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Sensitive and selective detection of trace copper in standard alloys, food and biological samples using a bulk optode based on N,N'-(4,4'-ethylene biphenyl) bis(3-methoxy salicylidine imine) as neutral carrier

Majid Arvand*, Zahra Lashkari

Department of Chemistry, Faculty of Science, University of Guilan, Namjoo Street, P.O. Box 1914, Rasht, Iran

HIGHLIGHTS

G R A P H I C A L A B S T R A C T

 An optical sensing film has been proposed for sensitive determination of Cu(II) ion.

- ► The copper sensing membrane works on the basis of a cationexchange mechanism.
- Satisfactory analytical sensing characteristics for determining Cu(II) ion were obtained.
- ► The optode membrane has been applied to determine Cu(II) in various real samples.

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Absorption spectra of ionophore in the solution at the presence of different cations.

ABSTRACT

In this paper, an optical sensing film has been proposed for sensitive determination of copper (II) ion in aqueous solutions. The copper sensing membrane was prepared by incorporating N,N'-(4,4'-ethylene biphenyl) bis(3-methoxy salicylidine imine) as ionophore in the plasticized PVC membrane containing bis(2-ethylhexyl) sebacate (DOS) as plasticizer. This proposed membrane works on the basis of a cation-exchange mechanism and shows a significant absorbance signal change on exposure to acetate buffer solution of pH 4.0 containing copper ion. The proposed sensing film displays a linear range of 0.01–32.0 μ g mL⁻¹ with a limit of detection 0.008 μ g mL⁻¹. Moreover, upon the introduction of a negatively charged lipophilic additive (oleic acid) into the membrane, the optode displayed enhanced sensitivity. In addition, satisfactory analytical sensing characteristics for determining copper (II) ion were obtained in terms of the selectivity, stability and reproducibility. The response time of the optode was less than 3 min, depending on the concentration of Cu(II) ions. The optode membrane has been applied to determine Cu(II) in various real samples.

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Introduction

In recent years, there has been a growing need for constructing chemical or electrochemical sensors for fast and economical monitoring of environmental samples especially for heavy metal ions in real time [1–18]. In this connection, chemical sensors based on

* Corresponding author. Tel./fax: +98 131 3233262. *E-mail address:* arvand@guilan.ac.ir (M. Arvand). optical signal measurement are those of the advanced techniques in analytical chemistry and they have been accepted as advantageous because they can be miniaturized and manufactured at low cost [19].

For the preparation of an ion-selective optode membrane, an ionophore can be incorporated into a hydrophobic membrane such as a plasticized poly(vinylchloride) (PVC) membrane, and utilized in contact with a sample solution containing a primary ion. Ionophores are lipophilic complexing agents having the capability of binding ions reversibly and to transport them across organic membranes

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by carrier translocation. Ideally, selective ion carriers render a membrane permeable for one given sort of ion I only. For an analytically relevant application of ionophores in solvent polymeric membranes for bulk optodes or ion selective electrodes some requirements such as sufficient lipophilicity, sufficiently reversible exchange kinetics and high selectivity have to be met simultaneously [20–27].

Copper is one of the heavy metals, low concentration of which is essential for plants and animals whereas, it is highly toxic to aquatic plants and bacteria in a high concentration. Copper is an essential trace element for biological processes, even at an ultra trace level [28]. Due to its mobilization and redox activity, copper is believed to play a central role in the formation of reactive oxygen species, such as O₂ and OH radicals. These radicals bind very rapidly to DNA, and can cause damage by breaking the DNA strands or modifying the bases and/or deoxyribose, therefore leading to carcinogenesis [29]. Moreover, it is an essential constituent of about thirty enzymes and glycoproteins and is required for the synthesis of hemoglobin and for some biological processes. It also promotes iron absorption from the gastrointestinal system, is involved in the transport of iron from tissues into plasma, helps to maintain myelin in the nervous system, is important in the formation of bone and brain tissues and is necessary for other many important functions [30]. Thus, due to the urgent need for selective copper determination in many biological, geological, environmental and industrial samples, various analytical techniques have been proposed for determination of copper including spectrophotometry [31-38], atomic absorption spectrometry (AAS) [39], inductively coupled plasma-emission spectrometry (ICP-ES) [40], potentiometry [41-45], and anodic stripping voltammetry [46]. These methods usually have a sufficiently low detection limit and specificity, but also have drawbacks such as high costs for equipment and assays, time-consuming, and complicated operation. So these methods are unsuitable for simple, low cost, and remote determination of copper especially in field analysis strategy.

