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A novel optical membrane with extended detection range of pH

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A pH optical sensor was developed based on the use of a mixture of Malachite Green Oxalate and Bromocresol Green indicators immobilized in a triacetylcellulose membrane, which had been previously hydrolyzed. Requirements of the pH indicators were investigated, including the effects of the coupling pH, indicator ratio, and concentrations of the indicators. The pH sensor performed with a very fast response (10 s) and long term stability with no significant leaching of the dyes. The sensor had a linear response over a range of 6.6 pH units in the pH range between 0.4 and 7.0. A relative standard deviation of pH equal to 1.59% was obtained for 7 replicated pH measurements.

The procedure for preparation of this optical sensor is simple, inexpensive, and rapid.

Key Words: Mixed reagents, pH sensor, Bromocresol Green, Malachite Green Oxalate

Introduction

pH is one of the most common laboratory measurements because so many chemical, biological processes, and practical applications such as clinical analysis, environmental analysis, process control, and rate of chemical and biochemical reactions are dependent on pH.[1,2] In order to optimize the desired reaction and to prevent unwanted reactions, controlling the pH of solutions is also very important. Traditionally, the measuring methods for pH values fall roughly into 5 categories: indicator reagents, pH test strips, metal electrode methods (hydrogen

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electrode, quinhydrone electrode, and antimony electrode method), glass electrode, and optical sensor (optode) methods. Optical and fiber-optic pH sensors complement the glass electrodes for pH measurement and offer numerous advantages such as immunity from electrical interference, feasibility of miniaturization, and the possibility of remote sensing and in vivo measurement. In optodes, such indicators are chemically or physically immobilized in (or on) a solid support or polymeric matrices, covalent immobilization and spread as a thin layer or coating.[3−14] Optical pH sensors based on the use of pH indicators have 2 major limitations, i.e. non-linear response and narrow dynamic range for pH measurement. Most dyes are only sensitive to changes in pH values over the range of 2-4 pH units. Some attempts have been made to broaden the dynamic range of pH measurement by employing for example, fluorescent indicators,[15−18] indicators with 2 acidic groups,[19] or several signal processing techniques, such as the Fourier transform method[20] and multivariate calibration based on an artificial neural network.[18]

Immobilization of a mixture of 2 indicators with different pK_a values can extend the useful dynamic range of a pH sensor. In this study, we developed a simple and inexpensive method for producing a linear response over a broad range for optical pH sensors by immobilization of a mixture of 2 pH indicators, Malachite Green Oxalate (MGO) and Bromocresol Green (BCG), on optically transparent triacetylcellulose (TAC). Two of the advantages of the proposed membrane are that it responds linearly over a range of 6.6 pH units in the pH range between 0.4 and 7.0 and has a fast response time (10 s).

Experimental

Reagents

All reagents used in this work were of analytical grade and were used without any further purification. All aqueous solutions were prepared with deionized water. All chemicals were supplied by Merck Company. The general information about the pH indicators (BCG and MGO) is shown in Table 1.

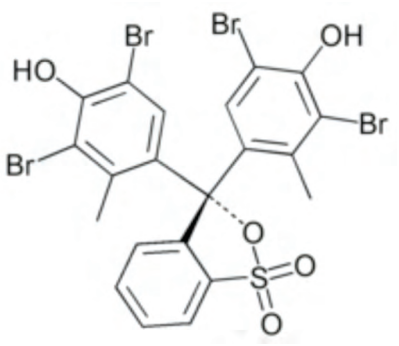
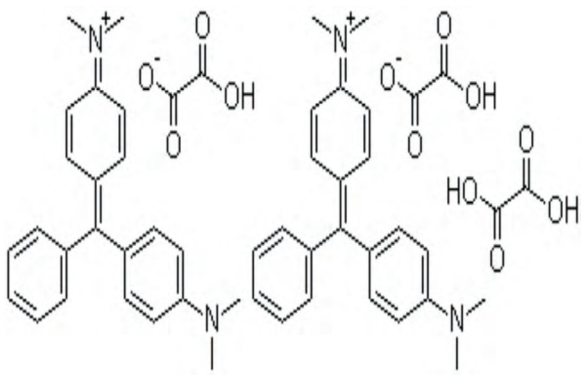
Universal pH buffer solutions were prepared from boric acid/citric acid/phosphoric acid (0.04 M each). A wide range of buffers covering pH from 1.0 to 8.0 was adjusted by the addition of 0.2 M sodium hydroxide or 4 M hydrochloric acid solutions. For low pH values, -log[H^+] was used to calculate the pH value of the solution.

The stock solutions of MGO and BCG were prepared by solving 0.05 g of BCG and 0.05 g of MGO in two 50 mL volumetric flask with methanol (0.1% m/v).

