

UV-VIS SPECTRAL PROPERTIES OF WATER-SOLUBLE 3,5-BIS-(2,4,6-TRI-HYDROXYPHENYL-AZO)BENZOIC ACID AND THEIR APPLICATION FOR pH DETECTION**

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We present a multiple water-soluble azo dye with a D- π -A- π -D conjugated structure, 3,5-bis-(2,4,6-trihydroxyphenylazo)benzoic acid (THPBA) developed using phloroglucinol as the electron-donating group, benzoic acid as the electron-accepting one, and the azo double bond as the bridging one. The UV-Vis spectral properties of the elaborated structure as well as its spectral response to the pH are investigated in detail. The results show that the present THPBA, combining carboxyl, azo, and multi-hydroxyl groups, possesses excellent UV-vis spectra ($\epsilon_{max} = 2.794 \times 10^4 - 2.115 \times 10^4$ L/mol·cm) with changing pH values and solvents. Especially in DMF-H₂O (v/v=1:9), there is an obvious spectral response for THPBA to pH in the linear range 5.2–7.5, which is successfully applied to detect pH values in aqueous and commercial fruit juice samples.

Keywords: 3,5-bis-(2,4,6-trihydroxyphenylazo)benzoic acid, UV-vis spectral properties, pH detection, environmental analysis.

СПЕКТРАЛЬНЫЕ СВОЙСТВА В УФ-ВИДИМОЙ ОБЛАСТИ ВОДОРАСТВОРИМОЙ 3,5-БИС-(2,4,6-ТРИГИДРОКСИФЕНИЛАЗО)БЕНЗОЙНОЙ КИСЛОТЫ И ИХ ПРИМЕНЕНИЕ ДЛЯ ОПРЕДЕЛЕНИЯ pH

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Представлен сложный водорастворимый азокраситель с сопряженной D- π -A- π -D структурой – 3,5-бис-(2,4,6-тригидроксифенилазо)бензойная кислота (THPBA), разработанный с использованием хлороглюцинола в качестве донорной группы, бензойной кислоты – в качестве акцепторной группы и азо-двойной связи – в качестве связующей группы. Исследованы спектральные свойства структуры в УФ-видимой области, а также ее спектральный отклик на изменение pH. Предлагаемый краситель THPBA, объединяющий карбоксильную, азо- и мультигидроксильную группы, обладает отличными УФ и видимыми спектрами: ϵ_{max} изменяется от 2.794×10^4 до 2.115×10^4 л/моль·см при изменении pH и замене растворителей. Особенно заметны изменения в спектрах при использовании DMF-H₂O (v/v=1:9), где наличие THPBA обуславливало отклик на изменение pH в диапазоне 5.2–7.5, что успешно применено для определения щелочности в некоторых водных и коммерческих образцах фруктовых соков.

Ключевые слова: 3,5-бис-(2,4,6-тригидроксифенилазо)бензойная кислота, спектральные свойства в УФ-видимом диапазоне, определение pH, анализ окружающей среды.

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Introduction. Ultraviolet-visible spectroscopy (UV-vis) investigates electronic transitions from the low energy state to the high energy one. Yet, a great change in the absorption intensity can be observed when some guest molecules or ions bind with the anions [1, 2], metal ions [3–5], and protons [6, 7] of the host medium. Owing to this, UV-vis spectra are widely applied in various analytic and sensing fields. A large number of organic conjugated molecules with large π -conjugated structures have been reported, such as Schiff base [8], azo [9–11], squaraine [12–14], fluorescein [15–17], luminol derivatives [18], and so on. Among them, azo and its derivatives are of great interest due to their convenient synthetic procedures, controllable molecular structures, and promising polydentate chelating ability [19, 20].

As we know, the pH plays a key role in various physiological processes, i.e., cell growth, cell generation and conversion, cell proliferation, calcium regulation, drug resistance, phagocytosis, endocytosis, and signal transduction. Too high or too low pH values will result in serious clinical problems, such as physical fatigue, memory loss, poor attention, lumbago, diarrhea, and even gout, hypertension, cancer, and hyperlipidemia [21, 22]. Great efforts have been devoted to develop efficient sensing methods to monitor the pH and discover its pathological and physiological processes [23–26]. However, there remains a challenge to construct some monitoring systems for on-site pH detection with the merit of simplicity, efficiency, and cost-effectiveness.

