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Development of optical pH sensors based on derivatives of hydroxyazobenzene, and the extended linear dynamic range using mixture of dyes

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Abstract

In order to fabricate efficient pH optical sensors, the characteristic features of the UV–vis spectra of six synthesized hydroxyazobenzene dyes were studied at different pH(s). Two optical pH sensors were developed using 4-[(*p*-nitrophenyl)azo]phenol and 2-methoxy-4-[(4-nitrophenyl)azo]phenol dyes immobilized on transparent triacetylcellulose (TAC) membrane, which showed reversible responses within the dynamic ranges of 8.5–10.5 and 6.5–8.5, respectively. A new optical pH sensor with a linear response over a range of 4.5 pH units (7–11.5) was also developed by immobilization of a mixture of the aforementioned dyes on a TAC membrane. All the fabricated optodes showed fast response time (less than 20 s), sharp and clear color change as well as excellent long-term stability (at least 3 months) under the applied conditions.
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Keywords: Optical sensor; Hydroxyazobenzene; pH determination; Spectrophotometry

1. Introduction

Optical sensors have been extensively investigated for the promising potential applications in areas such as clinical analysis, environmental analysis, and process control. They offer advantages with respect to cost, electrical noise, safety and remote sensing [1–4].

Optical pH sensors are usually made by immobilization of pH indicator(s) onto a solid support permeable to protons. Indicators are also usually selected from dyes which are weak acids or bases. As a result, they exhibit different absorption or emission bands for their dissociated and undissociated forms in the desired range of pH. The most commonly used pH indicators include nitrophenols, phthaleins, sulfonphethaleines and azo dyes [1–11]. Due to their chromophoric nature, azo dyes are valuable as pH indicators and optical pH sensor.

Several reports have been published on the synthesis of azo dye derivatives and their subsequent applications in optical pH measurements. It has been shown that minor structural modification of an indicator could bring about remarkable changes in the optical features of the fabricated sensor such as its pK_a, linearity and stability [12,13].

One of the major limitations of pH optodes concerns the pH range in which the optode responses. Most of the applied dyes show linear sensitivity in a narrow range of pH, approximately two units. Therefore, attempts have been made to extend the pH response range of the optodes, for instance indicators with two acidic groups or a mixture of two indicators with different acidic constants have been applied in the construction of optodes [14].

Literature survey reveals that little attention has been paid to the spectrophotometric features of the azo derivatives of guaiacol and phenol. Consequently, the possible application of these dyes in the construction of pH optodes has not been explored well. Therefore, the spectral features and pK_a values of six different azo derivatives of these compounds (Fig. 1) were studied. Based on the results of this study, two optical pH sensors

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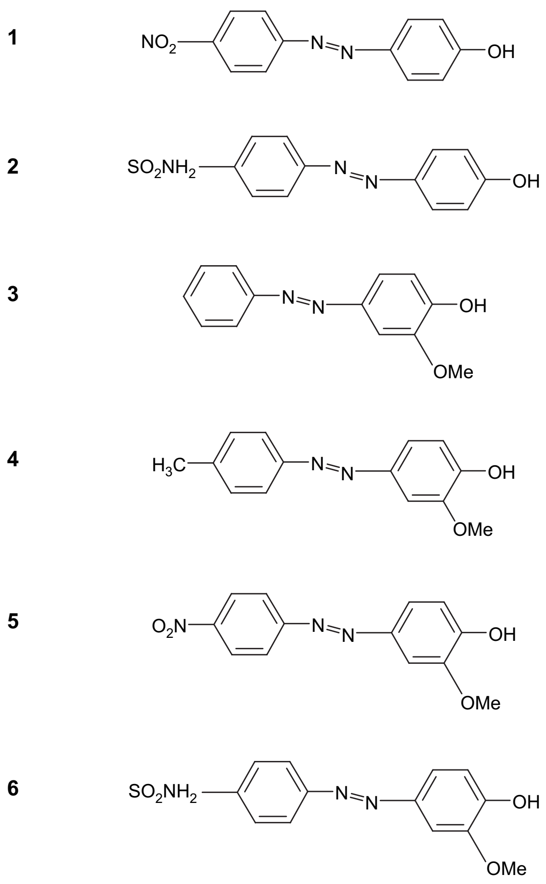

Fig. 1. Chemical structure of hydroxyazobenzene derivatives.

with different pH ranges of response were developed. A new pH sensor with an extended linear range of sensitivity was also fabricated through immobilization of a binary mixture of the selected dyes onto TAC film. The optical and chemical properties of the sensors as a function of pH were also evaluated.

