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A visible colorimetric pH sensitive chemosensor based on azo dye of benzophenone

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1. Introduction

The pH plays significant roles in agriculture [1], industry [2,3], human health [4,5], and, more particularly, bio-gland secretion of fluid [6]. So, the methods for visualizing H^+ ions would be powerful tools to examine the concentration of H^+ ionsignaling mechanisms in detail and might eventually be useful for diagnosis as well. Although over the past decade, fluorescence detection methods for pH have been well reported [7–11], visible ratiometric detection methods are widely welcome for pH sensing because the process of detecting the analyte can be observed with the naked eyes [12,13]. However, most colorimetric chemosensors have a weaker pH sensitivity than fluorescent probes [14–16] and can cause measurement errors due to poor color change. Therefore, a highly pH sensitive colorimetric chemosensor having a significant color change would be very useful in the field of physiological fluids.

This communication describes the design and synthesis of a visible colorimetric sensitive chemosensor (Dye A) derived from benzophenone (Fig. 1).

ABSTRACT

A visible colorimetric pH sensitive chemosensor (Dye A) based on a benzophenone azo dye was designed and synthesized. This chemosensor had a large wavelength shift (>170 nm) and showed excellent sensitivity in the range of pH from 7.9 to 9.3. In addition, the possible mechanism of the chemosensor with remarkable change of visible color was established by the ¹H-NMR- and ¹³C-NMR-titration experiments. This pH chemosensor has a fast response time and is fully reversible, which is important for pH chemosensors in practical applications. In this study, Dye A was successfully applied to determine the pH of simulated small intestine fluid.

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PIĞMĔNTS

2. Experimental

2.1. Materials

All chemicals were analytical grade and purchased from Sinopharm Chemical Reagent Co., Ltd., China. The purification of the dye was performed by conventional column chromatography on silica-gel absorbent (200–300 Å, Qingdao Makall Group Company, China). Ethyl acetate-petroleum ether (60–90) mixtures were used as the elution. Deionized water was redistilled before use. Dimethyl sulphoxide (DMSO) was HPLC grade.

2.2. Preparation and characterization

The preparation of Dye A based on azo dye of benzophenone was carried out by diazo-coupling reaction as shown in Scheme 1.

2.2.1. Procedure for the preparation of Dye A

A mixture of 4-nitroaniline (1.38 g, 10 mmol), 3.6 ml 37% or concentrated hydrochloric acid and 25 ml H₂O was stirred vigorously for 0.5 h at 70 °C. The resultant solution was cooled below 5 °C with the aid of an ice bath and stirred as NaNO₂ (0.73 g, 10.5 mmol) in 5 ml H₂O was added rapidly. The reaction mixture was stirred at 5 °C until a clear solution was formed. The solution was added to 50 ml H₂O containing compound 2-hydroxy-4-methoxybenzophenone (2.28 g, 10 mmol) and Na₂CO₃ (2.40 g, 45.4 mmol) while keeping the



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Fig. 1. UV/Vis absorption spectra of dye $(5.33 \times 10^{-5} \text{ mol } L^{-1})$ in DMSO solution containing 10% water.



Scheme 1. Synthesis of Dye A from 2-hydroxy-4-methoxybenzophenone.

temperature not higher than 5 °C during the addition, and the resulting solution was stirred for 2.5 h at 5 °C. Then, the pH of reaction solution was adjusted to 7.0 by adding 10% Na₂CO₃ aqueous solution. The solid was filtered, washed with 50 ml H₂O, and dried to give a yellow solid. The Dye A was obtained by column chromatograph silica gel using ethyl acetate-petroleum ether (60–90) mixtures as the eluent, yield: 77.8%. ¹H-NMR (400 MHz, DMSO-*d*₆, ppm): δ = 4.06 (s, 3H, CH₃), 6.86 (s, 1H, CH), 7.54–7.58 (t, 2H, CH), 7.65–7.69 (t, H, CH),



Fig. 2. (a) Nonlinear fitting of the pH-dependent value of absorbance ϵ 402 nm; (b) Nonlinear fitting of the pH-dependent value of absorbance ϵ 571 nm; (c) The relationship of absorbance intensity ratio (Vis_{571 nm}/Vis_{402 nm}) and pH; (d) UV/Vis absorption spectra of dye (5.33 × 10⁻⁵ mol L⁻¹) with different pH in DMSO solution containing 10% water; Inset: the color change of Dye A (5.33 × 10⁻⁵ mol L⁻¹) with different pH in DMSO-H₂O 9:1 (V/V).



7.72–7.74 (d, 2H, CH), 7.85 (s, H, CH), 7.93–7.95 (d, 2H, CH), 8.38–8.40 (d, 2H, CH), 11.86 (s, H, OH); ¹³C-NMR (400 MHz, DMSO- d_6 , 25 °C, TMS): δ = 196.94, 164.84, 162.43, 155.57,147.80, 137.50, 134.64, 132.70, 129.08, 128.50, 125.00, 123.17, 119.63, 116.40, 100.87, 56.56; Q-TOFMS: M⁻¹ calculated: 377.0933, measured: 377.0941.

2.3. Methods

All absorption measurements of solution were performed in a $1 \times 1 \times 4$ cm quartz cuvette (sight optical path length, 1 cm) (Starna), using a HP-8453 spectrophotometer. The pH measurements

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Fig. 3. The pH $^1H\text{-}NMR$ titration of dye $(3.0\times10^{-6}\ mol\ L^{-1})$ in DMSO: (a) dye only; (b) 1 + 1.00 equiv of TEA.



