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A fluorescent colorimetric pH sensor and the influences of matrices on sensing performances

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Abstract

A fluorescent colorimetric pH sensor was developed by a polymerization of a monomeric fluorescein based green emitter (**SM1**) with a monomeric 2-dicyanomethylene-3-cyano-4,5,5-trimethyl-2,5-dihydrofuran derived red emitter (**SM2**) in poly(2-hydroxyethyl methacrylate)-*co*-polyacrylamide (PHEMA-*co*-PAM) matrices. Polymerized **SM1** (**PSM1**) in the polymer matrices showed bright emissions at basic conditions and weak emissions at acidic conditions. Polymerized **SM2** (**PSM2**) in the polymer matrices exhibited a vastly different response when compared to **PSM1**. The emissions of **PSM2** are stronger under acidic conditions than those under basic conditions. When **SM1** and **SM2** were polymerized in the same polymer matrix, a dual emission sensor acting as a ratiometric pH sensor (**PSM1,2**) was successfully developed. Because the **PSM1** and **PSM2** exhibited different pH responses and separated emission windows, the changes in the emission colors were clearly observed in their dual color sensor of **PSM1,2**, which changed emission colors dramatically from green at pH 7 to red at pH 4, which was detected visually and/or by using a color camera under an excitation of 488 nm. In addition to the development of the dual color ratiometric pH sensor, we also studied the effects of different matrix compositions, crosslinkers, and charges on the reporting capabilities of the sensors (sensitivity and pK_a).

Keywords

pH sensors; colorimetric sensors; matrix; charge; crosslinkers

1. Introduction

Measuring pH is a widely applied principle, however, is important for environment, ecology, agriculture, industry, food, and biology. Optical pH sensors based on changes of emission intensity and lifetime have been intensively studied as they can be non-invasive or minimally invasive, disposable, easily miniaturized (down to sub-micrometer), and simple to process (as a coating or solid layer on optical fibers and certain surfaces) for investigation of biology, environmental analysis, medical diagnosis, and process control [1 – 4]. Some

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Supplementary Data: Detailed schematic drawing of the preparation of the sensor films, stability and reversibility of the sensors, and the mechanism of tautomerization of the fluorescein units were given.

optical pH sensors have been integrated with other sensors for simultaneous monitoring of biological and environmental (multi)-parameters [5–9]. In order to fulfill the requirements for pH sensing in environment, agriculture, industry, and biology for extracellular pH values, optical pH probes needed to be physically incorporated or chemically immobilized with a proton permeable polymer matrix, such as collagen, polyurethane hydrogel, or poly(2-hydroxyethyl methacrylate) [5–9]. Leaching of the physically doped sensors from the matrices is a significant problem [5] which may result in signal instability, inaccuracy of the resultant measurements, decreased long-term applicability, and potential cytotoxicity for cells. To alleviate the leaching problem, optical sensors have been conjugated into suitable matrices through either chemical grafting of the probes onto matrices [9] or polymerization of the probes with the precursors/monomers of the matrices [6, 7, 8, 10]. We have been investigating thin film (membrane)-based sensors derived from polymerizable monomeric pH probes [10] and their dual sensors [7,8] for extracellular pH analysis. The pH probe was polymerized with other necessary monomers including a crosslinker to form a stable gel membrane (Figure 1). Thus the probes were chemically immobilized in the matrices formed by the polymerization of the monomers and crosslinkers. It is a generally accepted fact that if the chemical structures of the probes are different, their sensing performances are different even if they are in the same polymer matrix. However, little information is available on how the structures of the matrices for the same sensing probes in terms of matrix compositions, charge densities, and crosslinker concentrations and structures affect the sensing capabilities of the sensors.

