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New Journal of Chemistry

ARTICLE

A Novel pH Sensor which Could Respond Multi–Scale pH Changes *via* Different Fluorescence Emissions

Received 00th January 20xx,
Accepted 00th January 20xx

DOI: 10.1039/x0xx00000x

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This paper designed and synthesized a multi–scale pH chemosensor **L6** based on ICT–TICT (an intramolecular charge transfer and a twisted intramolecular charge transfer) state change mechanism. The sensor **L6** exhibited two obvious pH jumps (pH ranged from 3.0 to 5.0 and 8.5 to 10.5, respectively) among different pH ranges. Thus, the sensor **L6** could indicate over a broad pH range including acidic, neutral and alkaline ranges by performing different fluorescence emissions (blue at pH 1.0–3.0, green at pH 4.5–8.5 and quenching at pH 10.5–13.5). Interestingly, owing to the relatively high water solubility, the whole sensing process could be observed successfully in DMSO/H_2O (v/v, 1/9) aqueous solution. These properties made the sensor **L6** have the potential to monitor pH variations over the entire physiological pH range (4.5–8.5).

1. Introduction

In the past decade, the development of fluorescent sensors for hydrogen ion, has attracted more and more interest of researchers,[1-9] because hydrogen ion, as one of the most important intracellular species, is closely related to cell growth and metabolism,[10] ion transport and homeostasis,[11] as well as neuronal excitability.[12] The disruption to the balance of the pH in different organelles may lead to the dysfunction of the affected organelle and even a diseased state, such as tumor,[13] renal failure,[14] lysosomal storage disorders and even cancer.[15] When a diseased state occurs, the pH related to many diseases lies in the acidic range. By contrast, in non-diseased state, the pH of the cytosol and the nucleus is typically 7.2–7.4.[16,17] However, there are few studies that have reported fluorescent sensors for discerning the above two ranges.

On the account of the aforementioned purpose and the previous research work,[18-21] this paper designed and synthesized an efficient fluorescent pH chemosensor which was sensitive to pH in both extremely acidic and neutral biological systems. The strategy for obtaining the pH chemosensor was illustrated as follows: firstly, in order to achieve high sensitivity, the coumarin group was introduced as fluorescent signal group. Secondly, owing to the fact that the benzimidazole group could act as a proton donor and acceptor at the same time, the benzimidazole was introduced to the sensor as proton response site. Finally, the ICT–TICT mechanism was employed during the response process of the pH sensor. The results showed that the obtained pH chemosensor **L6** could respond pH changes *via* different fluorescence emissions, blue at pH 1.0–3.0, green at pH 4.5–8.5 and quenching at pH 10.5–13.5. Meanwhile, the sensor **L6** exhibited two dramatic pH jumps (pH 3.0–4.5 and 8.5–10.5, respectively) among a broad pH scale. Furthermore, the relatively high water solubility allowed **L6** to make the same response to pH successfully in DMSO/H_2O (v/v, 1/9) aqueous solution. Thus, **L6** was an excellent fluorescent chemosensor to respond pH change.

2. Experimental

2.1. Materials and instruments

^{1}H NMR spectra were recorded on a Bruker Digital RF spectrometer (300MHz), Varian Mercury spectrometer (400MHz) and Agilent (Varian) INOVA spectrometer (600MHz). ^{1}H chemical shifts are reported in ppm downfield from tetramethylsilane (TMS, δ scale with the solvent resonances as internal standards). Mass spectra were recorded on a Bruker Esquire 6000 MS instrument. The infrared spectra were performed on a Digilab FTS-3000 Fourier transform-infrared spectrophotometer. Melting points were measured on an X-4 digital melting-point apparatus (uncorrected). Fluorescence spectra were recorded on a Shimadzu RF-5301PC spectrofluorophotometer. Ultraviolet-visible (UV-vis) spectra were recorded on a Shimadzu UV-2550 spectrometer. pH values were recorded on a Sartorius PB-10 acidometer.

The tetra-n-butyl ammonium salts and perchlorate salts were purchased from Alfa Aesar Chemical Reagent Co. (Tianjin, China) and stored in a vacuum desiccator. All solvents and other reagents were of analytical grade, commercially purchased and were used without further purification.

2.2. General procedure for fluorescence spectroscopy

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Electronic Supplementary Information (ESI) available: [details of any supplementary information available should be included here]. See DOI: 10.1039/x0xx00000x

All the fluorescence experiments were carried out on a Shimadzu RF-5301PC spectrofluorophotometer. Any changes in the fluorescence spectra of the synthesized sensors were recorded on the addition of HEPES buffered solution with various pH values ranged from 1.0 to 13.5. The host concentration was kept constant in all experiments.

