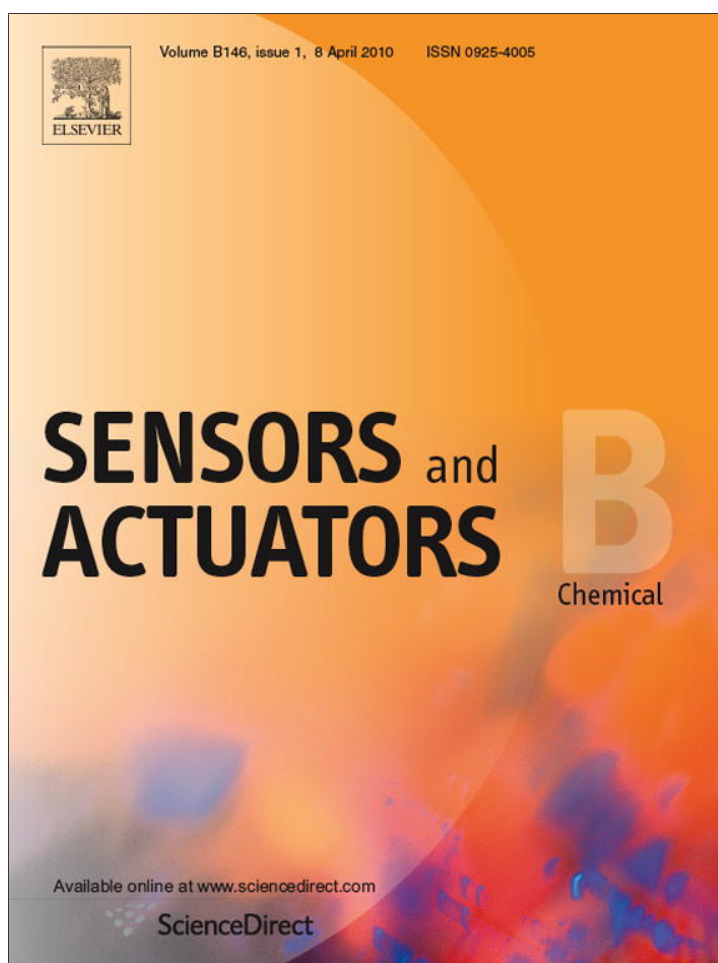


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Colorimetric optical pH sensor production using a dual-color system

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ABSTRACT

Utilizing a dual color design, a rapid, convenient and in situ applicable pH sensor was constructed based on a dual light-emitting diode (LED) system. One LED lamp with a maximum emission wavelength of 465 nm was used to excite the pH fluorescent indicator, 5(6)-carboxyfluorescein (CF), and the other LED lamp with red color emission (λ_{\max} 660 nm) was selected to supply a stable color background (the reference light). The dual light from the fluorescent emission of CF and the background light were captured using a commercial charge coupled device camera. Under constant and stable background light, the change of the CF fluorescence intensity in solutions at different pH values appeared as different colors. The sensor characteristics, including the effects of temperature, co-existing ions, and the sample analysis performance, were investigated.

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1. Introduction

Because of its important role in numerous areas, such as life science, environmental analysis and industry applications [1–4], pH determination has rapidly developed in recent decades. At present, three approaches, namely pH test paper strips, electrochemical pH sensors and photochemical pH sensors [3,4], have been widely applied in practice. The pH test paper strip emerged early and has gained widespread application in numerous fields due to its simplicity and convenience, but compared with pH sensors, it still has many disadvantages, such as irreversibility, contamination of the environment, unfeasibility of online use, and no high throughput analysis or automatic detection. In addition, the pH test paper strip cannot be applied in a dark environment since it is based on a light absorbance mechanism.

Many pH sensors have been constructed based on electrochemical or photochemical principles. Compared with the pH test paper, although they lack convenience and handy detection, they overcome many drawbacks, such as reversibility and accurate determination. Until now, there have been many commercialized electrochemical pH sensors and successful applications. However, many problems such as signal interference and drift after long-term usage limit their further development. Photochemical pH sensors, as a favorable supplement to electrochemical pH sensors, have had

more emphasis placed on them in recent years. However, both of these sensors are based on complicated data processing and are sophisticated instruments, and this limits their application in various fields. For instance, optical pH sensors are always made based on the analysis of fluorescence intensity, lifetime and wavelength shift [5,6], which generally requires complicated scientific instruments.