Consequently, scientists are currently focusing on the development and use of fluorescence - based colorimetric approach for sensing studies [11–17] either through fluorescence intensity ratios [11–16] or by fluorescence lifetime referencing method [17]. The materials suitable for colorimetric imaging, which can be taken by conventional digital color cameras, should possess at least two separated emissions either from the same fluorophore or from different fluorophores. The colorimetric imaging is believed to be able to (1) act as an alternative scientific instrument of spectrofluorophotometer for ratiometric imaging with one excitation wavelength and multiple (three wavelengths of red-green-blue (RGB)) readouts and (2) produce small and inexpensive analytical devices.

Herein, we will address a few universal aspects for pH sensor study. (1) To investigate the influences of the matrix compositions on the sensing performance. We chose to study two monomeric pH probes, **SM1** [18] and **SM2** [19] (Figure 1). **SM1** is based on the mechanism of intramolecular charge transfer to manipulate the responses [20]. **SM1** exhibits a strong green emission at basic conditions, while producing a weak emission at acidic conditions. **SM2** is based on a photo-induced electron transfer (PET) mechanism [20]. **SM2** shows strong emission at acidic conditions, while weak emission is produced at basic conditions. (2) To prepare ratiometric sensors with high sensitivity and a broad dynamic range for pH value measurement. We integrated **SM1** and **SM2** into the same matrix. The dual color pH sensor (**PSM1,2**) will possess two emission colors suitable for ratiometric measurements and will achieve high sensitivity because of their opposite pH responsive trends. (3) Because of the well separated emission spectra of the individual **SM1** and **SM2**, the emission changes of the integrated dual color sensor can not only be measured using spectrofluorophotometer, but can also be visualized by eyes or cameras. Therefore, this dual color sensor is also an emission-based colorimetric sensor. This study divulges mechanics behind the development of advanced small and inexpensive analytical devices, which may broaden the application of these sensors in the future.

2. Experimental Section

2.1 Materials and reagents

All chemicals and solvents were of analytical grade and were used without further purifications. *N,N*-dimethylformamide (DMF), trimethylsilylpropyl acrylate (TMSPA), 2-hydroxyethyl methacrylate (HEMA), acrylamide (AM), 2-(methacryloyloxy)ethyltrimethyl ammonium chloride (METAC), poly(ethylene glycol)-dimethacrylate (PEGDMA, $M_n = 550$), and azobisisobutyronitrile (AIBN), were commercially available from Sigma-Aldrich (St. Louis, MO) and were used without further purification. **SM1** and **SM2** were synthesized according to previous publications [18, 19]. Ethyloxylate trimethylolpropane triacrylate (SR454®) is a commercial product of Sartomer Company (Exton, PA). 2-(Methacryloyloxy)ethylsulfonic acid sodium salt (MESA) was purchased from TCI America (Portland, OR). Doubly distilled water was used for the preparation of the buffer solutions. Britton-Robinson (B-R) buffers composed of acetic acid, boric acid, phosphoric acid, and sodium hydroxide were used for tuning pH values. For fluorescence measurements, quartz glass from University Wafer (South Boston, MA) was cut into squares of 1.31 cm × 1.31 cm using a dicing saw (Microautomation, Billerica, MA).

2.2 Polymerization – the preparation of PHEMA-co-PAM copolymer sensor thin films

Thin films were prepared according to our published protocols [7, 8, 10, 21]. The preparation of the membrane is given in Figure 1. More detailed schematic drawing of the film preparation is given in Supplementary Data (S-Figure 1). The components for the films, possessing different ratios of polyHEMA (PHEMA), polyAM (PAM), polyPEGDMA (PPEGDMA), polyS454 (PSR454), poly(METAC) (PMETAC), and/or poly(MESA) (PMESA) were given in Table 1 and Table 2. Film of F5 is described here as a typical example. 1 mg of the monomeric **SM1**, 800 mg of HEMA, 150 mg of AM, 50 mg of PEGDMA, and 10 mg of AIBN were dissolved in 1 mL DMF as a stock solution. 15 μ L of the stock solution was added onto the surface of the TMSPA-modified quartz glass and covered with a clean but untreated cover slip to make a sandwich structure. Using TMSPA to modify the quartz glass was to enable the sensors and matrices to be chemically grafted onto a quartz substrate [7, 8, 10, 21]. The thickness was controlled using 25 μ m Kapton tape (DuPont, Wilmington, DE). The sandwich set-up was placed into a vacuum oven, which was then evacuated and refilled with nitrogen three times. Polymerization was carried out under nitrogen at 80 °C for 1.5 hours in the oven. The quartz glasses with polymer membranes were removed from the oven, with Kapton tape and non-surface modified glass being removed from the polymerized membrane surface. The polymer membranes on the quartz glasses were washed three times using methanol to remove any remaining non-polymerized monomers and residual DMF. The films were dried and stored in the dark at room temperature.