2. Experimental

2.1. Reagents

Except for the hydroxyazobenzene dyes, which were synthesized according to the previously reported methods [15], all other chemicals and solvents were taken from the authentic samples. The buffers used were prepared in doubly distilled water. Dye solutions were prepared by dissolving 0.01 g of each dye in 25 ml of methanol. For the purpose of spectrophotometric measurements, the desired concentration of the dye solution was obtained through diluting the appropriate quantities of the dye solution in suitable buffer solutions.

2.2. Instrumentation

UV–vis spectra were recorded on a Shimadzu Multi Spec-1501 spectrophotometer. A fiber optic probe was used for evaluation of spectrophotometric data. A Metrohm 713 pH-meter with a double junction glass electrode was used for pH adjustment.

2.3. Preparation of the sensor membrane

The transparent TAC membranes were produced from waste photographic films (KODAK ULTRA MAX 400), which were previously treated with commercial sodium hypochlorite in order to remove colored gelatinous layers. Then, the TAC films were treated with ethylenediamine (EDA) solution for 5 min at ambient temperature and finally washed with water to remove excess EDA. The resulting transparent TAC films were placed in the selected dye solutions and stirred for 3 h at room temperature. To remove the loosely immobilized dye molecules, films were finally washed by detergent solution and stored in water for further use [16,17].

2.4. pK_a measurements

Dyes reversibly respond to H^+ ions both in solution and after immobilization on TAC membrane. Absorption spectra of dyes were obtained as follows.

Methanolic solutions (0.04% (w/v)) were diluted to 0.001% (v/v) with an appropriate buffer solution at a suitable pH. Absorption spectra of the membranes were recorded after fixing the membrane at the end of a fiber optic probe and placing it in the appropriate solution at the desired pH.

The pK_a values of the studied dyes were determined by measuring the pH-dependent absorption of the dye at a fixed wave length chosen from the maximum optical density of the dye at two extremes represented by totally protonated or totally deprotonated forms of the compound. Plots of the absorbance vs. pH gave a sigmoidal curve with one reflection point. To evaluate the dissociation constants of the free and immobilized dyes, the absorbance vs. pH (Figs. 2 and 3) data was treated by KINFIT, a non-linear least squares curve fitting program [10]. The dissociation equilibrium between the acidic (HS) and basic (S^-) forms of the dye can be defined by:

$$\begin{aligned} &\mathrm{HS} = \mathrm{H}^+ + \mathrm{S}^- \\ &K_a = [\mathrm{H}^+][\mathrm{S}^-]/[\mathrm{HS}] \end{aligned} \qquad (1)$$

which can be written as:

$$[\mathrm{S}^-]/[\mathrm{HS}] = (A - A_{\mathrm{HS}})/(A_{\mathrm{S}^-} - A) \qquad (2)$$

and

$$A = \left([\mathrm{H}^+]A_{\mathrm{HS}} + K_a A_{\mathrm{S}^-}\right)/\left(\mathrm{H}^+ + K_a\right) \qquad (3)$$

where K_a, A_{HS} and A_{S^-} represent acidity constant, the maximum absorbance of the molecule in highly acidic and basic solutions, respectively. The pK_a values of the free and immobilized dyes were calculated using Eq. (3). Results are illustrated in Tables 1 and 2.

