at room temperature under the protection of argon for 3 days. The dark purple solution was washed with water and extracted with CH_2Cl_2. The solvent was removed, and the residue was purified with a column chromatography on silica gel (CH_2Cl_2/MeOH = 10/1) to obtain a red powder (110 mg, yield 98%). Mp > 280 °C. ^{1}H NMR (400 MHz, $CDCl_3$, ppm): $\delta = 8.79$ (s, 1H, naphthalene-H), 8.48 (s, 1H, naphthalene-H), 4.17 (t, $J = 7.6$ Hz, 4H, $-NCH_2C_3H_7$), 3.58 (t, $J = 4.4$ Hz, 4H, $-NC\boldsymbol{H_2}CH_2NCH_3$), 2.71 (t, $J = 4.4$ Hz, 4H, $-NCH_2C\boldsymbol{H_2}NCH_3$), 2.42 (s, 3H, $-NCH_3$), 1.74−1.67 (m, 4 H, $-NCH_2C\boldsymbol{H_2}C_2H_5$), 1.49−1.41 (m, 4H, $-NC_2H_4$-$C\boldsymbol{H_2}CH_3$), 1.01−0.96 (m, 6H, $-NC_3H_6C\boldsymbol{H_3}$). ^{13}C NMR (100 MHz,

$CDCl_3$, ppm): δ = 162.07, 161.84, 161.43, 161.37, 153.24, 137.92, 129.54, 126.33, 125.92, 124.76, 123.13, 123.03, 122.17, 107.46, 55.03, 52.21, 46.01, 41.16, 40.78, 30.25, 30.00, 20.38, 20.34, 13.84, 13.80. HRMS (TOF−ESI$^+$), *m*/*z*: calcd for $C_{27}H_{32}BrN_4O_4$, 555.1607 [M(^{79}Br) + H$^+$]; found, 555.1604; calcd, 557.1586 [M(^{81}Br) + H$^+$]; found, 557.1589.

Synthesis of Compound 2. A mixture of compound **1** (110 mg, 0.2 mmol), piperidine (20 mg, 0.22 mmol), triethylamine (0.5 mL), and NMP (0.5 mL) was stirred at room temperature for 3 days under argon. The dark purple solution was washed with a mass of water and extracted with CH_2Cl_2. The solvent was removed and the residue was purified with a column chromatography on silica gel (CH_2Cl_2/MeOH = 10/1) and a purple powder was collected for 80 mg with a yield of 72%. Mp > 280 °C. ^{1}H NMR ($CDCl_3$, 500 MHz, ppm) δ: 8.38 (s, 1 H, naphthalene-H), 8.36 (s, 1 H, naphthalene-H), 4.10 (s, 4 H, piperidine-H), 3.39 (m, 8 H, piperidine-H and -$\mathrm{NC}\boldsymbol{H}_2\mathrm{C}_3\mathrm{H}_7$), 3.23 (s, 4 H, piperidine-H), 2.68 (s, 4 H, piperidine-H), 2.36 (s, 3 H, $-\mathrm{NCH}_3$), 1.63−1.59 (m, 4 H, $-\mathrm{NCH}_2\mathrm{C}\boldsymbol{H}_2\mathrm{C}_2\mathrm{H}_5$), 1.39−1.35 (m, 4 H, $-\mathrm{NC}_2\mathrm{H}_4\mathrm{C}\boldsymbol{H}_2\mathrm{CH}_3$), 0.91 (t, *J* = 7.3 Hz, 6 H, $-\mathrm{NC}_3\mathrm{H}_6\mathrm{C}\boldsymbol{H}_3$). ^{13}C NMR (400 MHz, $CDCl_3$, ppm): δ = 162.96, 162.68, 161.84, 161.70, 151.97, 151.75, 125.70, 125.46, 125.25, 124.85, 124.73, 124.49, 111.46, 110.31, 55.01, 52.32, 52.07, 51.54, 45.98, 40.65, 30.28, 29.68, 20.37, 13.87. HRMS(TOF−ESI$^+$), *m*/*z*: calcd for $C_{31}H_{41}N_6O_4$ [M + H]$^+$, 561.3189; found, 561.3192.