2.3 Instruments and characterization

An oxygen plasma cleaner (Harrick Plasma, Ithaca, NY) was used for quartz glass surface activation. A Shimadzu UV-3600 UV-VIS-NIR spectrophotometer (Shimadzu Scientific Instruments, Columbia, MD) was used for absorbance measurements. A Shimadzu RF-5301 spectrofluorophotometer was used for fluorescence measurements. For easy measurement of the films in liquid solutions, quartz glass was cut with a dicing saw into squares of 1.31 cm × 1.31 cm, which can fit diagonally into a quartz fluorescence cuvette to enable the sensing membrane be positioned at an angle of 45° to the excitation light. The pH values were determined with a digital pH meter (Thermo Electron Corporation, Beverly, MA) calibrated at room temperature (23 ± 2 °C) with standard buffers of pH 10.01, 7.00, and 4.01.

For getting color images of the dual color pH sensor using a camera, a 488 nm laser under a Nikon Eclipse Ti Microscopy was used to excite the sensor film. A Nikon D3000 camera was connected to the microscope for taking pictures.

3. Results and discussions

3.1 pH responses of the sensing membranes derived from SM1 (PSM1)

3.1.1 Film preparation—PHEMA, PAM, and their composites were chosen as the matrices, since they are excellent ion permeable hydrogels [22–25]. PEGDMA and SR454 were used as the crosslinkers with different hydrophilicity and hydrophobicity. PMETAC was used a positive charge polymer and PMESA was used as a negative charge polymer. Fourteen films (Table 1) were prepared for the investigation of the influence of various weight ratios of PHEMA, PAM, crosslinkers, and charge densities on the sensing performances. F1 is a PAM sensing film without PHEMA components. F2 is a PHEMA sensing membrane without PAM segments. F3, F4, and F5 are three sensing films with different weight ratios of PHEMA to PAM. F6 to F10 are PHEMA-co-PAM derived films with tunable charge densities by the copolymerization of the neutral HEMA and AM with the charge-containing METAC or MESA. F5, F11, and F12 are the films using different weight percentages of PEGDMA in the PHEMA-co-PAM matrix. F13 and F14 are the films using SR454 as the crosslinkers.

3.1.2 Influences of matrix compositions on sensing performance—Figure 2 showed the typical UV-Vis absorption, emission spectra of F5 at various pH values, and its sensing mechanism. The F5 film showed stronger emissions at basic conditions and weaker emissions at acidic conditions. All these changes are due to the differences of the intramolecular charge transfers of the fluorescein moieties from a conjugated dianionic tautomer at basic conditions to a non-conjugated lactone tautomer at acidic conditions (Figure 2C) [26]. More detailed structures of the tautomers of the fluorescein moieties by pH were given in Supplementary Data (S-Figure 2). The dianionic tautomer is a conjugated moiety, which exhibited strong absorption and strong green emissions. While the lactone tautomer is a non-conjugated segment, which neither absorb the light around 488 nm significantly nor emit light strongly.

Fluorescence intensity change by ratio at 520 nm was plotted at Figure 2D. The sensor has a dynamic range from pH 5.5 to pH 7.5, indicating its suitability for biological application. The fluorescent intensity at pH 9 is 100 fold larger than that at pH 3, showing the high sensitivity of the pH sensor.