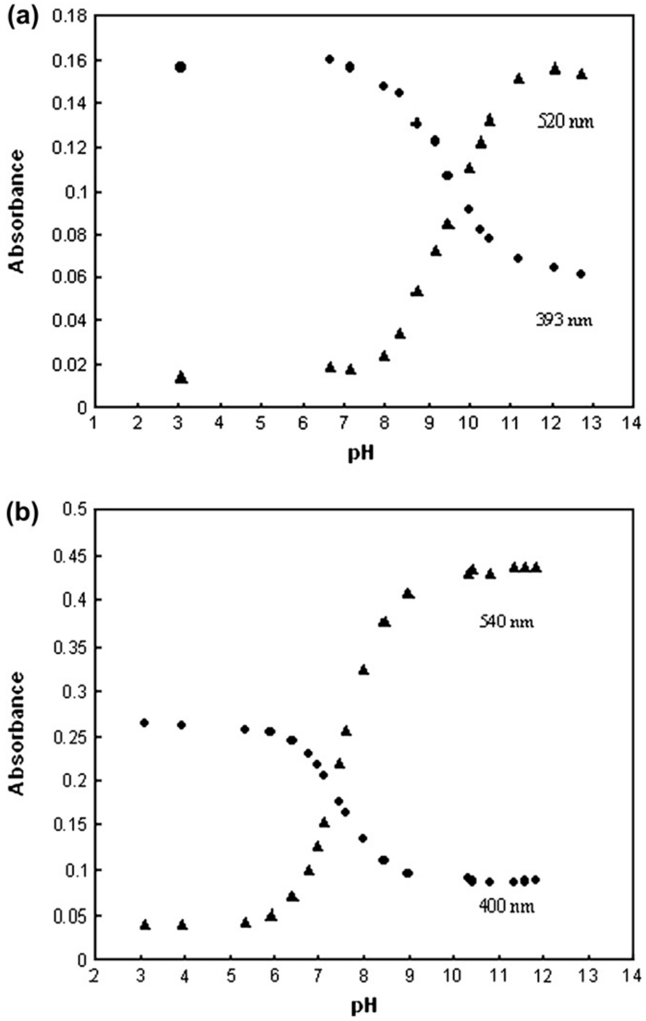

Fig. 2. Absorbance as a function of pH for membrane sensors of (a) **1** at 520 and 393 nm and (b) **5** at 540 and 400 nm.

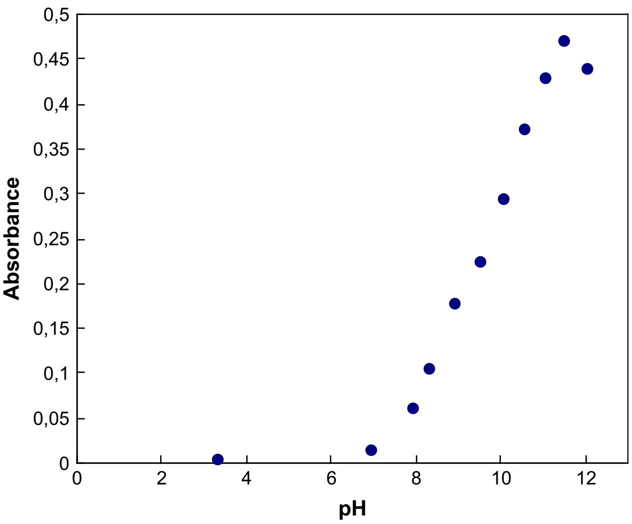

Fig. 3. Absorbance as a function of pH for mixed optode (at 529 nm).

Table 1
Absorption maxima of acid and conjugated base forms, maximum wave length differences, color changes and pK_a value of dyes in solution at 25 °C

Compound	Acidic λ_{max}	Basic λ_{max}	$\Delta\lambda$ (nm)	Color change	pK_a
1	375	485	110	Yellow to deep red	7.94 ± 0.01
2	356	442	86	Deep yellow to orange	8.21 ± 0.01
3	363	452	89	Yellow to deep yellow	7.23 ± 0.01
4	365	454	89	Yellow to deep yellow	7.62 ± 0.01
5	393	510	117	Yellow to deep violet	7.21 ± 0.01
6	377	466	89	Yellow to deep orange	8.15 ± 0.01

3. Results and discussion

3.1. Spectrophotometric study of hydroxyazobenzene derivatives

Many dyes act as pH indicators through the reversible action of acids and bases which has led to the concept of acid–base indicators [12,13]. Organic weak acids and bases are usually targeted for this purpose and aromatic azo dyes are attractive choices due to their extensive conjugated π system sharp and fast color change in the visible range. Azo derivatives of aromatic amines which can be considered as weak bases normally show blue shifts upon protonation and a decrease in the corresponding optical densities. However, azo derivatives of hydroxy aromatic compounds, display red shifts after dissociation at higher pH(s) without losing their corresponding optical intensities [8]. Therefore, spectrochemical properties of six derivatives of two different hydroxyazobenzene systems were investigated in this study.