Following to our recent studies concern to the application of a new class of ionophores containing nitrogen atoms such as 6-(4-nitrophenyl)-2-phenyl-4-(thiophen-2-yl)-3,5-diaza-bicyclo[3.1.0]-hex-2-ene [47], 6-(4-nitrophenyl)-2,4-diphenyl-3,5-diaza-bicyclo [3.1.0] hex-2-ene [48], 6-(4-nitrophenyl)-2-phenyl-4,4-dipropyl-3,5-diaza-bicyclo [3,1,0] hex-2-ene [49], 2-(4-methoxy phenyl) 6-(4-nitrophenyl)-4-phenyl-1,3-diazabicyclo [3.1.0] hex-3-ene [50], (2E, 4E)-5-(2,4-dinitrophenyl amino)penta-2,4-dienal [51] and N⁵-(2,4-dinitro-phenyl)-N¹,N¹-diethyl-penta-1,3-diene-1,5-diamine [52] as carrier in developing of ion-selective electrodes [47–50] and optodes [51,52], we have been motivated to investigate the application of N,N'-(4,4'-ethylene biphenyl) bis(3-methoxy salicylidine imine) (EBSI, Scheme 1) as complexing agent for sensitive determination of copper (II) ion in aqueous solutions. This iono-phore can extract Cu(II) from aqueous sample solution into organic



Scheme 1. Structure of EBSI used as ionophore.

membrane phase and form a complex, which results a decrease in the ionophore absorbance and produce a new absorbance peak at a higher wavelength. The sensor has longer linear dynamic range than the previously reported copper optical sensors [30–37], plus lower limit of detection. In addition, selectivity of the film optode based on EBSI was investigated. As a result, EBSI shows a striking selectivity for Cu^{2+} complexation and is more selective than the previously reported sensors [31–34]. To the best of our knowledge, this compound has not previously been used in the development of a Cu(II) optical sensing film or in any other optical sensors.

Experimental

Reagents

All chemicals were of the best available analytical reagent grade and all solutions were prepared with double distilled water. Relatively high-molecular-weight poly(vinylchloride) (PVC), nitrobenzene (NB), acetophenone (AP), dibuthyl phthalate (DBP), bis(2-ethylhexyl) sebacate (DOS), dibuthyl sebacate (DBS), tetrahydrofuran (THF), triethylammonium phosphate (TEAP), oleic acid (OA), sodium tetraphenyl borate (NaTPB) and all other reagents were obtained from Merck (Darmstadt, Germany) or Fluka (Buchs, Switzerland). A stock solution of 100 $\mu g \, m L^{-1}$ copper (II) solution was prepared by dissolving appropriate amount of Cu(NO₃)₂·3H₂O (Merck) in 100 mL volumetric flask and diluted with doubly distilled water. Working standard solutions of lower concentrations were prepared by suitable dilutions of the stock solution with water. The pH adjustments were made with acetate buffer solution $(1 \times 10^{-4} \text{ M} \text{ CH}_3\text{COOH} 5.5 \times 10^{-4}$ M CH₃COONa) to achieve the desired pH, but in studying of pH effect, the pH of solutions was adjusted with either HCl or NaOH solutions.

Synthesis of the N,N'-(4,4'-ethylene biphenyl) bis(3-methoxy salicylidine imine) (EBSI) as ionophore

