

Design and evaluation of a copper (II) optode based on immobilization of 1-(2-pyridylazo)-2-naphthol (PAN) on a triacetylcellulose membrane

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Abstract: An optical sensor was established to determine of copper (II) based on the immobilization of 1-(2-pyridylazo)-2-naphthol (PAN) on a triacetylcellulose membrane. Copper (II) ions react with the immobilized PAN and cause an increase in the absorbance of the membrane at 549 nm. The response time of the optode is within 10-12 min depending on the concentration of Cu (II) ions. This sensing phase had a dynamic linear range of 0.10-180 $\mu\text{g mL}^{-1}$ of copper ions with a limit of detection of 0.025 ng mL^{-1} . The selectivity of the optode to Cu (II) ion in the presence of other metal ions including Pb (II), Mg (II), Ca (II), Ba (II), Zn (II), Ni (II), Co (III), Mn (II), Cd (II), Bi (III), Pd (II) and Al (III) is high, with the exception of Hg (II) and Pd (II) ions as the main interferences which can be eliminated by iodide as the masking agent. The sensor could be fully regenerated by ethylene diamine, and the color is fully reversible. The membrane was applied for determination of Cu^{2+} in some real samples which gave satisfactory results.

Keywords: Optode; 1-(2-pyridylazo)-2-naphthol (PAN); Copper (II); Triacetyl cellulose membrane.

1. Introduction

Copper participates in many biological processes, such as hemoglobin synthesis (in Fe utilization and Hb regeneration), connective tissue development, normal functions of the central nervous system, and oxidative phosphorylation [1-3]. Many methods have already been developed for the determination of copper in real samples. These are based on atomic absorption spectrometry (AAS) and extractive spectrophotometric methods. The optochemical sensors can offer advantages in terms of size, cost, not requiring a reference element, and transmitting without the influence of an electromagnetic field. Most of these optodes are based on absorption or reflectance

measurements of immobilized colorimetric reagents in various matrices. Although, a few sensor exist for determination of copper but they have some difficulties such as pretreatment, higher detection limits and no simplicity. We before employed PAN as an ionophore for determination of Pd (II) [4] and in the present work it immobilized on a triacetyl cellulose membrane for the determination of low levels of copper (II) ion. The membrane responds to copper (II) ion by changing in absorbance, it can be applied as a relatively selective reagent for Cu (II) ions at $\text{pH}=2.0$.

2. Results and Discussion

2.1. Properties of PAN as an Ionophore and Spectral characteristics

The complexation reactions of PAN with metal ions are well known to be strongly dependent on the pH, as illustrated in Fig. 1. In its neutral form, PAN is nearly insoluble in water, while it is quite soluble in organic solvents [5]. Coo et al [6] reported a spectrophotometric study of the solubility and protolytic properties of PAN in different ethanol-water mixtures. The complexation reaction of PAN with metal ions has been reported in the literature [7-10], and the results revealed the formation of a complex with a 1:1 stoichiometry. In order to investigate the leaching of PAN from the membrane into test solution under the experimental conditions employed (i.e. in buffered solution of pH=2.0), the absorbance measurements at 549 nm of buffered solutions in the

presence of the membrane was carried out with elapse of time. No measurable leaching was observed even at a time period of 24 h. It should be noted at pH=2.0, the ligand exists its form (LH_2^+ , yellow- green).

Fig.2 shows the absorption spectra of free and immobilized PAN which were obtained after being equilibrated in buffer solution (pH=2.0) containing different concentrations of copper (II). The spectral change is a result of addition of Cu (II) ions and complex formation. The absorbance maxima of the immobilized PAN are located at 549 and 467 nm and those of the free dye at 548 and 450 nm. As can be seen from Fig. 2b the increase in the absorption band at 549 nm is more pronounced in the membrane as the copper concentration increases. The wavelength of 549 nm was selected for further studies because of higher selectivity and sensitivity at this wavelength.