With this goal in mind, a multiple water-soluble azo dye with a D- π -A- π -D conjugated structure, 3,5-bis-(2,4,6-trihydroxyphenylazo)benzoic acid (THPBA), was prepared, which incorporated phloroglucinol, benzoic acid, and the azo double bond into one molecule. After its UV-vis spectra had been investigated in detail, the proposed THPBA was applied for selectively monitoring pH in some aqueous and commercial fruit juice samples with satisfactory results.

Experimental. All the chemical reagents utilized in this work were of AR grade from Sinopharm Chemical Reagent Co., Ltd., and used directly without further purification. The water applied in this work was doubly deionized.

FTIR spectra of THPBA with a KBr disc were acquired using a Nicolet NEXUS 870 FTIR spectrophotometer at room temperature from 4000–500 cm^{-1} . ^1H NMR and ^{13}C NMR spectra were recorded on a Bruker AVANCE 600 using tetramethylsilane (TMS) as an internal standard and D_2O as a solvent. Elemental analyses were conducted using a Vario EL-III elemental analytical apparatus. UV-vis spectra were recorded on a Lambda 35 UV/Vis spectrometer from 300 to 600 nm, and the band-slit was set as 2.0 nm using a 1-cm square quartz cell. The pH was measured by a PHS-25 pH meter.

THPBA was synthesized as mentioned below, and its stock solution with a concentration of 2.213×10^{-4} mol/L was prepared using doubly deionized water as a solvent, which was stored at 4°C in a fridge. Phosphate buffers (PB) were prepared by mixing 0.01 mol/L H_3PO_4 solution, 0.01 mol/L K_2HPO_4 solution, 0.01 mol/L KH_2PO_4 solution, or 0.01 mol/L KOH solution in a proper ratio to obtain the desired pH values (pH 4.0, 5.0, 6.0, 7.0, 8.0, 9.0, 10.0).

For preparing environmental water and fruit juice samples, the aqueous sample was obtained randomly from tap water on the campus. Vinegar, orange juice, and lemon juice were purchased from the local farm product market near Qufu Normal University, China. The prepared samples were filtered several times by a 4 μm microporous membrane and stored at 4°C in a fridge for the subsequent analysis.

For the preparation of THPBA, 0.0501 g (3.292×10^{-4} mol) 3,5-diaminobenzoic acid was dissolved in a mixture of ethanol/ H_2O (v/v 1:1) containing 0.0270 g (3.913×10^{-4} mol) of NaNO_2 . When the mixture was cooled down to 0°C , the appropriate HCl solution was dropped into it to maintain its pH between 1–2. After the mixture was stirred for 30 min, 0.0035 g (6.21×10^{-5} mol) of urea was added to destroy the excess nitrous sodium. Then the obtained mixture was added into 10 mL of saturated Na_2CO_3 solution (pH 8) containing 0.0825 g (6.542×10^{-4} mol) phloroglucinol and stirred continuously for another 2.0 h at 0 – 5°C . The resulting precipitate was filtered and recrystallized three times from ethanol to get a red THPBA crystal in a yield of 84.6%.

IR (KBr), ν (cm^{-1}): 2510–3520 (-OH & -COOH), 1702 (C=O), 1621 (N=N), 1598, 1514, 1453 (Ar ring), 1178 (C-O); ^1H NMR (D_2O , 600 Hz) δ (ppm): 11.5 (s, 1 H, COO-H), 8.7 (t, 3 H, Ar-H), 7.4 (m, 4 H, Ar-H), 5.12 (w, 6 H, -OH). ^{13}C NMR (D_2O , 150 MHz) δ (ppm): 95.6, 118.4, 123.4, 126.6, 132.7, 152.3, 155.9, 163.1, 165.2. Anal. Calcd for $\text{C}_{19}\text{H}_{14}\text{N}_4\text{O}_8$: H, 3.31; C, 53.53; N, 13.14. Found: H, 3.25; C, 53.66; N, 13.30.