Scheme 2. Representative mechanism of the chemosensor based on the phenolic hydroxyl-group providing electron and the nitro-group absorbing electron.



Fig. 4. The pH $^{13}\text{C-NMR}$ titration of dye $(3.0\times10^{-6}$ mol $L^{-1})$ in DMSO: (a) dye only; (b) 1 +1.00 equiv of TEA.

were made with a pHS-25 digital pH-meter (Shanghai Lei Ci Device Works, Shanghai, China) with a combined E-201-C electrode. All experiments were performed at 293 K. The experimental curves were analyzed to extract the pKa values according to a literature method [15].

3. Results and discussion

The UV/Vis titrations for pH with triethylamine (TEA) were performed in a DMSO solution containing 10% water with a chemosensor concentration of 5.33×10^{-5} mol L⁻¹. Dye A showed an obvious color change visible to naked eyes from yellow (pH = 7.9) to Lyons blue (pH = 9.3) (Figs. 1 and 2d). As the pH increased, the absorbance intensity decreased at 401 nm (Fig. 2a), and increased at 571 nm (Fig. 2b). The isobestic point observed in both cases indicated equilibrium between two species as pH increased. Fig. 2c shows clearly that a significant change appeared for the ratiometric absorption measurements with the increase of pH, and the ratio of the absorbance intensity at 571 nm vs 402 nm was nearly a constant ($R^2 = 0.98152$). Compared with common colorimetric dyes [17,18],

the ratio was 0.42 by changing the pH value of 0.1 in the range of pH from 8.5 to 10.0. Therefore, the new Dye A had a higher sensitivity in the narrow pH range in terms of pH detection. The pKa value of Dye A was 9.20 calculated according to a literature method [15], which was suitable for alkaline fluid *in vivo*.

As shown in Fig. 2, there were some important features in the spectra of this new dye: a remarkably visible color change and no mirror image relationship between peaks. These features may result from an intramolecular charge transfer or rearrangement versus isomerization. To elucidate the mechanism of the remarkably visible color change, ¹H-NMR-titration experiments were conducted. As shown in Fig. 3, a single peak corresponding to the hydroxybenzene proton (α position) at 11.84 ppm disappeared with the increase of TEA in the range of 0.0–1.0 equiv. Concomitantly, the hydrogen in the β -position gradually displayed an apparent highfield shift from 6.86 ppm to 6.13 ppm. This indicated that the opened-ring form (Scheme 2) of Dye A became Dye B or Dye C in the examined solution. To further elucidate the structural mechanism of mutual transformation of Dye A (Scheme 2), the ¹³C-NMR-titration (Fig. 4) and HMBC (Fig. 5a and b) was conducted.

As shown in Fig. 4, the peak corresponding to No. 7 at 164.83 ppm disappeared, however, from HMBC a new peak appeared at 175.40 ppm with the increase of the TEA (1.0 equiv), which was No. 7 by the analysis from HMBC. Therefore, the peak of No. 7 displayed an apparent downfield shift (from peak centered at 164.83–175.40 ppm, the $\Delta \delta = 10.57$ ppm, upon addition of 1.0 equiv of TEA) which further proved that the coordination was changed from hydroxybenzene to benzoquinone. Concomitantly, these carbons of No. 6, 10, 12 and 15 also displayed an apparent shift ($\Delta \delta \ge 2.32$ ppm). The peak of No.15 was shown in Fig. 5 with increasing the volume of TEA to 1.0 equiv. One important structural change accompanying an oxygen anion



Fig. 5. (a) ${}^{13}C{}^{-1}H$ HMBC of Dye A (Dye A: NEt₃ = 1:1); (b) ${}^{13}C{}^{-1}H$ HMBC of Dye A.



Fig. 6. Reversibility of protonation and color change.



Fig. 7. Titration of 5.33×10^{-5} mol L⁻¹ of Dye A in DMSO-H₂O (9:1, v/v) with a simulated fluid of small intestine (8.0 g NaCl, 0.2 g KCl, 0.1 g MgCl₂, 0.2 g CaCl₂, 1.0 g NaHCO₃, 0.05 g NaH₂PO₄, 1.0 g glucose in 1 l of water). Inset: absorbance at 571 nm versus number of equivalents of a simulated fluid of small intestine (v) added.

intramolecular charge transfer and the nitro absorbing electron showed that the arrangement of the azo bond was transferred from pH = 7.9 to pH = 9.3 (Dye C, Scheme 2).

In a test of reversibility, the pH value was changed from 8.1 to 9.0 and then returned to 8.1 for four cycles, and the UV/Vis absorption ratio reached the expected value in all cases (Fig. 6 Dye $A = 5.33 \times 10^{-5}$ mol L⁻¹). The results showed that the pH chemosensor was fully reversible and fast in terms of response time, which makes it practical in real applications.

The UV/Vis pH determination of simulated small intestine fluid was shown in Fig. 7 (Dye A = 5.33×10^{-5} mol L⁻¹) in DMSO-H₂O (9:1, v/v). An obvious color change from yellow to purple was observed as the volumetric concentration of the fluid was increased from 0 to 0.1%. This suggests that Dye A is an excellent candidate as a pH chemosensor for alkaline fluid *in vivo*.

4. Conclusions

A benzophenone derivative has been synthesized as a visible colorimetic pH chemosensor. There was a remarkable red shift (>170 nm) when the pH value of solution was changed from 7.9 to 9.3. The color-changing mechanism was proposed on the basis of the ¹H-NMR- and ¹³C-NMR-titration experiments. Dye A is an excellent pH sensitive chemosensor for simulated intestinal fluid.

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