Instrumentation

A Metrohm 632 pH-meter with a Metrohm double junction glass electrode was used for monitoring pH adjustment. A UV–vis spectrophotometer (Analytik Jena AG, SPECORD S 100 spectrometer, Germany) with a photodiode array detector and a 1 cm standard quartz cell was used for recording the visible spectra and absorbance measurements. The size of the sensor membrane was 1.0 × 5.0 cm. In order to keep the sensor membrane standing vertically inside the sample cell, some upper part cut for opening was left bent against the wall of the cell and all measurements were performed in batch mode. In our experiment, the sensors' membranes were immersed in dilute hydrochloric acid solution of pH 2.0 only for 1 min to retrieve their acidic forms after each decaying measurement and dried by filter paper for the next use.

Table 1. Generalized scheme and information of Bromocresol Green and Malachite Green Oxalate.

BCG [a]	MGO [b]
$C_{21}H_{14}Br_4O_5S$	$2C_{23}H_{25}N_2,3C_2H_2O_4$
	

Indicator	Transition	pH range with transition
BCG	3.8 - 5.4	
MGO	0.2 - 1.8	
		0 1 2 3 4 5 6 7 8 9 10 11 12 13 14

[a]Bromocresol Green
[b]Malachite Green-Oxalate GR

Preparation of the sensor membrane

The immobilized pH indicators on triacetylcellulose were prepared according to the following procedure. The transparent triacetylcellulose membranes were produced from waste photographic film tapes that were previously treated with commercial sodium hypochlorite for several seconds in order to remove the colored gelatinous layers. The rectangular plate (1 cm × 5 cm) was placed in a 0.1 M sodium hydroxide for hydrolyzing of TAC membrane for 24 h.

The optical sensor membrane was treated as follows: 10 pieces of hydrolyzing TAC membranes were transferred into a beaker containing of a mixing of 3 mL of MGO and 5 mL of BCG stock solution (0.0375% m/v MGO and 0.0625% m/v BCG) for 2 min in a 60 °C water bath. The resulting optical sensor was thoroughly washed with water for removing loosely trapped dyes. The membranes prepared by this method were kept for 24 h in water prior to use. Prepared membranes were stable over several weeks of storage in pure water.

Results and discussion

Lin and Liu have explained that there are 3 factors to broaden the dynamic range by using mixture indicators for pH measurement; $\Delta \mathrm{p}K_a$ between indicators, colors of indicators, and concentrations of indicators.[21] They explained that when $\Delta \mathrm{p}K_a > 2$, a non-linear response is obtained. The second requirement is on the colors of the indicators, i.e. the colors of either the acid or base form of all the indicators must be similar to each other. The third requirement is on the concentrations of the indicators, i.e. they must be 'matched' to achieve a linear response with a minimum deviation of pH. In this study, we described that with matching of the last 2 factors, namely the colors of indicators and concentrations of indicators, the first factor is not a limitation for broadening the linear range of a pH optode.

Optical characteristics of the sensor membrane

The spectra of MGO with $\mathrm{p}K_a = 1.05$ and BCG with $\mathrm{p}K_a = 4.66$ in solution with different pH are shown in Figures 1a and 1b, respectively. At low pH, the absorbance maxima for MGO are at 619 nm and 427 nm, and absorbance for former peak increases as solution pH increases, while for the second peak absorbance decreases. There are reverse behaviors for the second peak. There is an isosbestic point at 494.5 nm for the pH range up to 2.0. The pH variation was linear for pH between 0.2 and 2.1. Moreover, the absorbance maximum for BCG is at 431 nm, and this peak decreases as solution pH increases and the peak at 619 nm continually increases with the increasing pH. There is an isosbestic point at 511 nm. The pH variation was linear for pH between 3.7 and 5.9. The spectral change is a result of acid–base equilibrium of the indicators.

The absorption spectra of MGO and BCG immobilized in TAC resemble the spectra of them in aqueous solutions (Figure 2a and 2b). Based on Figure 2a, at low pH, the absorbance maxima for MGO immobilized in TAC are at 631 and 435 nm, and absorbance for the former and latter peaks slightly increase and decrease, respectively, as solution pH increases. There is an isosbestic point at 574.5 nm for the pH range up to 2.0. Moreover, the absorbance maxima for BCG immobilized in TAC (see Figure 2b) are at 624 nm and 423 nm. The absorbance for the former peak ($\lambda_{\max} = 624$ nm) continually increases with the increasing pH between 3.5 to 6 and the absorbance for the second peak decrease as solution pH increases. There is an isosbestic point at 504 nm with increasing pH.