The fluorescence intensity changes can be described well by a sigmoidal function (Boltzmann fitting) as shown in equation 1 and plotted in Figure 2D.

$$\frac{I}{I_0} = \frac{m1 - m2}{1 + \exp(\frac{pH - pK'_a}{p})} + m2 \quad (1)$$

I and I_0 are the fluorescence intensities measured at varying pH values and that at the lowest pH value (pH 3) used during the titration, respectively. $m1$, $m2$, pK'_a , and p are empirical parameters describing the initial value ($m1$), the final value ($m2$), the point of inflection (pK'_a), and the width (p) of the sigmoidal curve. The apparent pK'_a value (pK'_a) for F5 was calculated to be 6.67 for F5. The fitting was highly reliable with a correlation coefficient (R^2) of 0.995.

Figure 3A showed the ratios of the fluorescence intensities at various pH values via the intensities at pH = 3 of the films F1 – F5. Exact ratios and pK'_a values were given in Table 1.

The sensor film of F5 with a ratio of HEMA to AM of 80:15 by weight exhibited the best sensitivity (e.g, the highest value of $I_{\text{pH}=9}/I_{\text{pH}=3}$) among the few compositions we studied. This result indicated that the chemical compositions of the PHEMA-*co*-PAM changed the H^+ permeability. It is known that both the PHEMA and PAM have good swelling properties and ion permeability. However, their swelling and ion permeability could be further improved by their copolymerizations with various ratios. Suitable composite materials can increase the swelling degree (W) and therefore increase the range of pore sizes and size distribution in the hydrogel film [27], which affects the pH responses of the sensors.

We found that charges of polymer matrices also affected the sensing activities of membranes (Figure 3B). Increasing the density of negative charge on the film by MESA did not change the sensitivity to pH. In contrast, increasing the density of positive charge by METAC decreased the response sensitivity and also shifted the $\text{p}K_a$ values to low pH values. This should be attributed to that ion permeability and the water swelling properties of the various composite materials were affected by the charged polymers.

Crosslinker densities and species also affected the sensing performances (Figure 3C). Increasing the crosslinker densities decreased the sensitivity and shifted the $\text{p}K_a$ to low values, which can be found by a comparison of F5, F11, and F12 or a comparison of F14 and F15. When there is more crosslinker present, the network becomes much tighter, which may result in the decrease of swelling ability of the hydrogel and therefore the difficulty of protonation and deprotonization of the pH sensor in the matrices. Using SR454 instead of PEGDMA decreased the sensitivity significantly. Most likely, the films with the hydrophobic and short crosslinker of SR454 possess much tighter networks and smaller swollen ratios than those with PEGDMA.

3.2 pH responses of the sensing membranes derived from SM2 (PSM2)

3.2.1 Membrane preparation and sensing mechanism—Table 2 lists the films of **PSM2** (F15 – F29) with various compositions. Sixteen films were prepared for investigation of the influence of various weight ratios of PHEMA, PAM, crosslinkers, and charge densities on the sensing performances.

Figure 4 shows the typical UV-Vis absorption and emission spectra of F16 at various pH values. The pH sensor 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. Because of the strong electron-donating and -withdrawing units conjugated within the sensing moiety, SM2, the fluorophore exhibits an absorption maximum around 500 nm and emits in the red spectral window. A quasi-isosbestic point from the absorbance spectra was observed at 528 nm, showing that the sensor response to pH is through a single acidification and basification mechanism. The emission maxima shifted from 640 nm at pH 9 to 610 nm at pH 3 with the emission intensity increases as the pH decreases. The emission intensity change follows a sigmoidal (Boltzmann fitting, equation 1). The fluorescence intensity changes and their curve fittings are shown in Figure 7C. The apparent $\text{p}K_a$ value ($\text{p}K_a'$) was 5.33 with a correlation coefficient of 0.995.