2.3. General procedure for UV-vis experiments

All the UV-vis experiments were carried out on a Shimadzu UV-2550 spectrometer. Any changes in the UV-vis spectra of the synthesized sensors were recorded on the addition of HEPES buffered solution with various pH values ranged from 1.0 to 13.5. The host concentration was kept constant in all experiments.

2.4. General pH adjustment procedures

The pH measurements were carried out in air at room temperature using a Sartorius PB-10 acidometer. The pH was adjusted by addition of TBAOH and $HClO_4$, respectively, in the HEPES buffered system. The emission and absorption spectra were recorded in 10 mm quartz cuvettes after equilibration.

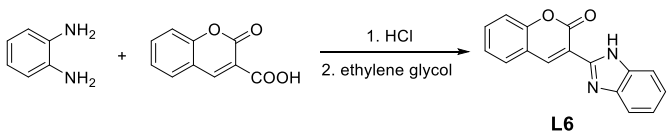
2.5. Synthesis and characterization of sensor L6

The synthetic route and molecular structure of sensor **L6** is shown in Scheme 1. *O*–phenylenediamine (1.0 mmol) was added to concentrated hydrochloric acid (2.2 mmol) and the reaction mixture was stirred at room temperature for 1 h to get *o*-phenylenediamine hydrochloride. Then, *o*–phenylenediamine hydrochloride (1.0 mmol) and coumarin-3-carboxylic acid (1.2 mmol) were added to ethylene glycol (10 mL). After the reaction mixture was stirred under refluxed conditions for 7 h, the precipitate of **L6** yielded and then was recrystallized with DMF to get solid of sensor **L6**.

L6: Yield: 75.0%.m.p. 256-258℃.IR: (KBr, cm^{-1}) *v*: 3442 (NH), 3285 (ArH), 3043 (ArH), 1793 (C-O-C), 1622 (C=C), 1485 (C=N). ^{1}H NMR (DMSO-d_6, 400 MHz): δ 11.14 (s, 1H, NH), 9.01 (s, 1H, C-H), 7.93-6.97 (m, 8H, ArH). ^{13}C NMR (DMSO-d_6, 150 MHz): δ 159.23, 153.23, 145.73, 142.24, 134.80, 130.91, 129.56, 125.03, 122.79, 122.14, 118.47, 116.11, 112.77. ESI-MS calcd for $[C_{16}H_{10}N_2O_2 + H]^+$ = 263.0742, found 263.0818.

3. Results and discussion

The structure of the sensor **L6** is shown in Scheme 1. The pH response properties of the sensor **L6** were primarily investigated in DMSO/H_2O (v/v, 1/1) HEPES buffered solution with various pH values (pH ranged from 1.0 to 13.0, the step-size is 1.0 pH unit). And the results with respect to the pH response properties are illustrated as follows. The sensor exhibited relative weak fluorescence at pH ranged from 1.0 to 3.0 in DMSO/H_2O (v/v, 1/1) HEPES buffered solution when excited at λ_{ex} = 371 nm (ESI,† Fig. S1). Interestingly, a sharp fluorescence intensity enhancement at 497 nm appeared when the pH raised up to 4.0–9.0. The apparent color change to brilliant green could be distinguished by the naked eye (Fig.1). However, the sensor **L6** showed the fluorescence quenching when the pH values ranged from 10.0 to 13.0. In addition, there was a narrow pH jump between 9.0 and 10.0.



L6

Scheme 1 The synthetic route and molecular structure of sensor **L6**.

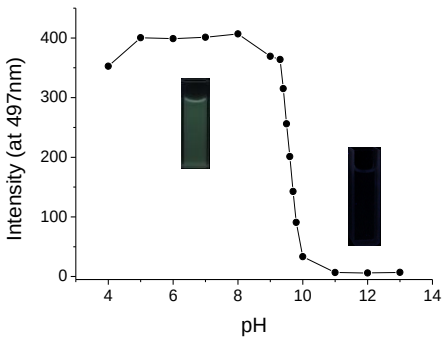


Fig.1 Fluorescence response of **L6** to pH variation (pH ranged from 4.0 to 13.0, DMSO/H_2O (v/v, 1/1) HEPES buffered solution, $c_{L6} = 2.0 \times 10^{-5}$ M, λ_{ex} = 371 nm, λ_{em} = 497 nm).