Results and discussion. As it is a polar and multi-protic molecule, its UV-vis spectral properties are greatly affected by the types of solvents. Here, the effect of some polar or protic solvents on the UV-vis spectra of THPBA was investigated with the same concentration of 2.2×10^{-5} mol/L for dimethyl sulfoxide (DMSO), N,N-dimethylformamide (DMF), acetonitrile, ethanol, and water, as shown in Fig. 1a. From Fig. 1a

we can see that in all the tested polar or protic solvents, the UV-vis spectral curves of THPBA possess almost the same profile with a maximum absorption peak (λ_{\max}) ranging from 432 to 448 nm and a molar absorptivity $\epsilon_{\max} = 2.794 \times 10^4 - 2.115 \times 10^4$ L/mol·cm. However, when the solvent changes from the strong polar and aprotic (DMF and DMSO) to the protic one (ethanol and H₂O), the λ_{\max} value is shifted a little to short wavelengths, and ϵ_{\max} decreases. Perhaps, this phenomenon is attributed to the fact that in strong polar and aprotic solvents such as DMF and DMSO, THPBA exists in a single molecular state. In protic solvents, especially in H₂O, strong intermolecular hydrogen bond interaction makes the conjugated system of THPBA distorted, and the conjugated electron cloud density is decreased [27]. Therefore, the mixed solvent of DMF/H₂O (v/v=1:9) was selected for all the following experiments.

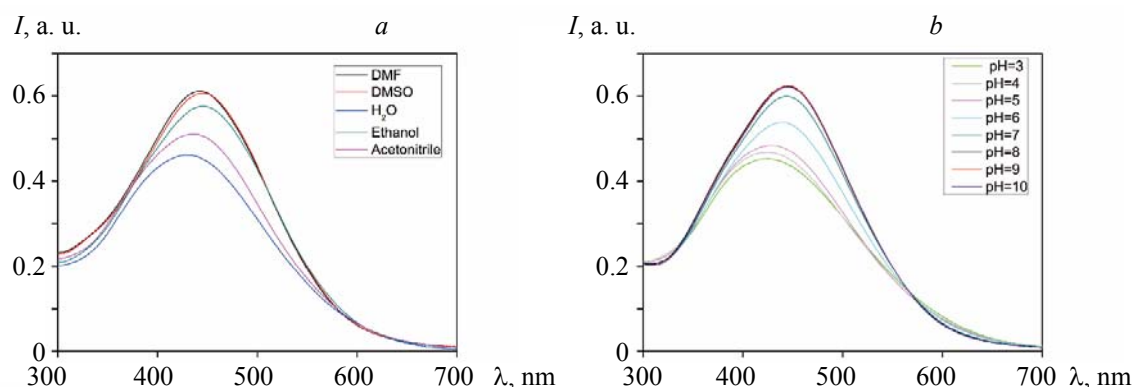


Fig. 1. a) The UV-vis spectra of THPBA in DMF, DMSO, acetonitrile, acetone, water, and ethanol; b) effect of pH on the UV-vis spectra of THPBA in DMF-H₂O (1/9, v/v; $c_{\text{THPBA}} = 2.2 \times 10^{-5}$ mol/L).

As THPBA is a protic molecule, the pH value affects its form of existence and alters its UV-vis spectrum. Accordingly, the effect of the pH on the UV-vis spectrum of THPBA was investigated from pH 3.0 to 10.0. As shown in Fig. 1b, when the pH increases from 3.0 to 8.0, the maximal absorption intensity improves from 0.4507 to 0.6245 a.u. with λ_{\max} shifting a bit from 425 to 447 nm. Meanwhile, we suggest that THPBA is a potential probe for pH detection in this pH range. While the pH reaches 8.0, both the absorption intensity and the absorption peak do not change any more. THPBA possesses quite a stable configuration when the pH is greater than 8.0. The observation can be attributed to the fact that hydroxyl (-OH) and carboxyl (-COOH) groups in THPBA molecules are gradually turned into phenol oxygen and carboxyl anions, improving the pH, which increases the electron cloud density of the conjugated system in THPBA. When the pH is greater than 8.0, all -OH and -COOH groups in THPBA are completely turned into phenol oxygen and carboxyl anions, so no changes happen anymore.