Fluorescence intensity change of **PSM2** was ascribed to photo-induced electron transfer (PET) in the pH sensor being suppressed by the protonation of the amino group. 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 (Figure 4C) [6–10]. In the piperazinyl group of **SM2**, the nitrogen atom in NCH_2CH_2 is not directly connected to the TCF-conjugated fluorophore, of which the NCH_2CH_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 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.

3.2.2 Influences of matrixes on sensing performance—Figure 5A showed the ratios of the fluorescence intensities at various pH values versus the intensities at pH = 9 of the films F15-F19. Exact ratios and pK_a values were given in Table 2. The sensor film of F16 with a ratio of HEMA to AM of 80:15 by weight exhibited the best sensitivity (e.g, the highest value of $I_{pH=3}/I_{pH=9}$). This phenomenon is consistent with that of **PSM1**, showing the influences of the chemical compositions of the PHEMA-co-PAM on the sensing behaviors.

Figure 5B plots the charge influences on the sensing performances. Increasing the density of negative charge on the film by MESA did not significantly change the sensitivity to pH, however, it did significantly shift the pK_a from 5.33 of F16 to ~7.7 of F20 and F21. Increasing the amount of positive charge segments of METAC decreased the sensitivity and shifted the pK_a to low pH.

Crosslinker densities and species also affected the sensing performances of **PSM2** (Figure 5C and 5D). Increasing the crosslinker densities decreased the sensitivity and shifted the pK_a to low values. Using SR454 instead of PEGDMA decreased significantly the sensitivity. This trend is consistent with those of the films with **PSM1**.

3.3. Dual color PSM1,2 sensor (F30, F31, F32)

A dual color sensor was prepared by copolymerization of **SM1** and **SM2** in the neutral PHEMA-co-PAM (80:15 by weight) film with 5% of PEGDMA, because the individual **PSM1** (F5) and **PSM2** (F16) have good sensitivity using the matrix with the above mentioned composition. Three films (F30, F31, and F32) with variable ratios of **SM1** and **SM2** were prepared (Table 3). Typical sensor's responses of F32 to pH at the excitation wavelength of 488 nm were given in Figure 6A. The green emission at 520 nm decreased with the decrease of pH. The red emission at 612 nm increased with the decrease of pH value. At pH 9, mainly green emission was observed; while only the red emission is dominated at pH 3.

A few methods were used to calculate the pK_a and the application range of the dual color sensor and compare the sensitivity of the dual color sensor with its corresponding individual sensors. Method 1: Only the intensity at 520 nm was concerned. The intensity ratios by pH were plotted in Figure 6B. The pH sensitive range is from 6 to 8 with a pK_a of 6.89, which is close to the individual **PSM1** sensor (F5). The comparison of the sensing performance is given in Table 3. Method 2: Only the intensity at 612 nm was considered. The intensity ratios by pH were plotted in Figure 6B. The pH sensitive range is from 4.5 to 7 with a pK_a of 5.85, which is close to the individual **PSM2** sensor (F16). Method 3: Ratiometric approach using the ratios at 520 nm to 612 nm or reverse was used. The data was plotted in Figure 6C. By using the ratios of 520 nm divided by 612 nm, the pK_a of 7.09 was calculated with a sensitive range of 6 to 8; by using the ratios of 612 nm divided by 520 nm, pK_a of 5.01 was calculated with a sensitive range of 4 to 7. Because the dual color sensor is composed of the two individual **PSM1** and **PSM2** sensors, this sensor has a pH dynamic range from 4 to 8. This dynamic range is broader than those of the individual sensors.

In order to discern whether the dual color sensor has a higher sensitivity than its individual sensors, we used method 4 to perform the calculation. The intensity ratios of I_{520}/I_{612} at various pH were plotted against the I_{520}/I_{612} at pH3. This plot reflected the changes of the fluorescence intensity ratios by pH. It was found that the ratio at pH 9 was 200 folds of that

at pH 3. The reverse order of I_{612}/I_{520} generated similar results. The calculated results were plotted in Figure 6D. The data shown in Figure 6D indicated that the dual color sensor exhibited higher pH sensitivity than its individual sensors, showing the advantage of the dual color sensor. Most likely, this improvement of the sensitivity is due to the fact that the dual color sensor is an integration of two individual sensors with opposite pH responsive trends.