Colorimetric detections [7–9] have attracted much attention due to their special advantages, including rapidly readable responses, and a feasible and simple detection approach. Although colorimetric electrogenerated Chemiluminescence (ECL) sensor fabricated by Chen group [9] is with the potential to be applied in pH measurement, the necessary applied potential using electrodes in ECL might become a bar for its application in a microenvironment such as cells or living organs. Different from ECL, fluorescence sensing approaches based on photoexcitation reduce the limitation, and have made great progress in cell image. Recently, a sensitive pH probe using multicolor micellar complexes was reported [7]. In that research, different pH values can be distinguished by micellar complexes assembling two kinds of fluorescent polymers, and the multicolor micellar can be applied to measure the pH value quickly and so is favorable for application in bioanalysis.

Derived from the inspiration of the traditional pH test paper and the newer colorimetric detection, we made a colorimetric optical pH sensor which presented sensitive color change in the pH range 1.0–7.7. This sensor retained the traditional functions of the pH test paper, and had characteristics such as simple structure, small size, feasibility, and low-cost, but combined them with the abilities of real-time and in-situ application.

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2. Experimental

2.1. Chemical reagents and materials

5(6)-Carboxyfluorescein (CF) and triton X-100 were purchased from Sigma–Aldrich (St. Louis, MO, USA), and tetramethoxysilane (TEOS) was obtained from the Silicone New Material Co. of Wuhan University (Wuhan, China). Buffer solutions were prepared with potassium dihydrogen phosphate/disodium hydrogen phosphate ($\text{KH}_2\text{PO}_4/\text{Na}_2\text{HPO}_4$) and the pH values of the buffer solutions were measured using a commercial digital pH meter (CyberScan pH 510, EUTECH). All chemicals were used as received and without further purification, and all the aqueous solutions were prepared using deionized distilled water. Mineral water, pure water and white vinegar were purchased from a local supermarket, rain samples were collected around Xiamen University, and the acidic wastewater was from our laboratory.

2.2. Preparation of the pH sensing film

In the preparation of the pH sensing film, a glass slide was selected and used as a base for the immobilization of the pH sensing film. Before the film preparation, the glass slide was submerged into 1 mol L^{-1} NaOH for 24 h, then, purged thoroughly with anhydrous ethanol and pure water. The pH sensing film was prepared following previous descriptions [10–13]. Typically, 1.0 mL TEOS, 1.0 mL dehydrated ethanol, 0.5 mL of 0.1 mol L^{-1} HCl and 20 μL Triton X-100 were mixed to produce a sol–gel solution in a glass vial, which was stirred for 24 h but with magnetic stirring. This sol–gel solution and 1 mg mL^{-1} CF ethanol solution (v/v, 5:1) were mixed and stirred to ensure homogeneity, and then a clean glass slide was dipped into the mixture. The prepared pH sensing film was dried for 24 h in darkness at room temperature, and stored in phosphate buffer solution (pH 5.8) ready for use.

2.3. Setup of colorimetric pH sensing system

Based on the dual color mechanism, we produced an optical colorimetric pH sensor based on a dual light-emitting diode (LED) system that is economically efficient and easy to use. In the pH sensor preparation, CF was chosen as the optimal pH indicator since it has a strong fluorescent emission and sensitivity towards pH variation. Two reciprocally vertical LED lamps with maximum wavelengths of 465 nm and 660 nm were set in the colorimetric system (Fig. 1). The blue LED ($\lambda_{\text{em,max}}$ 465 nm) acted as a light source

to excite the pH indicator dye, and the red one ($\lambda_{\text{em,max}}$ 660 nm) was selected as the color background.

The prepared pH sensing film was installed in a 4-way cuvette holder (Ocean Optics Co., USA) and a commercial charge coupled device (CCD) camera (Canon Co. Ltd, Japan) was used to capture the images. In order to obtain optimal images, the parameters of the CCD camera were set as follows: white balance, cloudy; focus, macro-mode; aperture, $F=4.5$; ISO sensitivity, ISO 200; and shutter speed, $1/5 \text{ s}$. A Hitachi F-4500 Fluorescence Spectrometer (Hitachi Co. Ltd, Japan) was used to record the fluorescence spectra.

3. Results and discussion

3.1. Optimization of experimental conditions

In the configuration of the colorimetric optical pH sensor, the background light with the maximum wavelength of 660 nm played two roles in the sensing system. It supplied a stable and optimal light intensity to mix with the CF fluorescence, which transformed the intensity signal into color. In the second role, the background light could also be applied as a reference light [14] to calibrate the effects of temperature and excitation fluctuation.