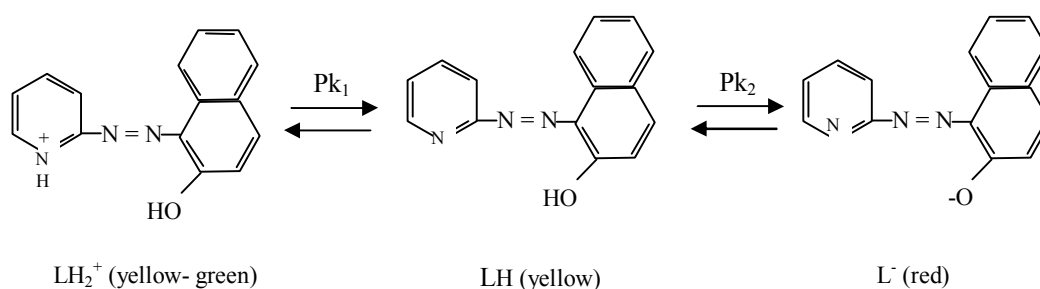


Fig. 1. Protolytic reactions and color change of PAN

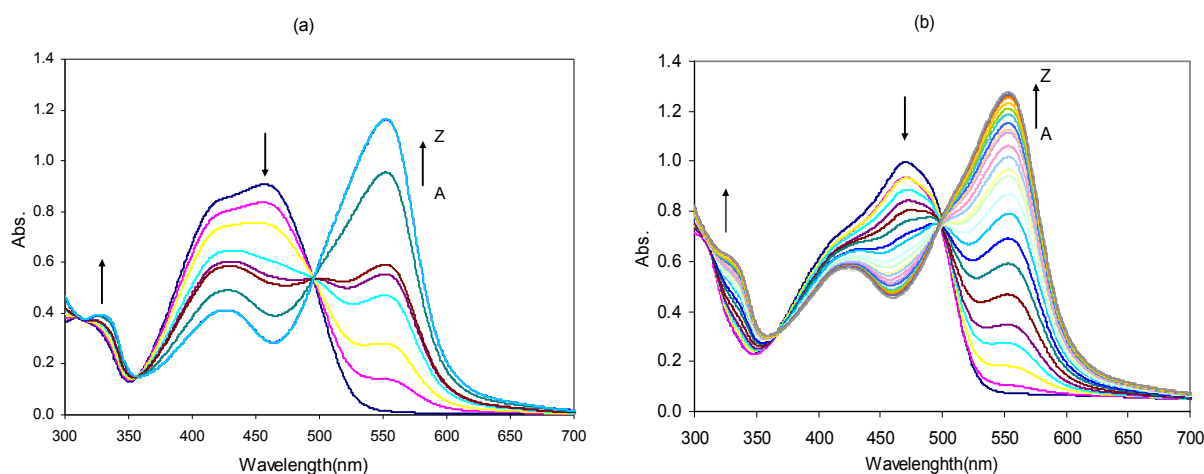


Fig. 2. (a) Absorption spectra for a PAN solution in range of 0 - 20 $\mu\text{g mL}^{-1}$ of Cu^{2+} at pH 2. (b) Absorption spectra of optode film in the presence of 0- 18 $\mu\text{g mL}^{-1}$ Cu(II) at pH 2. (A-Z indicate the spectra trends in changing Cu(II) concentration from 0 - 20 $\mu\text{g mL}^{-1}$ in (a) and 0-18 $\mu\text{g mL}^{-1}$ in (b))

2.2. Effect of pH

Fig.3a shows the effect of pH values on the absorbance intensity of the optode membrane. The absorbance measurements were made for 1.0×10^{-5} M ($2.0 \mu\text{g mL}^{-1}$) copper (II) ion at different pH values at 549 nm. The blank membrane (membrane without PAN in buffer solution) was taken as the reference. The absorbance measurement were expressed as absorbance difference, which was defined as the difference between the absorbance of the immobilized PAN alone and the absorbance of the Cu (II)-PAN complex at 549 nm. As pH increase from one to nine, the difference in absorbance reaches a maximum at pH=2.0, and then decrease sharply. This phenomenon might be due to the fact that at low pH values ($\text{pH} < 2$), complexation is weak. At pH values higher than 2, Cu (II) forms different hydroxide species ($\text{Cu}(\text{OH})_n$ ($n=2$)-) which make complex formation with PAN impossible.

2.3. Dynamic range

Fig. 3b shows the absorption signals of the optode film to the various concentrations of Cu (II) ions in the range 0 – $20 \mu\text{g mL}^{-1}$. In this case, $18 \mu\text{g mL}^{-1}$ was found as the concentration of Cu (II) ions that saturates the film. The absorbance measurements were expressed as the absorbance difference, which is defined as the difference between the absorbance of the membrane with immobilized PAN alone and the absorbance of the membrane in the presence of Cu (II). As can be seen in Fig. 3b, the plot of the absorbance difference versus Cu (II) concentration exhibits a linear range between $1 \mu\text{g mL}^{-1}$ and $18 \mu\text{g mL}^{-1}$. The regression equation is:

$$\text{Response} = 0.0478 C + 0.0151$$

Where response is the increase in the absorbance of the film at 549 nm for a fixed time of 12 min, the C is the concentration of Cu (II) in $\mu\text{g mL}^{-1}$, with a correlation coefficient of 0.994.