The comparison of the sensing performance of the three films with variable ratios of **SM1** and **SM2** in their matrices was given in Table 3. It was found the pK_a values calculated by the three methods as described above were not affected significantly by the compositions of the fluorophores of **SM1** and **SM2** in the dual color sensor films. This observation means most likely no significant fluorescence resonance energy transfer occurred between the two kinds of fluorophores (**SM1** and **SM2**) in the thin films at our experimental conditions.

3.4 Colorimetric sensor

Because the colorimetric sensor possesses two emission colors at green and red spectral windows, changes in the pH dependent emission colors by pH were observed. Figure 7 showed the emission colors of the dual color sensor (F32) at different pH, taken by a camera (Nikon D3000) using 488 nm light as the excitation light source. Green emission dominated in the pH range of 7 to 9. Significant change of the color was observed from pH 7 to pH 4. For quantifying the pH depended emission color, we used Adobe Photoshop CS3 to analyze the pictures. Under the RGB model, the intensities of red (R) and green (G) from the pictures were obtained and their ratios were plotted against pH (Figure 7H). It was determined that the intensity ratios of G/R were sensitive to pH from 4 to 7, and that the ratios of R/G were sensitive to pH in the range of pH 3 to 6. Therefore, the data analysis indicated that the colorimetric sensor can be used to quantify pH from 3 to 7.

4. Conclusion

Two different monomeric pH probes were polymerized into polymer matrices to form thin film sensors. Influences of the matrices including the compositions of the matrices, cross-linkers, and charges, on the sensing performance were studied. Results showed that PHEMA-co-PAM with a weight ratio of 80:15 is the best matrix for the two sensors, which exhibit the highest sensitivity among the few compositions that we studied. Hydrophobic crosslinker SR454 decreased the sensitivity and shifted the pK_a to low value as compared with the hydrophilic crosslinker of PEGDMA we used. The matrices with more cross-linkers exhibited lower sensitivity. Charges also affected the sensitivity and pK_a values. The studies on the fundamental aspects about the influences of the compositions of the sensor films on the sensing performances may provide insightful information for sensor design.

Because of the two individual sensors (**PSM1** and **PSM2**) possess different pH responsive trends and emission colors, their dual color sensor exhibited higher sensitivity to pH and broader responsive ranges than the individual sensors. The dual color sensor is also a colorimetric sensor. The colors can be easily visualized by the human eyes and commercially popular cameras, which showed plentiful and significant color differences in a pH range of 7 to 4. The color pictures can also be used to quantify pH values by the data analysis using the popular Photoshop program.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.
The pH probes and other monomers used for the sensor film preparation.

Figure 2. pH responses of F5 film. A) changes of absorbance by pH; B) changes of fluorescence by pH at an excitation wavelength of 488 nm; C) Simplified structural changes of the fluorescein moieties at strong basic and acidic conditions. Detailed intermediates between the two states were given in supporting S-Figure 2; D) fluorescence intensity (at 520 nm) ratios of F5, from pH 3 to pH 9. I_0 is the fluorescence intensity at pH 3.

Figure 3.

A) Fluorescence intensity (at 520 nm) ratios of films of F1 – F5, from pH 3 to pH 9. I_0 is the fluorescence intensity at pH 3; B) Influences of charges on sensing performances. I_0 is the fluorescence intensity at pH 3; C) Influences of cross-linkers and their concentrations on sensing performances. I_0 is the fluorescence intensity at pH 3.