Due to the narrow Stokes shift of CF, the excitation light from the blue LED affected the apparent color. To eliminate the disturbing signal, a favorable filter and a convex lens were assembled in the 4-way cuvette holder. The blending signals from the background light and fluorescence emission of CF were filtered and focused. Furthermore, the light pathway of the excitation light was specially considered. As shown in Fig. 1, the excitation from the upper side of the sensing film resulted in an excellent removal of the light influence from the blue LED.

In order to obtain a distinct apparent color, the intensity of the color background light was taken into consideration. In different pH buffer solutions, the responses of the pH sensor in terms of their fluorescence spectra are shown in Fig. 2(a). Favorable corresponding color images were captured by the CCD camera and the results are presented in Fig. 2(b). It was difficult to make colorimetric identification without the red background light, as shown in the upper row of Fig. 2(b), but when the red background light was applied, as shown in the lower row, the sensor presented obvious color change in different pH solutions. This proved that a suitable background light intensity played a primary role in the colorimetric determination. Based on the results shown in Fig. 3, a different intensity of LED would contribute to a different color resolution. On the second row, obviously, satisfactory color resolution was obtained under suitable background light intensity. Under the selected intensity, the colorimetric optical pH sensor gave an ideal resolution up to 0.3 pH unit and distinguishable apparent color in the pH range 1.0–7.7.

3.2. Reversibility, reproducibility and duration of usage

Compared with traditional pH test paper, as we noted above, the colorimetric optical pH sensor presented reversible and reproducible responses to pH change. Response time was just several seconds and in the experiments, a 15-s interval was selected for individual sample measurement. After each circulation (from pH 1.0 to 7.7), the sensing film was immersed in a pH 5.8 buffer solution to test its reproducibility. As shown in Fig. 4, homogeneous and good reversible color images could be obtained. In addition, after 10 continuous measurements in a period of 2.5 h, the relative standard deviation of the fluorescence intensity of the pH sensing film was less than 3.5% in pH 5.8 buffer solution at 25°C . After storage in the buffer solution (pH 5.8) at room temperature for one month, the fluorescence intensity of the pH sensing film still remained at 90.5% of the original intensity, resulting in stable color changes in

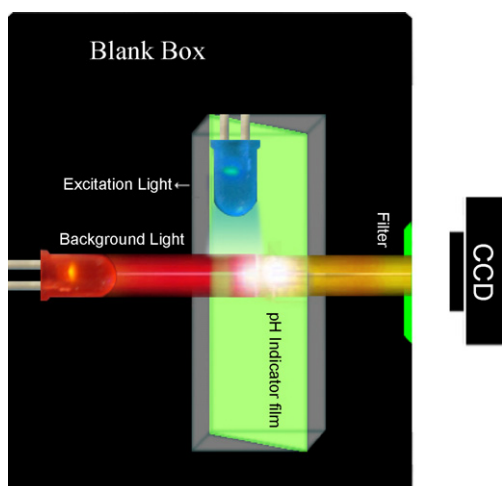


Fig. 1. Schematic diagram of the colorimetric optical pH sensor system.

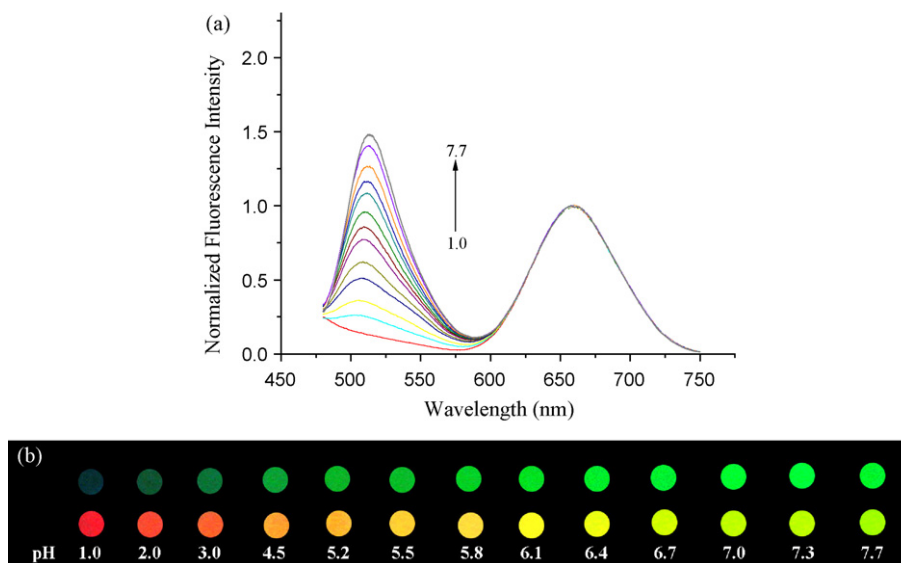


Fig. 2. (a) Responses of the pH sensor towards pH values ranging from 1.0 to 7.7. (b) Color images with background light (lower row) and without background light (upper row) captured by a CCD camera. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