Table 1, shows the absorption maxima of both acid and base forms of these compounds as well as their corresponding color change in the applied solutions. Because of larger differences in the absorption maxima, it is clear that the undissociated and dissociated forms of **1** and **5** exhibited distinct color changes in comparison with the other four derivatives. Compounds **1** and **5** have nitro group at the *para*-position of the second ring which effectively changes the conjugated system of the whole molecule and affects the π–π^* transition through both induction and resonance phenomena. Therefore, further studies were carried out on **1** and **5** for construction of pH optodes.

3.2. Spectrophotometric performance of immobilized 1 and 5

The optical properties of **1** and **5** in solution and immobilized on hydrolyzed cellulose acetate films are shown as a function of pH in Figs. 4 and 5. There are fixed at isosbestic points,

Table 2
Absorption maxima of acid and conjugated base forms, maximum wave length differences and pK_a value of immobilized forms of **1** and **5** dyes at 25 °C

Optode	Acidic λ_{max}	Basic λ_{max}	$\Delta\lambda$ (nm)	pK_a
1	393	520	127	*9.22 ± 0.01*
5	400	540	140	*7.56 ± 0.01*

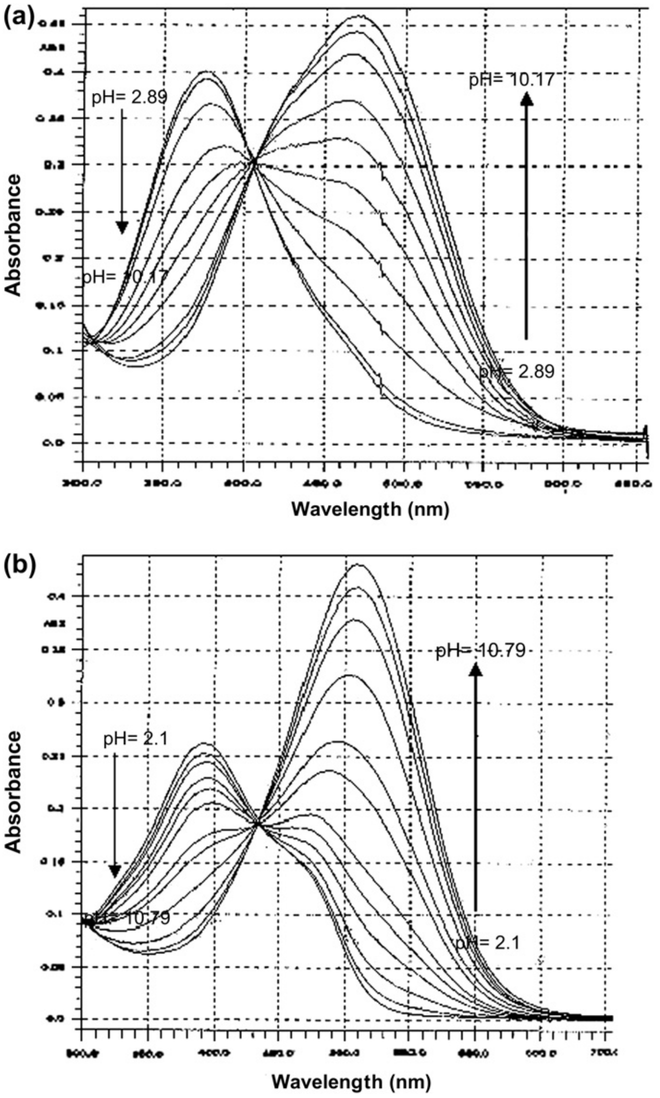

Fig. 4. Absorption spectra of solutions of (a) **1**, pH: (1) 2.89, (2) 6.18, (3) 7.16, (4) 7.64, (5) 7.90, (6) 8.15, (7) 8.40, (8) 8.96, (9) 9.40, (10) 10.17 and (b) **5**, pH: (1) 3.10, (2) 5.92, (3) 6.40, (4) 6.76, (5) 6.97, (6) 7.13, (7) 7.48, (8) 7.64, (9) 8.00, (10) 8.46, (11) 8.96, (12) 10.79. Arrows indicate the spectral trends in changing pH from 2.89 to 10.17 in (a) and from 2.10 to 10.79 in (b).