The detection limit, which was estimated as the concentration of analyte producing an analytical signal equal to three times the standard deviation of the blank signal was found to be 0.025 ng mL^{-1} .

2.4. Response time

An important analytical feature of any optode film is its response time. In this work, the optode film was found to reach 95 % of the final signal at 10–12 min depending on the concentration of Cu (II) ions. The response time of the present optode film is controlled by the time required for the analyte to diffuse from the bulk of the solution to the membrane interface and to associate with the indicator.

2.5. Life time and stability

The life time of membrane was determined by adding a buffer solution ($\text{pH}=2.0$) in a cuvette including a film. The signal was recorded at wavelength of 549 nm over a period of about 24 h. No significant loss of the indicator occurs during this time. When the membrane was exposed to light, no drift in signal occurred and the membrane was stable over the experiment with no leaching of the indicator. However, prepared membranes were kept under water when not in use to prevent them from drying out. Additionally the stability of response of the film was investigated over five weeks under ambient conditions, which indicated that the film was stable over this period.

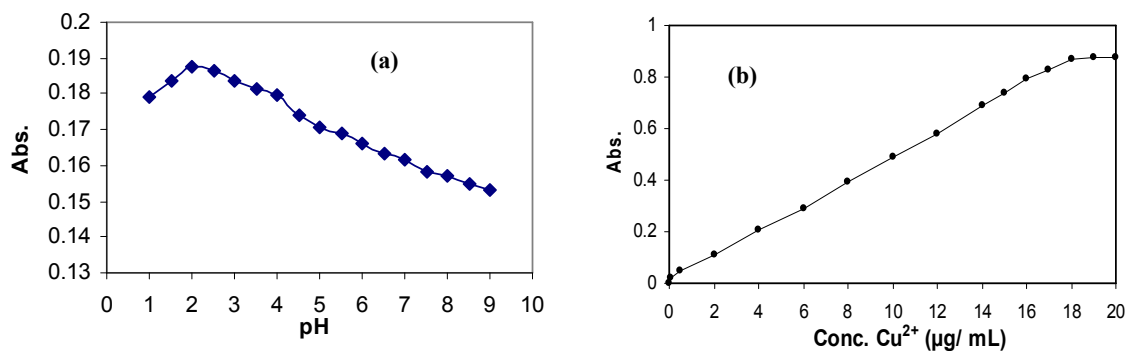


Fig.3. (a) Effect of pH on the optode film response (concentration of Cu (II) is $2 \mu\text{g mL}^{-1}$). (b) The optode film response vs. Cu (II) ion concentration in the range of 0 – $20 \mu\text{g mL}^{-1}$ at $\text{pH}=2.0$.

2.6. Regeneration of the optode film

For a membrane to perform suitably, the color change must be reversible. Anions such as SCN^- , NO_3^- , SO_4^{2-} , Cl^- and EDTA were studied as regenerating reagents. It was found that, none of them were not useful for this study. Regeneration with I^- takes long time to remove all traces of copper (II) from the membrane. The best result was obtained by applying ethylene diamine, which gave short membrane regeneration times (3-5 sec). After this and for the next copper concentration measurement, the optode should be placed in buffer (pH=2.0) for 15 min.

2.7. Reproducibility and reversibility

The reproducibility and reversibility of the optode membrane in the determination of copper (II) ions were evaluated by repeatedly exposing the optode membrane to a $5 \mu\text{g mL}^{-1}$ and ethylene diamine solution. The repeatability was evaluated by performing seven determinations with the same standard solution of Cu (II). The relative standard deviation (R.S.D) for the response of one membrane towards a $5 \mu\text{g mL}^{-1}$ of copper (II) ions solution was 1.3 %. The reproducibility of the response of different membranes was also studied. Seven different membranes were prepared from the same batch and they were evaluated by performing the determination of $5 \mu\text{g mL}^{-1}$ copper (II) ions. The relative standard deviation between membranes was 2.8 %. The results show that the reproducibility and reversibility are satisfactory and the membrane could be regenerated easily by using ethylene diamine solution.