Synthesis of Monomer NDI. A mixture of compound **2** (56 mg, 0.10 mmol), methylacryloyl chloride (104 mg, 1.0 mmol), triethylamine (4 drops) and CH_2Cl_2 (5 mL) was stirred at room temperature overnight. The solution was poured into water and extracted by CH_2Cl_2. The solvent was removed and the residue was washed with petroleum ether to give a purple dash powder (60 mg, yield 95%). Mp >280 °C. ^{1}H NMR ($CDCl_3$, 500 MHz, ppm) δ: 8.46 (s, 1 H, naphthalene-H), 8.39 (s, 1 H, naphthalene-H), 5.27 (s, 1 H, $=\mathrm{C}\boldsymbol{H}(\mathrm{H})$), 5.13 (s, 1 H, $=\mathrm{CH}(\boldsymbol{H})$), 4.18 (t, *J* = 6.6 Hz, 4 H, $-\mathrm{NC}\boldsymbol{H}_2\mathrm{C}_3\mathrm{H}_7$), 3.94 (s, 4 H, piperidine-H), 3.48 (t, *J* = 4.4 Hz, 4 H, piperidine-H), 3.37 (s, 4 H, piperidine-H), 2.73 (t, *J* = 4.4 Hz, 4 H, piperidine-H), 2.42 (s, 3 H, -NCH_3), 2.02 (s, 3 H, $=\mathrm{CCH}_3$), 1.73−1.66 (m, 4 H, $-\mathrm{NCH}_2\mathrm{C}\boldsymbol{H}_2\mathrm{C}_2\mathrm{H}_5$), 1.50−1.40 (m, 4 H, $-\mathrm{NC}_2\mathrm{H}_4$-$\mathrm{C}\boldsymbol{H}_2\mathrm{CH}_3$), 0.99 (t, *J* = 7.4 Hz, 6 H, $-\mathrm{NC}_3\mathrm{H}_6\mathrm{C}\boldsymbol{H}_3$). ^{13}C NMR ($CDCl_3$, 100 MHz, ppm) δ: 171.42, 162.82, 161.84, 161.77, 152.15, 151.66, 140.25, 125.93, 125.91, 125.51, 125.28, 124.66, 124.31, 115.92, 111.61, 109.52, 55.01, 52.16, 46.01, 40.69, 30.28, 30.25, 20.57, 20.37, 20.36, 13.84. HRMS(TOF−ESI$^+$), *m*/*z*: calcd for $C_{35}H_{45}N_6O_5$ [M + H]$^+$, 629.3451; found, 629.3466.

Synthesis of Copolymer P(NDI−HEMA). A mixture of monomer NDI (31 mg, 0.05 mmol), HEMA (0.78 g, 6.0 mmol), 2,2′-azodiisobutyronitrile (AIBN, 48 mg), and NMP (1.2 mL) in a Schlenk tube was subjected to repeated freeze−thaw cycles, before heated at 70 °C for 20 h. After polymerization, the product was precipitated by dropping the resulted solution into ethyl acetate. The crude product was dissolved in methanol and precipitated from ethyl acetate or CH_2Cl_2 in turns for 4 times in all. The solid was dried under vacuum to give a purple copolymer P(NDI−HEMA) (0.68 g). The weight-average molecular weight of the resulting copolymer determined by GPC was 400,700 with a polydispersity of 6.2. Absorption spectroscopic analyses confirmed the ratio of NDI monomer to HEMA monomer for about 1:1100 in copolymer P(NDI−HEMA).

Methods for Spectral Determination of Copolymeric Film. The sensor film based on fluorescent P(NDI−HEMA) was obtained by spin-coating a 5 wt % methanol solution of copolymer P(NDI−HEMA) on quartz substrate, which was washed thoroughly with deionized water. The film-coated quartz as sensor patches was inserted and firmly attached on the quartz cuvette inner-wall. In order to eliminate the effect of total-reflected light in fluorescence determination of P(NDI−HEMA) film, the angle of incident light of about 35 degree was chosen to determine the fluorescent response to pH (Figure 2) with a miniature fiber optic spectrometer and accessories from Ocean Optics to realize the convenient and noninvasive monitoring.

■ ASSOCIATED CONTENT

Supporting Information. ^{1}H NMR, ^{13}C NMR, and HRMS (TOF−ESI$^+$) spectra of compounds **1** and **2** and monomer NDI and characterization of P(NDI−HEMA). This material is available free of charge via the Internet at http://pubs.acs.org.

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■ DEDICATION

Dedicated to Professor Fosong Wang on the occasion of his 80th birthday.

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