Based on Table 1, the colors of either the acid or base form of MGO and BCG are similar to each other (note: the color of MGO in pH < 0.2 is yellow). The absorption spectra of a mixture of MGO and BCG (a mixture 3 mL of MGO and 5 mL of BCG from stock solution) in solution (water) and immobilized forms of these reagents on hydrolyzed cellulose acetate film are shown in Figure 3. The spectra for mixed MGO and BCG in solution are shown in Figure 3a. At low pH, the absorbance maximum for this mixed solution is at 437.0 nm, and this peak decreases as solution pH increases and a peak at 617.3 nm continually increases with the increased pH from 1.0 to 5.5. There is an isosbestic point at 511.0 nm. The spectral changes are completely reversible with variation in the pH.

The absorption spectra of MG and BCG immobilized in TAC reassembles to the spectra of them in aqueous solutions (Figure 3b). In acidic solution, the absorbance maximum for this optode is at 432.0 nm, and this peak decreases as solution pH increases and a new peak at 624.0 nm continually increases with the increased pH. A maximum absorbance was observed at pH 7.0 and was selected as optimum. This pH is quite

close to the optimum immobilization pH that was already obtained for BCG alone on the same support. Thus, the absorbance changes with variation in the pH of the mixed reagent in the optode at maximum wavelengths (624.0 nm) can be used for low pH values within 6.6 pH units between 0.4 and 7.0.