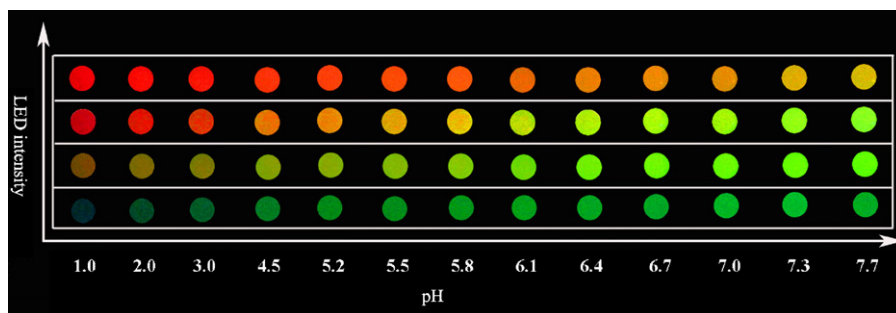


Fig. 3. Color images from pH 1.0 to 7.7 under different background light intensity. The second row (from up to down) shows ideal images with optimal LED intensity. For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

different pH solutions. These results indicated that the proposed colorimetric optical pH sensor had good reproducibility and a long life for practical applications.

3.3. Temperature effect

Generally, temperature affects the fluorescence efficiency of a fluorophor, and a favorable fluorescence sensor should possess the ability to function at different temperatures. Thus, we investigated the temperature effect ranging from 5 °C to 60 °C on the pH 5.8 solution. In Fig. 5, the first row reveals that temperature gener-

ated obvious effects on the color images and, with an increase in temperature, fluorescence intensity of the CF decreased sharply. In our research, all investigations were carried out below 25 °C. In order to reduce the temperature effect, several models generally were established to calibrate the detection results. Obviously, this requires an exclusive accessory temperature sensor and complicated data processing. However, for the colorimetric optical pH sensor, according to the standard color strip shown in Fig. 2(b), we could simply modulate the background light intensity corresponding to the change in the fluorescence of the pH probe and maintain the ratio of the background light intensity to pH indi-

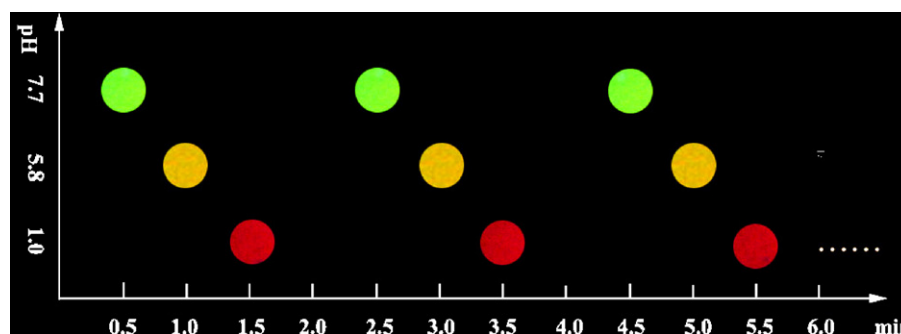


Fig. 4. Circular color images at pH values of 1.0, 5.8 and 7.7 captured by a CCD camera. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

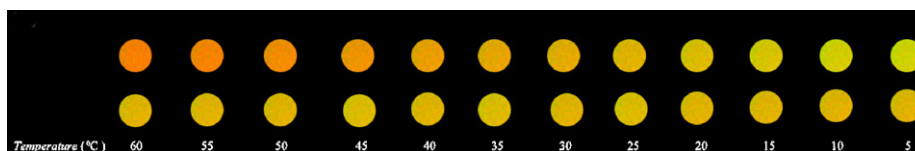


Fig. 5. Temperature effect on the CF in a pH 5.8 solution from 5 °C to 60 °C. The 1st row shows the result of the temperature effect on the color image; the 2nd row demonstrates the results after adjusting the intensity of LED light on the basis of Fig. 2(b). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.).