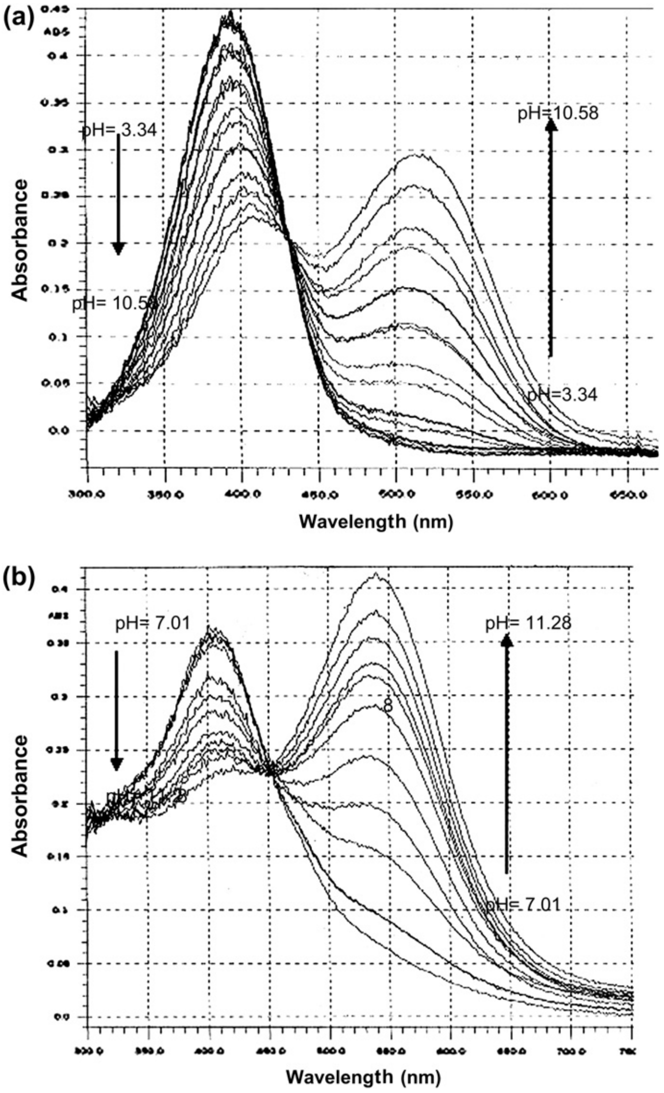

Fig. 5. Absorption spectra of immobilized forms of (a) **1**, pH: (1) 3.34, (2) 7.78, (3) 8.18, (4) 9.16, (5) 9.26, (6) 9.47, (7) 9.71, (8) 9.97, (9) 10.15, (10) 10.36, (11) 10.58 and (b) **5**, pH: (1) 7.01, (2) 7.76, (3) 8.54, (4) 8.84, (5) 8.96, (6) 9.27, (7) 9.46, (8) 9.60, (9) 9.85, (10) 10.18, (11) 11.28. Arrows indicate the spectral trends in changing pH from 3.34 to 10.58 in (a) and from 7.01 to 11.28 in (b).

indicating the presence of simple equilibrium between the protonated and deprotonated forms of the dyes both in solution and immobilized forms. As expected, the isosbestic points of the immobilized forms of **1** and **5** have shifted toward lower frequencies ($\Delta\lambda_{max} \geq 30$ nm) in comparison to those of their soluble forms (Tables 1 and 2). As previously reported, it was suggested that these shifts are possibly due to the change of the micro-environment and the structural conformation of the immobilized dyes that become more planar than that of their soluble forms [10,17,18]. As shown in Table 2, immobilization on TAC has raised the pK_a values of both **1** and **5**. The above results can be interpreted based on the influence of the immobilization on the behavior of the dyes. pK_a values are also affected by micro-environment interactions of indicator with medium. Similar observations were also reported by others [13,16,18]. The fact that immobilization changes the absorption maxima of the dye shows that the hydroxyl groups are auxochrome elements of the molecule and loss of a proton after immobilization influences the charge distribution during the dissociation of the immobilized dye. It seems that the oxygen adjacent to the hydroxyl functional group in **5** has a significant role in stabilizing the corresponding anion. Hence, the change in the pK_a value of the immobilized **5** is not as prominent as that for the immobilized **1**.

3.3. Calibration curve and the dynamic response range

The optical responses of **1** and **5** immobilized on TAC membrane to changes in pH are shown in Fig. 2. It is obvious that the curves can be suitably used as calibration plots for determination of pH in ranges 8.5–10.5 and 6.5–8.5, respectively. It is worth noting that the observed pH ranges for the optodes of **1** and **5** are the same as the safe pH ranges for phenolic and guaiacolic compounds [15]. Auto-oxidation of these compounds in the presence of molecular oxygen occurs at a faster pace beyond those for the mentioned pH ranges.