The ionophore EBSI (Scheme 1), used in this effort, was synthesized according to the reported method with some modification in the described procedure [53]. 0.7 g (3.3 mmol) of the 4.4'-diamino bibenzyl was dissolved in 20 mL ethanol (96% v/v). To this was added dropwise solution of (1.0 g, 6.6 mmol) o-vanilin in 2 mL ethanol over period of 10 min. This solution was stirred for 15 min at room temperature. The color of solution changed to orange. The crude precipitate was filtered and recrystallized from toluene. The precipitate was washed with cold ethanol. The melting point of the orange crystalline form of the EBSI ionophore was 224-226 °C; IR (KBr): 3450, 1620, 1470, 1260 cm⁻¹. ¹H NMR (500 MHz, $CDCl_3/DMSO-d_6$): $\delta = 13.4$ (s, 1H), $\delta = 8.3$ (s, 1H), δ = 6.91 (s, 4H), δ = 6.7 (dd, J = 1.1 Hz, J = 7.8 Hz, 1H), δ = 6.6 (d, J = 7.8 Hz, 1H), $\delta = 6.5$ (t, J = 7.8 Hz, 1H), $\delta = 3.58$ (s, 3H), $\delta = 2.64$ (s, 2H) ppm; ¹³C NMR (125 MHz, DMSO-d₆): 38.1, 57.9, 118.6, 120.1, 121.8, 123.4, 129.7, 139.5, 148.6, 148.8, 150.9, 161.1 ppm; C₃₀H₂₈N₂O₄ (480.56): calcd. C 74.98, H 5.87, N 5.83, O 13.32; found C 75.21, H 5.71, N 6.11, O 12.97.

Apparatus

A Cary 100 UV–Vis spectrophotometer (Agilent Technologies, Santa Clara, United States) with a 1 cm cell was used for recording all spectra and absorbance measurements. A Metrohm pH meter (model 827, Swiss made) with a combined double junction glass electrode was used for monitoring pH values. All measurements were made in the absorbance mode.

Membrane preparation

The optimized composition for the preparation of the optode membrane consisted of 2 mg of EBSI as ionophore, 61 mg of DOS as plasticizer, 4 mg of OA as additive and 30 mg PVC. The membrane components were dissolved in 1.5 mL freshly distilled THF in a glass vial. The solution was immediately shaken vigorously to achieve complete homogeneity. An aliquot of 60 μ L of this solution was poured and uniformly spread on a dust free glass plate (8 mm \times 25 mm) which mounted on a spin device (rotating frequency \sim 2600 rpm). After a spinning time of about 15 s, the glass support plate with sensing membrane was removed and allowed to stand in ambient air for 24 h before use. The thickness of dry membrane was estimated to be \sim 4 μ m.

Sample preparation and determination

Preparation of standard alloys

A 0.1 g sample of the standard alloy was completely dissolved in 6 mL of (1 + 1) hydrochloric acid by heating in a water bath and adding 1 mL of 30% (v/v) hydrogen peroxide. The excess peroxide was decomposed by heating the sample in the water bath. The solution was cooled, filtered if needed and diluted to 200 mL with distilled water in a standard flask after adjusting the pH to 4 [31]. An aliquot of this sample was taken and the copper content was then determined according to the recommended procedure.

Preparation of water sample

One liter of mineral water sample was adjusted to pH 4.0 with acetate buffer, filtered through a Millipore 0.45 μ m pore size membrane into previously cleaned polyethylene bottle and was analyzed within 6 h of collection [31]. A defined volume treated by the recommended procedure for the determination of copper.

Determination of copper in hair

A 1.0 g of sample was decomposed by heating with 30 mL of concentrated nitric acid and 3 mL of 60% perchloric acid in a Kjeldahl flask. The solution was cooled, filtered and diluted to 200 mL with water in a calibrated flask after adjusting the pH to 4 [54]. An aliquot of this solution was taken and copper was determined by the general procedure.

Determination of copper in multivitamin capsule

Five capsules of multivitamin was accurately weighed and placed into ceramic crucible. Five milliliter of concentrated nitric acid was added to it and heated to near dryness. After cooling, the residue was dissolved in another 5 mL of concentrated HNO_3 and the solution was gently evaporated using a water bath. The residue was again heated with 50 mL distilled water, filtered off and diluted to 100 mL in a calibrated flask after adjusting the pH to 4 [55]. A defined volume treated by the recommended procedure for the determination of copper.

Determination of copper in foods

20.0 g of each sample was accurately weighed and placed into quartz crucible. Ten milliliter of concentrated sulfuric acid was added to it and evaporated to near dryness; then 10 mL of nitric acid (1 + 1, volume ratio) was added and evaporated to dryness. Under the heating conditions, concentrated hydrogen peroxide was added by drop till the solution clearness and evaporated. Water was added and continued to heat to remove the hydrogen peroxide. The residue cooled and was transferred into a 50 mL calibrated flask and diluted to the mark with water after adjusting the pH to 4 [56]. A defined volume was taken for the determination of copper via the recommended procedure under the established optimum conditions.