Figure 4. pH responses of film of F16. A) changes of absorbance by pH; B) changes of fluorescence by pH at an excitation wavelength of 488 nm; C) Mechanism of the sensor of PSM2; D) fluorescence intensity (at 612 nm) ratios of F16, from pH 3 to pH 9. I_0 is the fluorescence intensity at pH 9.

Figure 5.

A) Influences of the ratios of HEMA and AM on sensing performances of PSM2; B) Influences of charges on sensing performances of PSM2; C) Influence of PEGDMA as the crosslinker on sensing performances of PSM2; D) Influence of SR454 as the crosslinker on the sensing performance of PSM2. For easy comparison, 5% of PEG was kept in D.

Figure 6.

A) pH responses excited at 488 nm; B) pH dependent fluorescence intensity ratios at 612 nm and 520 nm respectively; C) pH dependent fluorescence intensity ratios calculated by I_{612}/I_{520} and reverse; D) pH dependent ratios of I_{520}/I_{612} and reverse normalized at pH 3 or pH 9.

Figure 7. Color images of the colorimetric pH sensor PSM1,2 at different pH (A – G) from pH 9 to pH 3 and the ratios of the intensities at green and red channels at various pH (H).

Table 1

Compositions of the films of PSM1 (F1 to F14), their sensitivity, and pK_a values.

Films	Polymer compositions and their weight ratios							Sensitivity ($I_{pH=9}/I_{pH=3}$) ^{d)}	pK _a
	Main matrices		Crosslinkers			Charges			
	PHEMA	PAM	PEGDMA	SR454	PMETAC (+)	PMESA (-)			
F1 <i>a)</i>		95		5				57	6.58
F2 <i>a)</i>	95		5					35	6.24
F3 <i>a)</i>	15	80	5					59	6.41
F4 <i>a)</i>	50	45	5					60	6.36
F5 <i>a)</i>	80	15	5					102	6.67
F6 <i>a)</i>	80	15	5		5			53	5.62
F7 <i>a)</i>	80	15	5		10			28	5.10
F8 <i>a)</i>	80	15	5		20			25	4.66
F9 <i>a)</i>	80	15	5			10		102	6.86
F10 <i>a)</i>	80	15	5			20		108	6.89
F11 <i>b)</i>	80	15	10					107	6.63
F12 <i>c)</i>	80	15	20					68	6.03
F13 <i>d)</i>	80	15		5				31	6.31
F14 <i>e)</i>	80	15		10				12	5.53

a) Each film contains a 5% PEGDMA crosslinker

b) The film contains a 10% PEGDMA crosslinker

c) The film contains a 20% PEGDMA crosslinker

d) The film contains a 5% SR454 crosslinker

e) The film contains a 10% SR454 crosslinker

f) The sensitivity of the sensing films to pH values was calculated according to the ratios of fluorescence intensities (I at 520 nm) at pH = 9 and pH = 3 for comparison.

Table 2

Compositions of the films of PSM2 (F15 to F29), their sensitivity, and pK_a values.

Films	Polymer compositions and their weight ratios							Sensitivity ($I_{pH=3}/I_{pH=9}$) ^{d)}	pK _a
	Main matrices		Crosslinkers		Charges				
	PHEMA	PAM	PEGDMA	SR454	PMETAC (+)	PMESA (-)			
F15 <i>a)</i>	95	0	5				3.5	5.63	
F16 <i>a)</i>	80	15	5				5.7	5.33	
F17 <i>a)</i>	50	45	5				4.0	5.93	
F18 <i>a)</i>	15	80	5				3.3	6.17	
F19 <i>a)</i>	0	95	5				3.2	6.00	
F20 <i>a)</i>	80	15	5			5	5.3	7.73	
F21 <i>a)</i>	80	15	5			20	4.5	7.67	
F22 <i>a)</i>	80	15	5		5		4.1	5.87	
F23 <i>a)</i>	80	15	5		20		4.1	5.13	
F24 <i>b)</i>	80	15	10				4.1	5.57	
F25 <i>c)</i>	80	15	20				3.2	4.60	
F26 <i>d)</i>	80	15	40				1.7	--- <i>h)</i>	
F27 <i>e)</i>	80	15		5			2.7	6.61	
F28 <i>f)</i>	80	15		20			2.1	4.81	
F29 <i>g)</i>	80	15		40			1.3	--- <i>h)</i>	