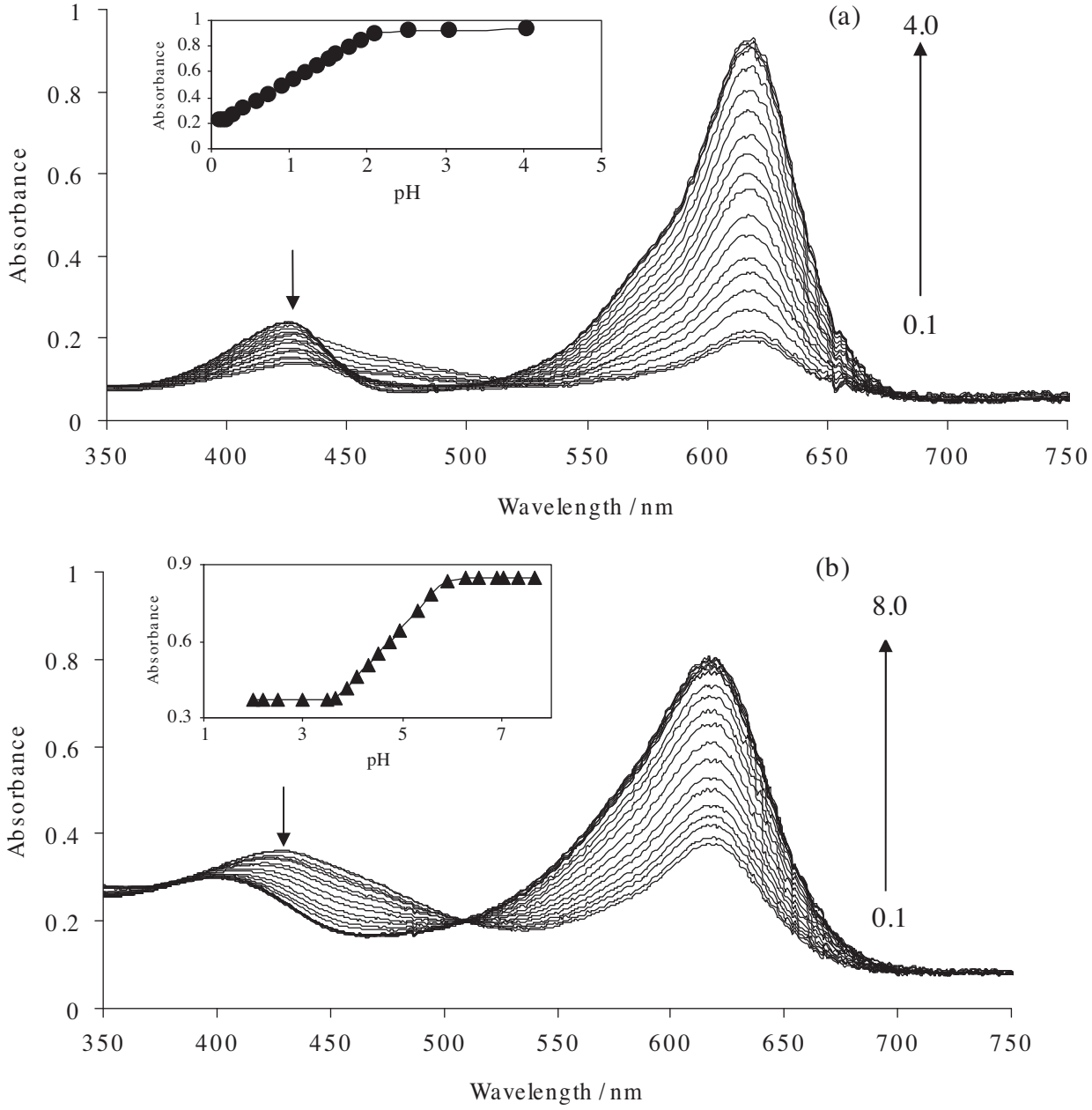


Figure 1. Absorption spectra in different pH values for the dissolved forms of (a) MGO, the insertion shows linear regressions of absorbance vs. pH with the equation: $Abs_{(\lambda=619nm)}$ =0.354pH + 0.161 for pH range 0.2-2.2, r = 0.9998. The indicator concentration was 0.001% (m/v) and (b) BCG, the insertion shows linear regression of absorbance vs. pH with the equation: $Abs_{(\lambda=619nm)}$ = 0.210pH -0.395 for pH range 3.8-6.0, r = 0.9996. The indicator concentration was 0.0006% (m/v). The arrows show the direction of pH decrease and increase.

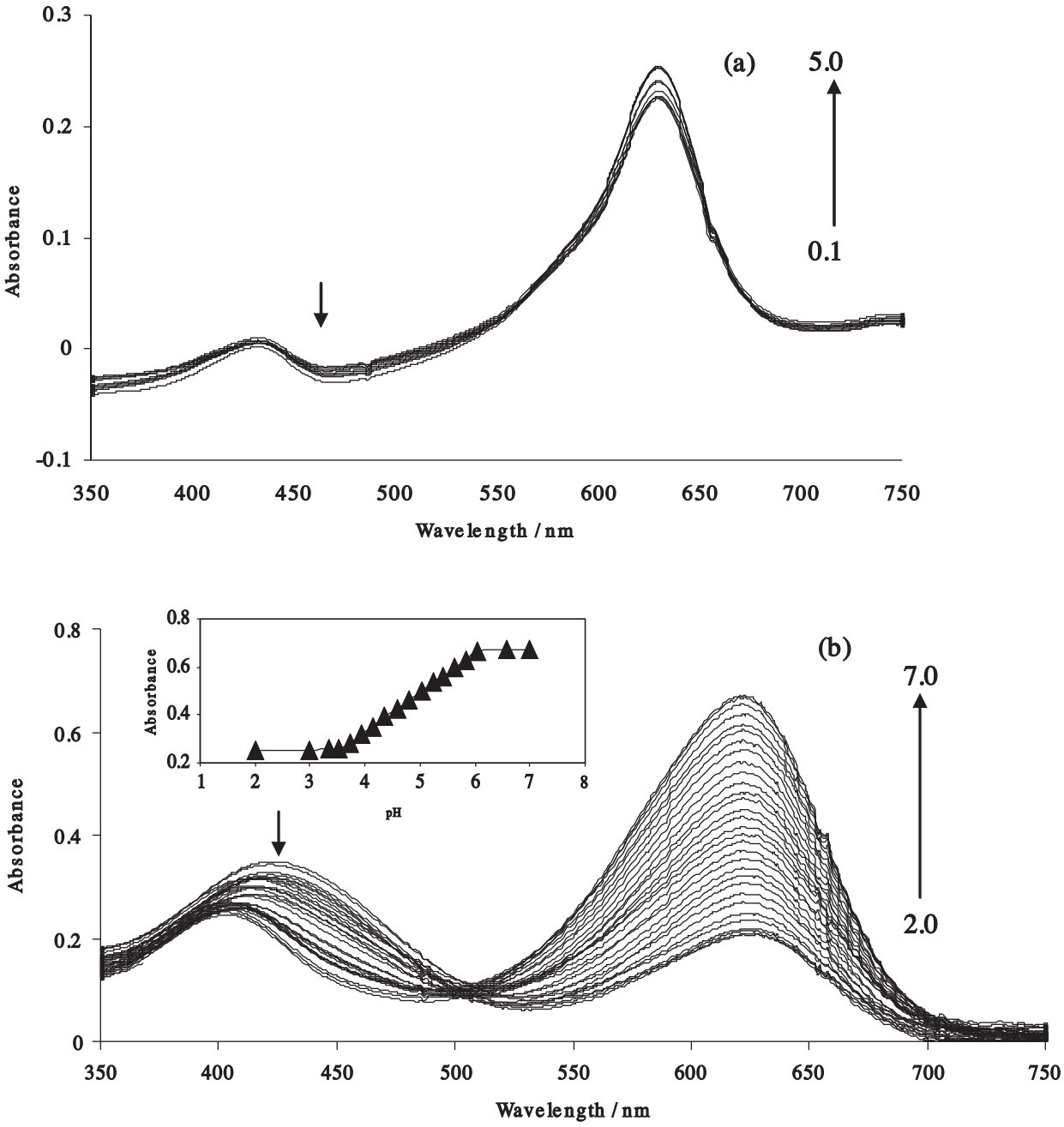


Figure 2. Absorption spectra in different pH values for immobilized forms on TAC (a) MGO and (b) BCG. The insertion shows linear regression of absorbance vs. pH with the equation: $Abs_{(\lambda=624nm)} = 0.162pH - 0.315$ for pH range 3.8-6.0, r = 0.9996. Conditions for preparation of membrane, solution contains 0.1% (m/v) for each, preparation time, 2 min and preparation temperature, 60 °C).