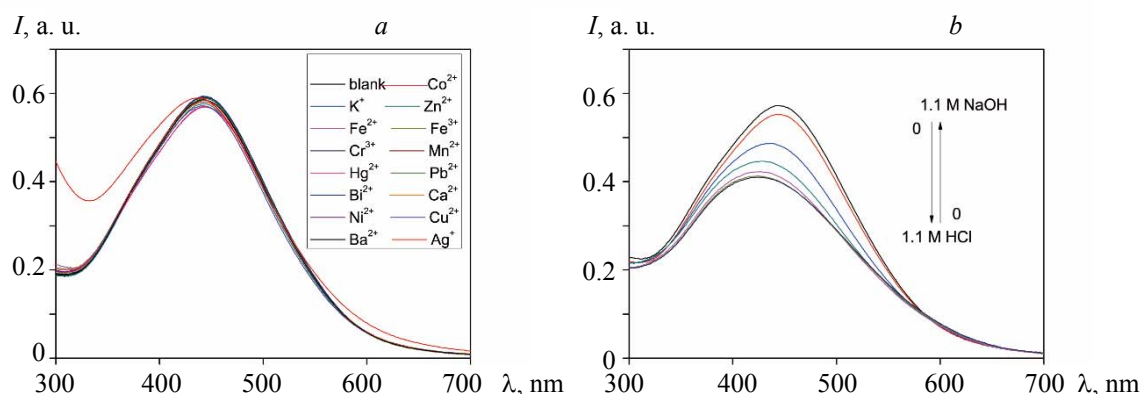


Fig. 2. a) Effects of different metal ions on the UV-vis spectra of THPBA (i.e., Ag⁺, Ba²⁺, Bi³⁺, Fe²⁺, Fe³⁺, Cd²⁺, Ca²⁺, Cr³⁺, Cu²⁺, Hg²⁺, K⁺, Mn²⁺, Ni²⁺, Pb²⁺, Zn²⁺ respectively) in DMF-H₂O, respectively (v/v = 1:9, pH 7.0, $c_{\text{THPBA}} = 2.2 \times 10^{-5}$ mol/L, $c_{\text{ions}} = 1.0 \times 10^{-5}$ mol/L); b) UV-vis spectral changes of THPBA by repeated addition of NaOH and HCl from 0 to 1.1 equiv.

The selectivity of THPBA to the pH was investigated at pH 7.0 upon addition of some common metal ions such as Ag^+ , Ba^{2+} , Bi^{3+} , Fe^{2+} , Cd^{2+} , Ca^{2+} , Cr^{3+} , Cu^{2+} , Fe^{3+} , Hg^{2+} , K^+ , Mn^{2+} , Pb^{2+} , Ni^{2+} , and Zn^{2+} with the same concentration of 1×10^{-5} mol/L, respectively. As shown in Fig. 2a, there is no obvious influence of all the tested ions on the UV-vis spectrum of THPBA, i.e., the change in the absorption intensity at 446 nm is less than 5.0% compared to that of THPBA itself at pH 7.0.

The reversibility of THPBA responding to the pH was investigated by repeated addition of NaOH and HCl from 0 to 1.1 equiv. As shown in Fig. 2b, the maximal absorption intensity of THPBA increases with a small shift to the long wavelength direction upon addition of NaOH, which gradually recovers after repeated addition of HCl, and vice versa. The observation suggests that there is an excellent reversibility for the UV-vis spectrum of THPBA upon addition of NaOH and HCl alternately.

To evaluate the potential application in practice, a series of UV-vis spectral titration experiments was carried out with different pH values in the range from 3.0 to 8.0 using different amounts of HCl or NaOH aqueous solutions. As shown in Fig. 3a, the maximal absorption intensity of THPBA increases with the gradual improvement of the pH from 3.0 to 8.0 and reaches the maximum when the pH is 8.0 (inset in Fig. 3a). Especially, there is a nice linear relationship between the maximal absorption intensity of THPBA and pH values between pH 5.2 and 7.5 (Fig. 3b). Also, from Fig. 3b, the regression equation and detection precision for pH detection can be obtained, i.e., $A_{\text{max}} = 0.224 + 0.053 \text{ pH}$ with a correlation coefficient $R^2 = 0.9851$.