a) Each film contains a 5% PEGDMA crosslinker

b) The film contains a 10% PEGDMA crosslinker

c) The film contains a 20% PEGDMA crosslinker

d) The film contains a 40% PEGDMA crosslinker

e) The film contains a 5% SR454 crosslinker

f) The film contains a 20% SR454 crosslinker

g) The film contains a 40% SR454 crosslinker

h) no reliable pK_a can be calculated

i) The sensitivity of the sensing films to pH values was calculated according to the ratios of fluorescence intensities (*I* at 612 nm) at pH = 3 and pH = 9 for comparison.

Table 3
Comparison of the sensitivity and pK_a values of the films of **PSM1,2** and their corresponding **PSM1** and **PSM2**.

Films	Sensor	$I_{pH=9}/I_{pH=3}$ (520 nm) ^{d)}	$I_{pH=3}/I_{pH=9}$ (612 nm) ^{e)}	$I_{520\text{ at }pH9}/I_{612\text{ at }pH9}$ $[(I_{520}/I_{612})_{pH9}]/[(I_{520}/I_{612})_{pH3}]$	$I_{612\text{ at }pH3}/I_{520\text{ at }pH3}$ $[(I_{612}/I_{520})_{pH3}]/[(I_{612}/I_{520})_{pH9}]$	pK_a [dynamic range]
F5	PSM1	102				6.58 [5.5–7.5]
F16	PSM2		5.5			5.50 [4.0–7.0]
F30 ^{a)}	PSM1,2	110	1.8	50 ^{f)}	4.3 ^{g)}	6.75 [6–8] ^{d)}
				[209] ^{h)}	[210] ⁱ⁾	5.41 [4.5–7] ^{e)}
F31 ^{b)}	PSM1,2	94	2.0			7.16 [6–8] ^{j)}
						4.60 [4–7] ^{g)}
						7.15 [5.5–8] ^{h)}
						4.67 [4–6] ⁱ⁾
				38 ^{f)}	5.6 ^{g)}	6.76 [6–8] ^{d)}
				[213] ^{h)}	[213] ⁱ⁾	5.26 [4.5–7] ^{e)}
F32 ^{c)}	PSM1,2	44	4.5			7.10 [6–8] ^{j)}
						4.72 [4–7] ^{g)}
						7.24 [5.5–8] ^{h)}
						4.60 [4–6] ⁱ⁾
				12 ^{f)}	13 ^{g)}	6.89 [6–8] ^{d)}
				[200] ^{h)}	[200] ⁱ⁾	5.85 [4.5–7] ^{e)}
						7.09 [6–8] ^{j)}
						5.01 [4–7] ^{g)}
						7.02 [5.5–8] ^{h)}
						4.69 [4–6] ⁱ⁾

^{a)} The weight ratio of SM1:SM2 is 1:1

^{b)} the weight ratio of SM1:SM2 is 1:5

^{c)} the weight ratio of SM1:SM2 is 1:10

- d) fluorescence intensity at 520 nm was used for ratio calculation
- e) fluorescence intensity at 612 nm was used for ratio calculation
- f) fluorescence intensity ratios were calculated by using I_{520nm} divided by I_{612nm}
- g) fluorescence intensity ratios were calculated by using I_{612nm} divided by I_{520nm}
- h) the ratios were calculated by using the fluorescence intensity ratios of I_{520nm}/I_{612nm} at pH 9 by I_{520nm}/I_{612 nm} at pH 3
- i) the ratios were calculated by using the fluorescence intensity ratios of I_{612 nm}/I_{520 nm} at pH 3 by I_{612 nm}/I_{520 nm} at pH 9.