3.4. Equilibrium response time

The response time is defined as the time required for 95% of the total signal to change. It has been pointed out that the hydrolyzed acetylcellulose membrane has the advantage of a short response time mainly because of its good permeability for small ions [11]. Fig. 6 shows a typical curve for the optical property changes of the optode **1** as a function of time. The response time of the sensors **1** and **5** was less than 20 s in a pH range of 7.01–11.09 and at wavelengths of 520 and 540 nm, respectively.

3.5. Storage, stability and reproducibility

The stability of the **1** and **5** optodes was measured over a period of 80 days. It was found that the sensor showed only an 8% decrease in absorbance. Fig. 7 shows a typical curve for the stability of the optode **1**. When not used, the optodes were kept in water to prevent them from drying out.

The reproducibility of the **1** and **5** optode responses at 520 and 540 nm and alteration of pH from 3.70 to 10.70 was investigated. The relative standard deviation for both sensors was about 0.2% (Fig. 8 shows a typical curve for the reproducibility of optode **1**).

3.6. Effect of ionic strength

Optodes show variable sensitivities due to the different ionic strengths of the real samples. Thus, it is necessary to study the effect of the ionic strength on the optical properties of the desired sensor. The influence of the ionic strength on the optical response of the optode **1** is shown in Fig. 9. The calculated pK_a values for the immobilized indicator dyes decrease in the order of 9.98 ± 0.01 and 9.58 ± 0.01 for **1**, 9.76 ± 0.01 and 9.54 ± 0.01 for **5**, in the presence of NaCl (0.1 M) and in absence of it. The results showed that the change in the ionic strength could affect the pK_a value of the dye. As previously reported with increasing ionic strength, the acidity of the optode decreases [8,13,19].

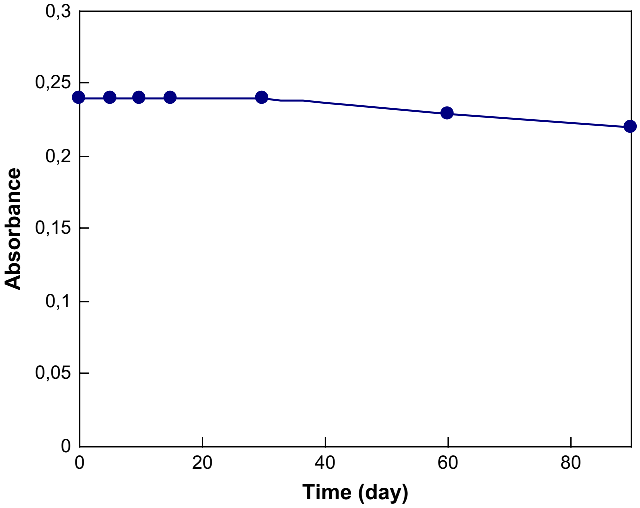

Fig. 7. Absorbance as a function of time (long-term stability) for the optode **1** at 520 nm (pH 6.5).

4. Extension of the linear dynamic range

Optical pH sensors containing pH indicators usually have two major limitations, a narrow dynamic range and non-linear response for pH measurements [1]. Sensors containing one indicator can normally measure pH over a range of about two pH units, i.e. p$K_a \pm 1$. Beyond this range, the change in absorbance with pH becomes small and the error will be large. It was shown that the dynamic range for pH measurement can

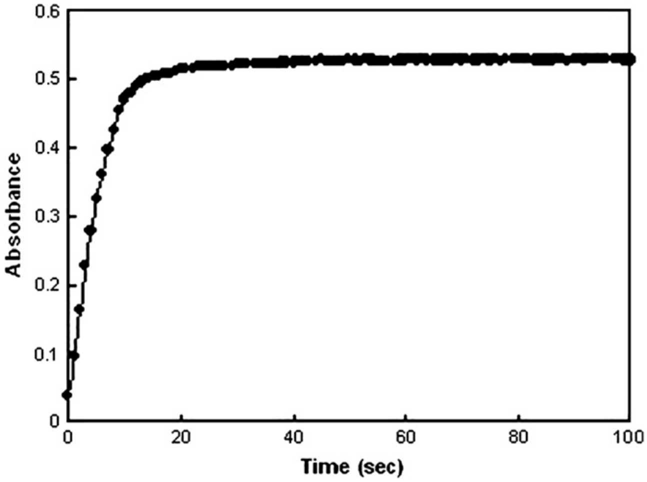

Fig. 6. Absorbance as a function of the time for **1** immobilized membrane when pH was changed from 7.01 to 11.09 at 520 nm.