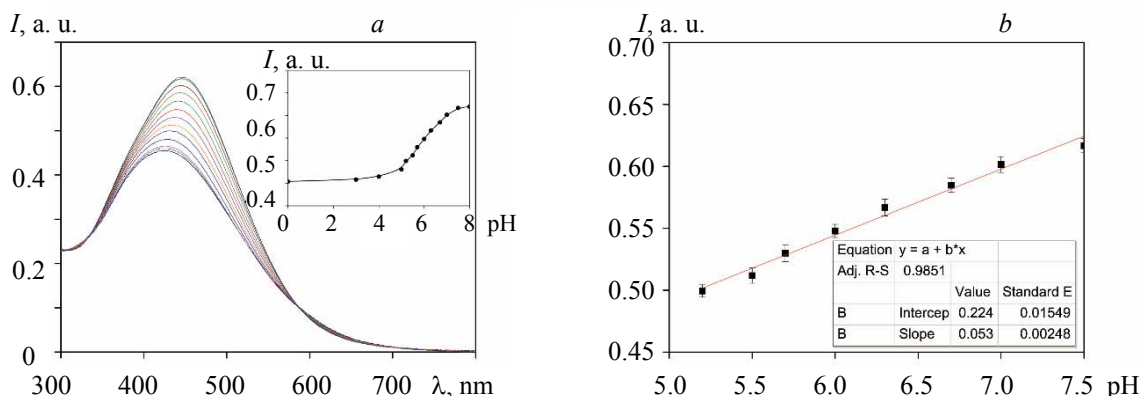


Fig. 3. a) UV-vis spectral titration curves of THPBA at different pH values between pH 3.0 and 8.0 (inset: changes in A_{max} over the pH range tested with a nonlinear fit); b) linear relationship between A_{max} and the pH.

Based on the conclusion above, the present THPBA was successfully applied for pH analysis in some aqueous and commercial fruit juice samples, namely, tap water, vinegar, orange juice, and lemon juice. For this, the pH values of all the above four samples were deduced from the above linear regression equation of $A_{\text{max}} = 0.224 + 0.053 \text{ pH}$, which were compared with the data obtained from a commercial PHS-25 pH meter as well. As illustrated in Table 1, the pH values deduced from $A_{\text{max}} = 0.224 + 0.053 \text{ pH}$ are almost consistent with those directly recorded from the commercial PHS-25 pH meter, confirming the reliability of the present THPBA as a pH sensor for pH detection in real samples.

TABLE 1. Results of pH Spectral Determination for Some Samples ($n = 5$)

Sample	UV-vis method	PHS-25 meter
Tap water	6.9 ± 0.15	6.7
Vinegar	5.3 ± 0.23	4.8
Orange juice	5.8 ± 0.30	6.1
Lemon juice	6.1 ± 0.35	5.7

Note. $c_{\text{THPBA}} = 2.2 \times 10^{-5}$ mol/L.

Conclusions. A water-soluble D- π -A- π -D conjugated azo dye, 3,5-bis-(2,4,6-trihydroxyphenylazo)benzoic acid (THPBA), was identified and synthesized. Combining an electron-donating phloroglucinol group, an electron-accepting benzoic acid group, and a bridging azo double bond, THPBA exhibited a strong and sharp absorption peak at ca. 446 nm with a molar absorptivity (ϵ_{\max}) from 2.794×10^4 to 2.115×10^4 L/mol·cm, contingent on pH values or the types of solvents. Importantly, THPBA expressed an excellent spectral response to the pH in the range from pH 5.2 to 7.5 with a linear regression equation of $A_{\max} = 0.224 + 0.053 \text{ pH}$ and a correlation coefficient $R^2 = 0.9851$. There was no obvious effect on pH detection in the presence of some other coexisting metal ions. When applied for pH detection in practice, the deduced pH values were well consistent with those recorded directly from a PHS-25 pH meter, implying that the present THPBA can be a potential pH sensor.

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