Besides, prompted by the fact that the life system is composed of large amount of water, the performance of the sensor **L6** in aqueous solution was further investigated. As the sensor **L6** could not completely dissolve in water solution because of the solubility problem, the pH response properties of **L6** were investigated in DMSO/H_2O (v/v, 1/9) HEPES buffered solution with various pH values (pH ranged from 1.0 to 13.5, the step–size is 0.5 pH unit). In the UV–vis spectrum (ESI,† Fig. S2), the pH values of the **L6** solution ranged from 10.5 to 11.0, there was a slight ca. 5 nm bathochromic-shifted of the max absorption wavelength upon deprotonation of the **L6**. And the absorption band at 361 nm was red-shifted to 379 nm eventually, with increasing of the pH is to 12.0 in this process. Meanwhile, In the corresponding fluorescence spectrum (ESI,† Fig. S3), the emission of **L6** showed brilliant blue color at the maximum emission wavelength 440 nm in acidic environment (pH ranged from 1.0 to 3.0) of DMSO/H_2O (v/v, 1/9) HEPES buffered solution (Fig.2). And a sharp fluorescence intensity decline at 497 nm appeared when the pH raised up to 4.5–8.5 (Fig.3). The apparent color change from brilliant blue to green could be distinguished by the naked eye. However, the sensor **L6** showed the fluorescence quenching at strong alkaline environment (pH=10.5–13.5). Furthermore, the sensor **L6** possessed two obvious pH jumps (pH 3.0-4.5 and 8.5-10.5, respectively) among a wide pH scale (pH 1.0–13.5). In summary, the sensor **L6** responded to pH change *via* different fluorescence emissions (blue at pH 1.0–3.0, green at pH 4.5–8.5 and quenching at pH 10.5–13.5 ranges). The sensor possessed high specificity, and it could monitor pH change in a wide scale of 1.0–13.5.

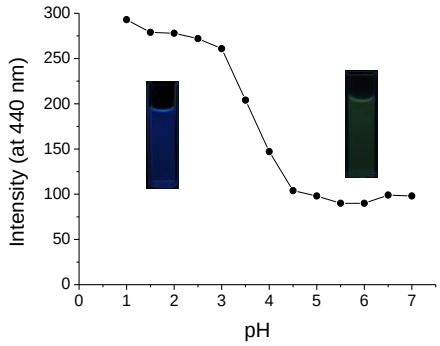


Fig.2 Fluorescence response of **L6** to pH variation (pH ranged from 1.0 to 7.0, DMSO/H_2O (v/v, 1/9) HEPES buffered solution, $c_{L6} = 2.0 \times 10^{-5}$ M, λ_{ex} = 371 nm, λ_{em} = 440 nm).

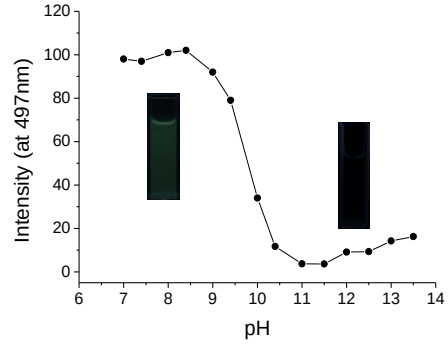


Fig.3 Fluorescence response of **L6** to pH variation (pH ranged from 7.0 to 13.5, DMSO/H_2O (v/v, 1/9) HEPES buffered solution, $c_{L6} = 2.0 \times 10^{-5}$ M, λ_{ex} = 371 nm, λ_{em} = 497 nm).

In order to validate the reversibility of the sensor **L6**, the pH response properties of **L6** were also investigated by gradually decreasing of the pH (from 13.0 to 1.0, the step-size is 1.0 pH unit). The sensor **L6** could reversibly indicate pH change at pH ranged from 10.0 to 4.0. These properties made the sensor **L6** act as a pH controlled "on-off-on" fluorescent switch. By alternating addition of tetra-n-butyl ammonium hydroxide (TBAOH) and $HClO_4$, the switch could be reversibly performed several cycles with little fluorescent efficiency loss (Fig.4).

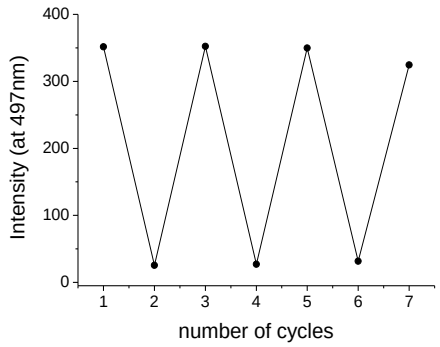


Fig.4 Fluorescence switching cycles of **L6** ($c_{L6} = 2.0 \times 10^{-5}$ M, λ_{ex} = 371 nm), controlled by alternating addition of TBAOH and $HClO_4$.