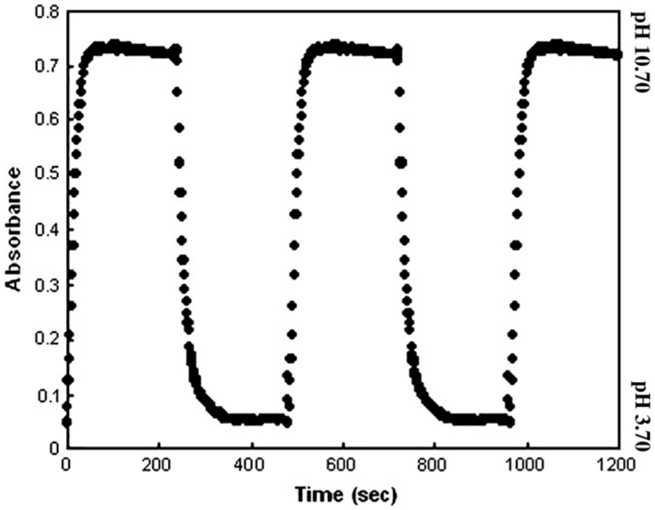

Fig. 8. Response reproducibility of the optode **1** at 520 nm for the alternative change in pH from 3.7 to 10.70.

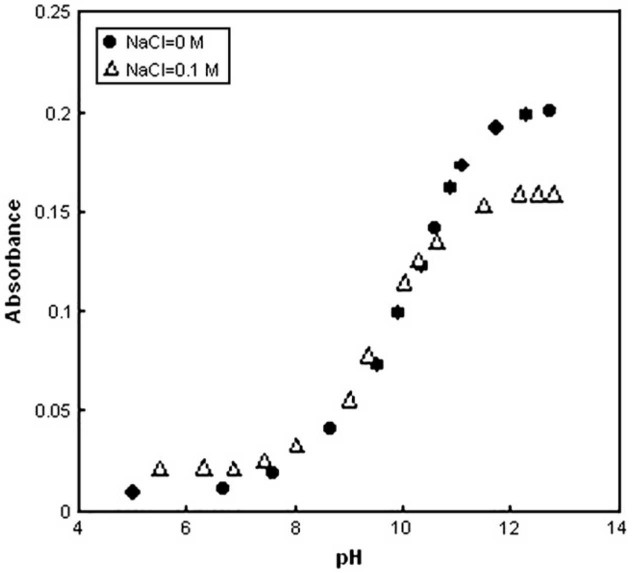

Fig. 9. pH titration plot of the optode **1** with NaCl (0.1 M) and without it. Absorption was measured at 520 nm, showing the effect of ionic strength.

be broadened using multiple indicators which should have a large ΔpK_a but not more than 1.7 [14].

As seen in Tables 1 and 2, the dyes **1** and **5** display $\Delta pK_{a(\mathrm{solution})}$ of 0.73 and $\Delta pK_{a(\mathrm{immobilized})}$ of 1.66, therefore they are suitable for construction of a mixed optode. Fig. 5 illustrates that the dynamic range of the mixed optode has become wider (7−11.5) in comparison with those of the individual ones with a good linearity (RSD less than 0.3%).

5. Conclusion

Azo dye derivatives of hydroxyazobenzene are used as indicators for the spectrophotometric measurement of hydrogen ions. Spectrochemical properties of six azo derivatives of two different hydroxyazobenzene (phenol and guaiacol) compounds against changes in pH were studied in this research. It was demonstrated that compounds **1** and **5** both with a strong withdrawing electron group, when immobilized on a TAC membrane, can act as suitable pH sensors in 8.5−10.5 and 6.5−8.5 pH ranges, respectively. The optodes are easily made for the determination of pH. Besides, they also possess good durability (>3 months), high reproducibility and rapid equilibrium time (<20 s). In this study the linear dynamic range was extended using mixed indicators (7−11.5). It was also shown that the linear range of the new optode becomes two fold wider than those of the individual forms.

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