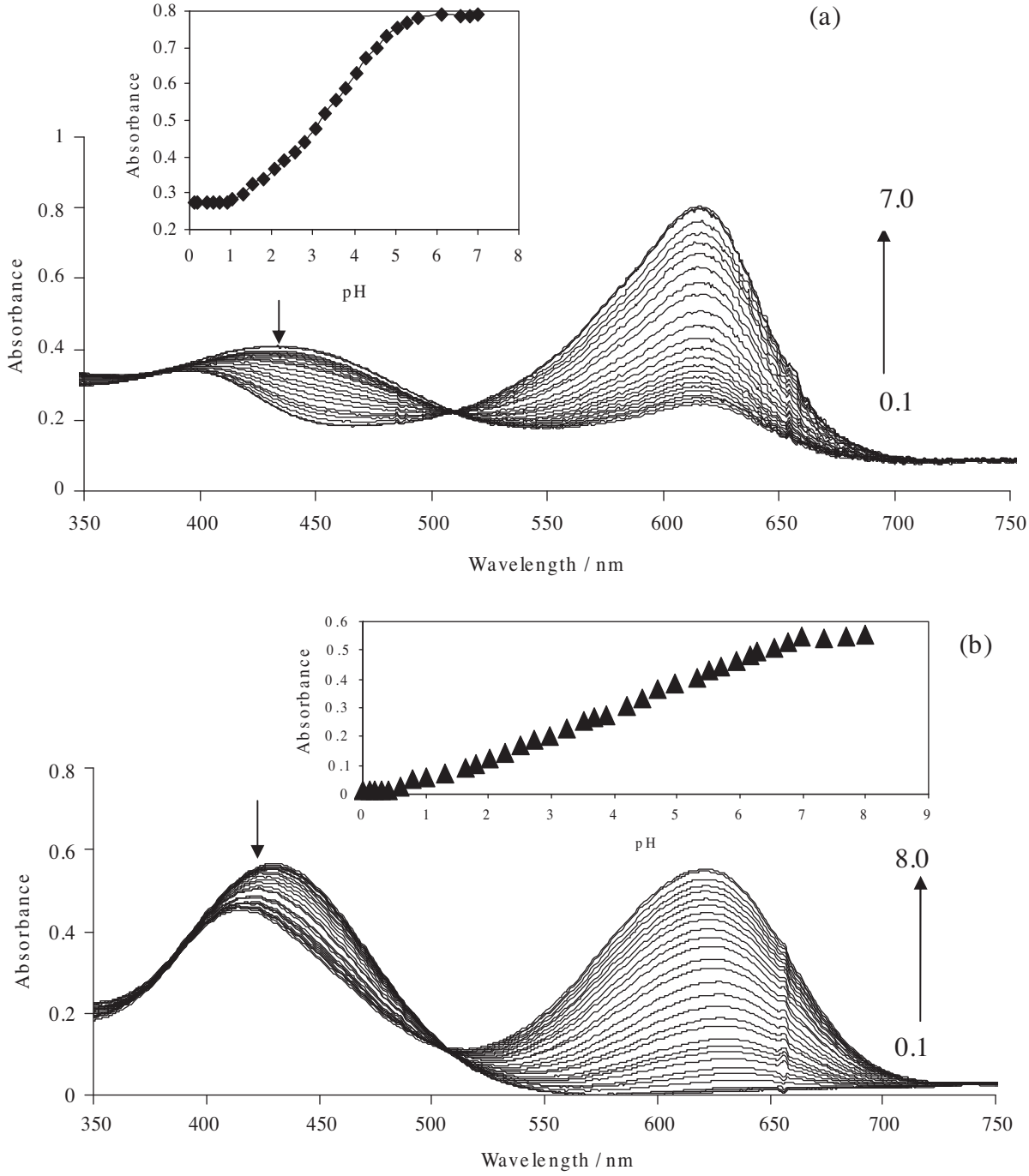


Figure 3. Absorption spectra in different pH values for the dissolved forms of (a) mixed indicators MGO (0.0375% m/v) and BCG (0.0625% m/v). The insertion shows linear regression of absorbance vs. pH with the equation: $Abs_{(\lambda=619nm)}$ = 0.120pH +0.132 for pH range 0.8-5.8, r = 0.9893 and (b) immobilized forms of mixed indicators on TAC. The insertion shows linear regression of absorbance vs. pH with the equation: $Abs_{(\lambda=624nm)}$ = 0.083pH - 0.034, for pH range 0.4-7.0, r = 0.998. (Conditions for preparation of membranes, solution contains 0.0375% m/v MGO and 0.0625% m/v BCG, preparation time, 2 min and preparation temperature, 60 °C).