The selectivity of the sensor **L6** to pH change was investigated in the presence of common ions. It is noticeable that the miscellaneous competitive anions (such as F^-, Cl^-, Br^-, I^-, AcO^-, $H_2PO_4^-$, HSO_4^-, ClO_4^-) and cations (such as Fe^{3+}, Hg^{2+}, Ag^+, Ca^{2+}, Cu^{2+}, Zn^{2+}, Pb^{2+}, Cd^{2+}, Ni^{2+}, Co^{2+}, Cr^{3+} and Mg^{2+}) did not lead to any significant interference in the pH response process of **L6** (ESI,† Fig. S4).

The pH response mechanism of the sensor **L6** was observed from ^{1}H NMR titration experiments (Fig.5). The ^{1}H NMR chemical shifts of N-H on the benzimidazole moiety showed single peak at δ 12.57 ppm. With the gradual addition of excess OH^-, the signal of the resonances for the N-H proton completely disappeared, which suggested that the N-H groups underwent a deprotonation process and induced the color change. This mechanism, together with an intramolecular charge transfer (ICT) process, caused the sensor **L6** to present dual–color and turn–on fluorescent emission (blue at pH 1.0–3.0, green at pH 4.5–8.5). In addition, the sensor **L6** exhibited no fluorescence at pH 10.5–13.5 because of a twisted intramolecular charge transfer (TICT) process. Thus, the fluorescent emission of sensor **L6** could be accurately controlled by pH changes under ICT–TICT state change mechanism (Fig. 6).

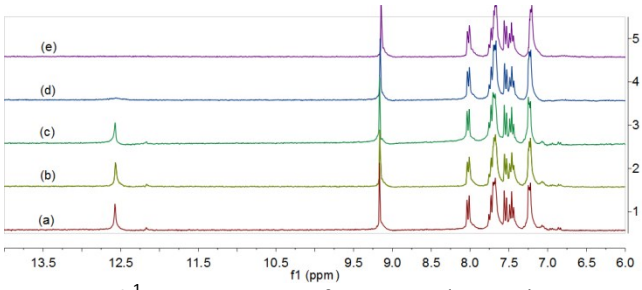


Fig.5 Partial ^{1}H NMR spectra of sensor **L6** (0.01 M) in DMSO-d_6 upon the addition of OH^-: (a) Free, (b) 0.04 equiv. of OH^-, (c) 0.2 equiv. of OH^-, (d) 0.6 equiv. of OH^-, (e) 1.0 equiv. of OH^-.

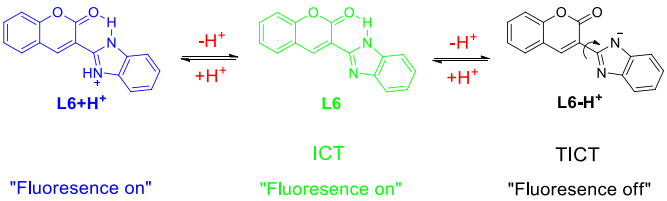


Fig.6 A possible mechanism of the sensor **L6** response to pH.

4. Conclusion

In this work, we have designed a multi–scale pH chemosensor (**L6**), which could respond pH *via* different fluorescence emissions based on ICT–TICT mechanism. **L6** could respond a broad pH range of 1.0–13.5 *via* different fluorescence emissions (blue at pH 1.0–3.0, green at pH 4.5–8.5 and quenching at pH 10.5–13.5). Common ions could not cause any obvious interference for the pH sensing process. Interestingly, **L6** exhibited excellent green fluorescent emission under the entire physiological pH conditions (pH 4.5–8.5) in the DMSO/H_2O (v/v, 1/9) aqueous solution. Besides, **L6** could act as a reversible pH fluorescence switch triggered by alternatively adding TBAOH and $HClO_4$. Thus, **L6** has lots of advantages including responding pH *via* different fluorescence emissions, fine reversibility, excellent anti-interference, high water solubility and a wide pH range, all of which made it possible to be used as an on-line chemosensor to bioimage for the investigation of microspecies from living cells and animals.

Acknowledgements

This work was supported by the National Natural Science Foundation of China (NSFC) (21574104; 21161018; 21262032), the Natural Science Foundation of Gansu Province (1010RJZA018) and the Program for Changjiang Scholars and Innovative Research Team in University of Ministry of Education of China (IRT1177).

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Graphical Abstract

A Novel pH Sensor which Could Respond Multi–Scale pH Changes *via* Different Fluorescence Emissions

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Sensor **L6** exhibited two obvious pH jumps and could respond multi–scale pH changes *via* different fluorescence emissions and colors.