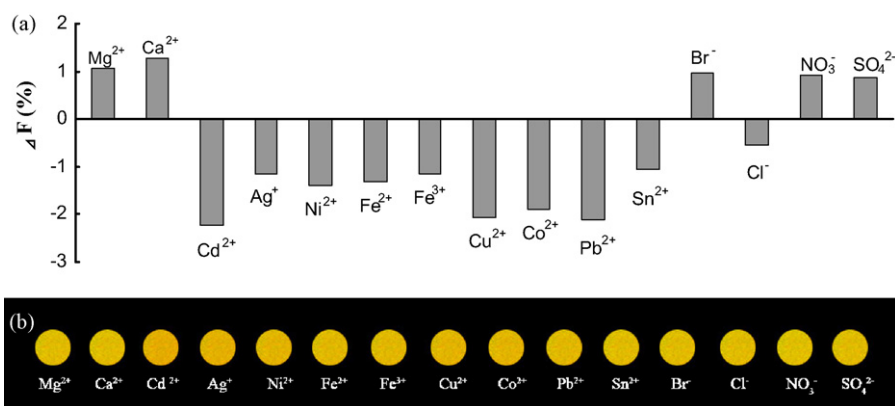


Fig. 6. Co-existing ion effect on the detection of pH value. (a) The fluorescence data for several typical ions in pH 5.8 solution. $\Delta F = F - F_0 / F_0 \times 100$, F is fluorescence intensity after adding co-existing ions and F_0 is fluorescence intensity without co-existing ions. (b) Color images of co-existing ion effect in the pH 5.8 solution. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.).

cator intensity (Fig. 2(a)). With the change of probe fluorescence intensity under different temperatures, the background light intensity of the red LED could be modified to obtain the most suitable color images (Fig. 2(b)), and this reduced the temperature effect. As shown in the first row of Fig. 5, without the modulation of the background light, the color images of the pH sensor in the pH 5.8 solution, changed from yellow-green to orange when the temperature changed from 5 °C to 60 °C. Using the intensity modulation of the background light, as shown in the second row of Fig. 5, no obvious color change could be found with the change in temperature from 5 °C to 60 °C.

3.4. Co-existing ion effect

The effects of common co-existing ions, including anion ($10^{-4} \text{ mol L}^{-1}$) and cation ($10^{-6} \text{ mol L}^{-1}$), were investigated in pH 5.8 buffer solution. As shown in Fig. 6(a), although Cd²⁺, Cu²⁺ or Pb²⁺ presented the most obvious negative effects on CF fluorescence intensity, less than a 2.5% decrease of only ΔF could be found. In addition, although the presence of Mg²⁺, Ca²⁺, Br⁻, NO₃⁻ and SO₄²⁻ slightly increased the fluorescence response of CF, the change of ΔF was less than 1.2%. Corresponding to these effects, it can be seen from Fig. 6(b) that no obvious color change could be found when the selected co-existing ion was present, which revealed that the pH sensor had a good response to the concentration change of H⁺.

3.5. Sample analysis

The colorimetric optical pH sensor was applied in water sample analysis. Rainwater, acid wastewater and some commercial water samples were selected, and the results for these samples were compared with results from a commercial electrochemical pH meter (Table 1). Referring to the standard color strip, as shown in Fig. 2(b), the results obtained were notable and favorable, and fitted well with the standard color strip. Comparing the electrochemical pH meter results with those obtained using the colorimetric optical pH sensor, we could judge the pH value of the selected samples well

Table 1

pH detection in water samples using the colorimetric optical pH sensor compared with a commercial pH meter.

Sample	pH Sensor	pH Meter	Color
Mineral water	7.3 ± 0.2	7.30 ± 0.05	
Pure water	6.7 ± 0.2	6.50 ± 0.02	
Rain water 1	6.1 ± 0.2	6.10 ± 0.08	
Rain water 2	6.7 ± 0.2	6.60 ± 0.05	
Rain water 3	6.1 ± 0.2	6.20 ± 0.03	
Acid effluent	3.5 ± 0.2	3.80 ± 0.03	
White vinegar	2.0 ± 0.2	2.10 ± 0.10	

with the pH sensor and the detection results are compatible with those obtained using the commercial electrochemical pH meter. Thus, we could easily discern the pH value ranges of targets within several seconds and such results proved that pH values could be read out directly using the colorimetric optical pH sensor.

4. Conclusions

In addition to the directly readable merit of the pH test paper, the colorimetric optical pH sensor which we developed based on a dual color mechanism exhibited good characteristics including reversibility, flexibility, low-cost and applicability in the dark. This sensor overcame the drawbacks of traditional pH sensors, including inconvenient and complicated data process using an instrumental

measurement mode. At the same time, the proposed colorimetric optical pH sensor exhibited good response in the pH measurement of water samples, utilizing the dual-color mechanism and nanotechnology, a kind of colorimetric pH probe could be conceived and great potential for its applications in biological sample analysis, especially in the cell environment could be foreseen.

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