The analytical signals' variation for the membrane at the λ_{max} = 624 nm is sharper than the former. Thus, this wavelength, 624.0 nm, was selected for further studies. The wavelength for the isosbestic point is at 509.0 nm. The spectral changes are completely reversible with variation in the pH. It is important to note that there is a red shift of the absorbency maximum of the new peak in the membrane compared to the aqueous solutions, e.g. the absorbance maximum are 624.0 nm and 617.3 nm in the membrane and in aqueous solutions, respectively. This may suggest that the structured conformation for chromophore groups of this wavelength in the immobilized dyes is more planar than that of its soluble analogue. Similar results have been reported by Safavei's group and Jones's group.[18,22] In addition, a reverse result has been obtained for the other peak. There is a blue shift of the absorbency maximum in the membrane compared to the aqueous solutions, e.g. the absorbance maxima are at 432.0 and 437.0 nm in the membrane and in aqueous solutions, respectively. However, at higher pH range this wavelength in the membrane shifts to 417.0 nm, indicating that the chromophore group for this wavelength in the membrane has 2 forms, possibly a free form and an adsorbed form.[23] Moreover, the wavelength for the isosbestic point shows a blue shift from 511.0 to 509.0 nm in the aqueous solutions compared to the membrane.

The effect of MGO and BCG concentration ratio

The effect of pH on the coupling of different mixtures of stock solutions of MGO and BCG for preparation of optodes is shown in Figure 4. These solutions were prepared by mixing of different volumes of stock solution with 0.1% m/v of MGO and BCG. As can be seen in Figure 4, the plot of the absorbance (at 624.0 nm) vs. pH exhibits no linear range up to 7.0 and the best concentration ratio of the 2 indicators for preparation of optode was 0.0375% m/v MGO (see Figure 3b). Therefore, a 0.0375% m/v MGO was selected for further studies. This result shows that in order to achieve a linear response with a minimum deviation of pH the concentrations of the 2 indicators must be matched. In addition, the effect of concentrations of MGO and BCG with a concentration ratio of $\frac{3}{5}$, m/m (MGO/BCG) on the absorbance of the sensor at 624.0 nm was also investigated. Increasing the indicators concentrations from 0.01% (m/v) to 1.0% (m/v) in individual stock solution increased the optode absorbance from 0.05 to 1.75 absorbance units. Since excessive absorbance can diminish the transparency of the optical sensor and increase the uncertainty of absorbance measurements, a concentration of 0.1% (m/v) with an absorbance of about 0.6 was used in subsequent experiments.

Analytical figures of merits

The major requirements for an ideal optode membrane are fast response time, high response sensitivity, long lifetime, and excellent reproducibility. Figure 5 shows a typical response for the transition process as a result of change in pH of the solution in contact with the optode from pH value of 5.90 to 3 successive pH values of 4.04, 2.01, and 0.68 at 624 nm. These results confirm the excellent reversibility of the sensor. The relative standard deviation was less than 1.59% for 7 measurements at 624.0 nm.

A further important feature of a sensor is its response time. The response time is defined as the time required for 95% of the total signal change. The response time of the optode was measured as 10 s in the pH transition interval between 1.34 and 5.01 at 624.0 nm. Typical response curves of the sensor as a function of