2.8. Effect of foreign ions on Cu (II)-PAN complex

To determine the selectivity of the proposed method, Interference of a number of ions is studied to estimate the absorbance of the Cu (II)-PAN complex by adding different amounts of other ions including, Ag (II), Mn (II), Co (II), Cd (II), Hg (II), Ba (II), Pb (II), B (III), Sn (II), Mg (II), Cu (II), Al (III), Ti (I), Ni (II), Cr (III), Pd (II) and Zn (II) to solution containing 5 ng mL^{-1} of copper (II) ion. The tolerance limit was taken as the concentration causing an error of $\pm 5\%$ in the analytical signal for determination of Cu (II). Ions such as Mn (II), Co (II), Cd (II), Hg (II), Ba (II), Pb (II), Mg (II), Al (III), Ti (I), Ni (II), Zn (II) and Cr (III) at the applied pH value of 2 no interference on the determination of copper even in a 1000 fold excess. Sn^{2+} ion shows some levels of interference, no interference was observed from even 66-fold excess. Bi (III), Ag (I) is participated at the pH. The main interferences in this case were Hg (II) and Pd (II) ions.

However, the interference from these ions can be eliminated by using potassium iodide as the masking agent. It is apparent from this study that discrimination between different metals can be achieved by the control of several parameters including the absorption wavelength, the pH and the sample contact time in addition to the use of masking agents.

3. Experimental

3.1. Reagents

All the reagents used in this work were of analytical grade. Ethylene diamine was supplied from Merck. Universal buffer solutions were prepared from boric acid / citric acid / phosphoric acid (0.04 M each). The final pH was adjusted by the addition of 0.2 M hydrochloric acid and sodium hydroxide. A stock solution of 0.004 M Cu (II) ion was prepared by dissolving 0.3802 g of $\text{Cu}(\text{NO}_3)_2$ (Merck) in double distilled water in a one liter volumetric flask and finally made up to the mark with double distilled water.

3.2. Apparatus and measurement procedure

A Shimadzu 1601 PC UV-visible spectro - photometer with 1.0 cm quartz cells was used for recording the visible spectra and absorbance measurements. The sensing membrane was placed and fixed in a disposable plastic cuvette and all measurements were performed in a batch mode. A Jenway pH-meter (model 3510) with a combined glass electrode was used for pH adjustment. A Sense AA GBC Scientific Equipment atomic absorption spectrometer was used to compare the result.

3.3. Preparation of the sensor membrane

The immobilized indicator on triacetylcellulose was 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 colored gelatinous layers. The films were treated with a clear solution of PAN (0.02 g) in 10 mL ethylene diamine for 7-8 min at ambient temperature [11]. Then they were washed with water for removing ethylene diamine and loosely trapped indicator. The membranes were finally washed with detergent solutions and water. Prepared membranes were kept under water when not in use.

Table 1. Determination of Cu (II) in vegetables and blood samples

Sample	copper (II) added ($\mu\text{g mL}^{-1}$)	copper (II) found ($\mu\text{g mL}^{-1}$)		Recovery %	RSD %
		AAS Method	Proposed Method		
Spinach	0.0	-	B.L.	-	-
	2.20	2.21	2.23	101.3	1.5
	4.50	4.49	4.51	100.2	1.2
Spearment	0.0	-	B.L.	-	-
	1.50	1.50	1.52	101.3	1.3
	2.50	2.51	2.53	101.2	1.1
Tarragon	0.0	-	B.L.	-	-
	5.50	5.50	5.54	100.7	1.2
	8.20	8.20	8.18	99.7	1.3
Blood 1	0.0	-	B.L.	-	-
	2.20	2.21	2.25	102.2	1.4
	4.50	4.49	4.48	99.5	0.9
Blood 2	0.0	-	B.L.	-	-
	1.50	1.50	1.52	101.3	1.3
	2.50	2.51	2.51	102.0	0.9
Blood 3	0.0	-	B.L.	-	-
	5.50	5.51	5.46	99.2	1.1
	8.20	8.21	8.23	100.3	0.8

B.L.Below linear range

3.4. Analytical Application

The developed optical method for Cu (II) was successfully applied for its determination in blood sample and vegetables sample. Various blood samples and various vegetables samples with different contents of Cu (II) were prepared and analyzed employing the recommended procedure. The results were in perfect agreement with those obtained by direct atomic absorption spectrometry. The results are presented in Table1. The results were in good agreement with those obtained by atomic absorption spectrometry.

From the result, the present optical membrane seems useful for the determination of copper in real samples.

4. Conclusion

The optode described in this work is easily prepared and provides a simple and inexpensive means

for the determination of Cu (II) ions. The membrane responds to copper ion by changing color reversibly from yellow - green to red. The sensor can be regeneration readily with a solution of ethylene diamine and has a long lifetime. The response of the optode was reproducible and the optode presented a good selectivity for Cu (II) over other metal ions except for Hg (II) and Pd (II) which can be omitted by using KI as masking agent. Since the sensor does not require solvent extraction, it can compete with standard optical fibers.

Acknowledgments

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