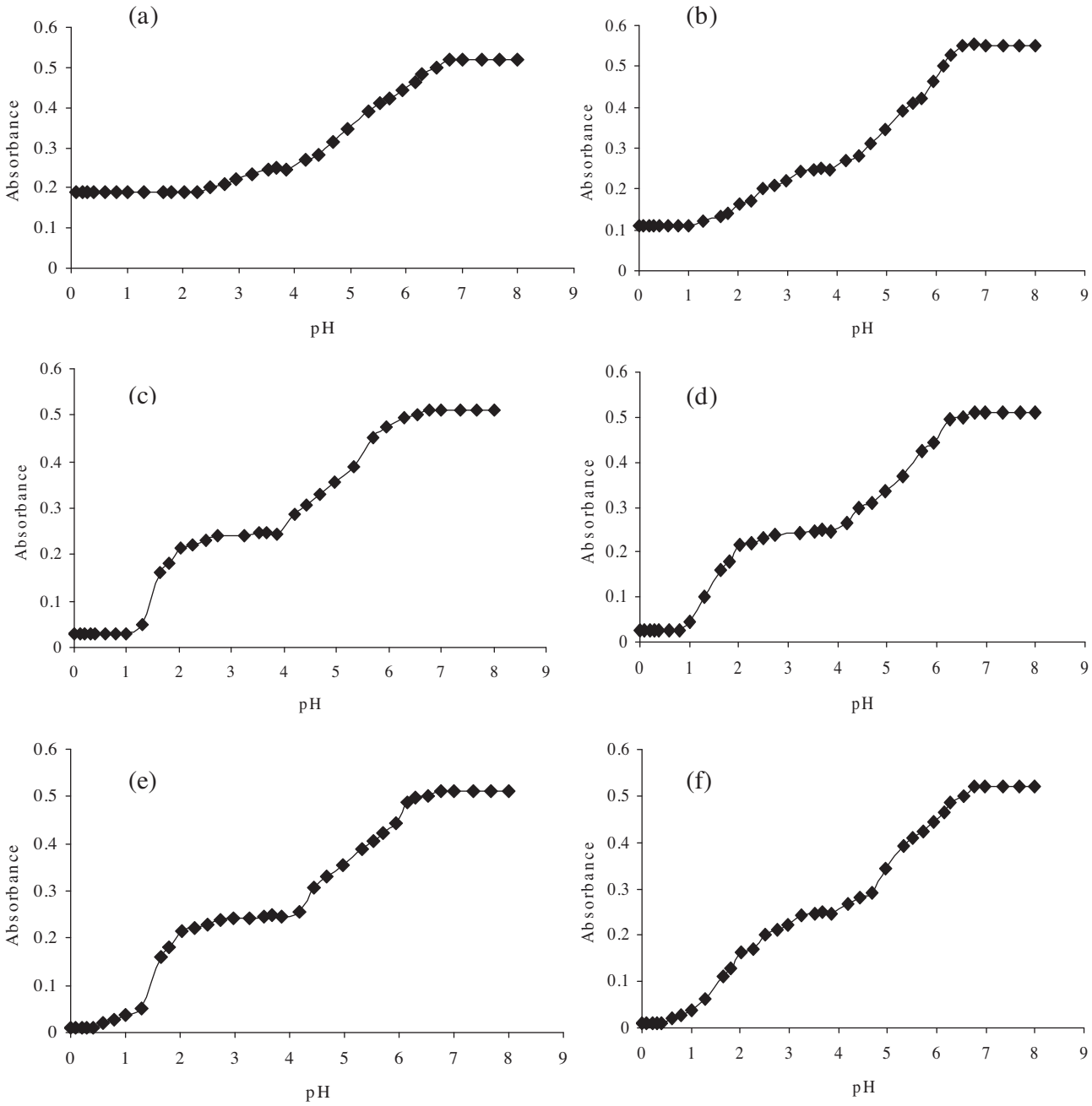


Figure 4. The effect of MGO % (m/v) in preparation solution of membranes on linear pH range of membranes a) 0.017%, b) 0.028%, c) 0.044% d) 0.050%, e) 0.0555%, and f) 0.0625% with a fixed BCG % (0.0625, m/v) for all membranes. (Absorbance was measured at 624 nm).

time when pH changes from 1.34 to 5.01 are shown in Figure 6. It should be noted that the signal leveled off after equilibrium and no drift in response was observed under the experimental conditions employed. The optode was

stable over successive pH measurements in the pH range of 0.4-7.0 and was described by the equation Abs. = 0.083pH - 0.034, r = 0.998 and n = 32, where Abs. is the absorbance measured by UV–vis spectrophotometer, r is the correlation coefficient, and n represents the number of determinations. The membrane was usually kept in water when not in use to prevent it from drying out. It was stable over the applied pH range with no leaching of the dyes.

Finally, the effect of temperature on the preparation time of optode was studied. The results are shown in Figure 7. An optimal temperature of 60 °C was selected for preparation of the optode after 2 min.

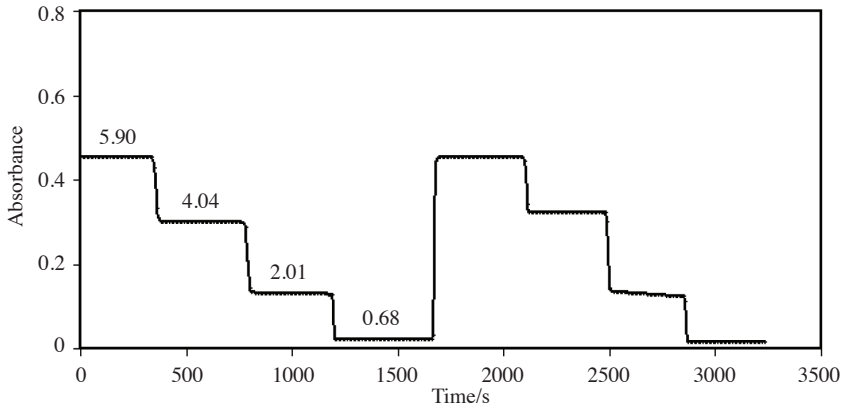


Figure 5. Response reproducibility of the membrane at 624.0 nm for the alternative change in pH from 5.90 to 3 different pH values of 4.04, 2.01, and 0.68, respectively. (Conditions for preparation of membrane, solution contains 0.0375% m/v MGO and 0.0625% m/v BCG, absorbance at 624 nm, preparation time, 2 min and preparation temperature, 60 °C).

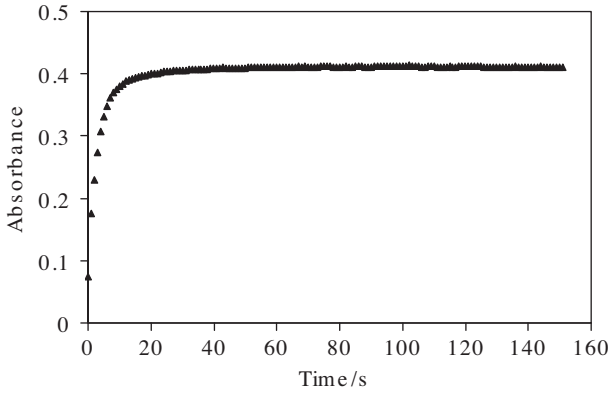


Figure 6. Absorbance as a function of the time for 1 immobilized membrane when pH was changed from 1.34 to 5.01 at 624 nm. (Conditions for preparation of membranes, solution contains 0.0375% m/v MGO and 0.0625% m/v BCG, absorbance at 624 nm, preparation time, 2 min and preparation temperature, 60 °C).

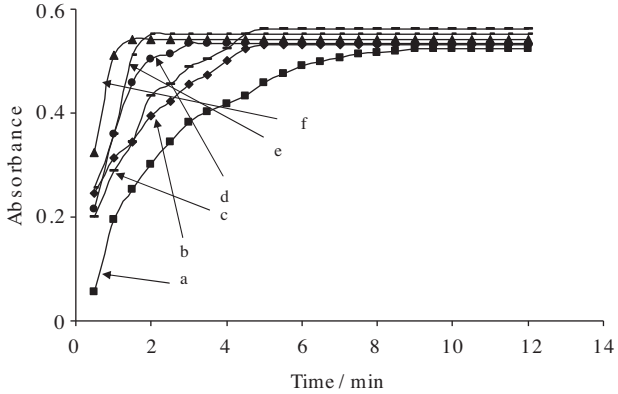


Figure 7. The effect of temperature on preparation time of membrane from a solution contains 0.0375% m/v MGO and 0.0625% m/v BCG a) 24 °C, b) 30 °C, c) 40 °C, d) 50 °C, e) 70 °C, and f) 60 °C. (Absorbance was measured at 624 nm).

Stability and life-time

The sensor requires an aging time of ca. 6 days after the indicators are immobilized onto the film. Within the first 6 days, no obvious loss of the dye into the solution was observed; as indicated there is no absorbance at 624.0 nm in the soaking solution, and no decrease in the film absorbance in the linear range of the optode. After further aging, the spectra remain unchanged. Absorbance values measured at days 30, 45, and 60 days are essentially the same and the relative standard deviation (R.S.D. %) in pH 6 buffer is less than 3.78% for n = 5.

Conclusion

The above results show that the application of a mixture of indicators of MGO and BCG immobilized on a triacetylcellulose membrane can offer a suitable pH sensor for wide range pH measurements. The membrane produced by this method is cheap, and has low immobilization time (about 3 min), high stability, short response time (10 s), and excellent spectral characteristics. One of the best features of such an optode is its ability to measure a linear response over a range of 6.6 pH units in the pH range of 0.4-7.0. This sensor exhibits advantages over many existing optical pH sensors including wider dynamic pH range, ease of fabrication, good reversibility, and stability (see Table 2). Requirements of the pH indicators were investigated, including the same colors of acid and base form of the indicators and the matching concentrations of the indicators, and the $\Delta \mathrm{p}K_a > 2$ between the indicators was not a critical limitation.

Table 2. Comparison the proposed optode with other optical sensor contains BCG for pH measurement.

Indicator	Support	Linear range / pH	Response time / s	Ref. q
BCG[a]	Sol-gel	5-8	60	24
BCG-BCP[b]-PR[c] (mixture of 3 indicators)	Sol-gel	6.3-9.8	-	21
BCG-BCP-PR-TB[d] (mixture of 4 indicators)	Sol-gel	4.3-8.8	-	21
BCG-MGO[e] (mixture of 2 indicators)	TAC	0.4-7.0	10	This work

[a] BCG: Bromocresol Green, [b] BCP: Bromocresol purple, [c] PR: Phenol red, [d] TB: Thymol blue, [e] MGO: Malachite Green-Oxalate.

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