Recommended procedure

For simple and easy use of the ion-sensing film optode, the PVC membrane was attached to the surface of an 8 mm \times 25 mm thin glass plate whose size fits into a conventional glass vessel used as a standard optical cell for a spectrophotometer as shown in Fig. 1. Consequently, the determination of an analyte ion using the film optode can be achieved by simply immersing it into the glass vessel for monitoring the absorbance change as the response of the optode.

The prepared membrane was put vertically inside the sample cell containing 3 mL acetate buffer solution with pH = 4.0, and a membrane (without ionophore) at the same conditions was used as a blank membrane. The standardized metal ion solutions were added to the sample cell and the absorbance value of the system was measured after 15 min (required to reach the equilibrium) at λ_{max} = 400 nm. The limiting absorbances A_0 and A_c were determined with the optode membrane in contact without and with 64.0 µg mL⁻¹ Cu²⁺. By plotting the calibration curve of the absorbance signal value α (Eq. (3)) versus the logarithmic concentration of Cu ion (*p*Cu), the unknown Cu concentration can be read. All measurements were carried out at room temperature.

Results and discussion

Spectral characteristics of ionophore in the solution

The membrane sensor proposed is based on the incorporation of a Schiff base, EBSI with two hydroxyl functional groups in its structure as an ion exchanger. This ionophore (EBSI) is insoluble in water in its neutral form. In preliminary experiments, the complexation of EBSI with different cations was investigated spectrophotometrically in ethanol solvent at 25 \pm 0.1 °C. A 1.0 \times 10 $^{-5}$ M ionophore solution was prepared by dissolving 0.24 mg of the ionophore in 50 mL of ethanol. The solutions of different cations with equal concentrations were selected. Then, 3 mL of them and 3 mL of ionophore solution were dropped into the glass vial. The solutions were immediately shaken vigorously to achieve complete homogeneity. Finally, obtained solutions were injected to the cells, and absorbance values were measured after 15 min (because of the time required to reach the equilibrium). The reference cell contains 3 mL cation solution and 3 mL ethanol. The absorbance was measured over the wavelength range of 300-700 nm (Fig. 2).

The spectral change (decrease in absorption band at 330 nm and increase in the absorption band at 400 nm) shows that the ionophore is a selective complexing agent for copper ions. As can be seen, the complexation was accomplished by a relatively strong shift of the absorption band of the ligand H₂L (EBSI), with λ_{max} at 330 nm toward higher wavelength, 400 nm, for the complex, at which the ligand has low absorbance. Such a pronounced effect on the electronic spectra of the ligand could be related to strong complexation with Cu(II) ions.



Fig. 1. Absorbance measuring system for the film optode.



Fig. 2. Absorption spectra of ionophore in the solution at the presence of different cations. Ionophore concentration was 1.0×10^{-5} M and absorbance values were measured after 15 min to reach the equilibrium.

In order to determine the stoichiometry of the resulting complex, the absorption spectra containing 1.0×10^{-4} M of the ligand solution at a fixed ionic strength of 0.05 M (maintained by TEAP) and varying amounts of the Cu ion were obtained at 400 nm in ethanol solution. The absorbance versus [Cu(II)]/[EBSI] mole ratio plot revealed an inflection point at [Cu(II)]/[EBSI] mole ratio of about 1, emphasizing the formation of a 1:1 (metal-to-ligand) complex in the solution.

Spectral characteristics of ionophore in the membrane

After EBSI incorporated in the PVC membrane, the spectral properties of the ligand in the membrane remained the same as those measured in ethanol solvent (Fig. 3). This is the basis of the optical sensing device in this investigation. Therefore, the wavelength 400 nm has been used in all subsequent measurements of absorbance. The absorption spectra of the optical sensor in buffer solution of pH 4.0 and in the presence of increasing concentration of Cu^{2+} ion is shown in Fig. 4. As it is obvious, the absorption intensity of ionophore is decreased considerably upon increasing concentration of Cu(II) ion in the buffer solution and a new absorption band was appeared at higher wavelength due to complex formation between ionophore and copper ion.

Measuring principle

Absorption spectrum of EBSI (H₂L) in Cu²⁺ solution showed a maximum at 400 nm. By addition of a metal ion, it forms a complex with H₂L and increases its absorption at λ_{max} = 400 nm. Under the experimental condition used and supposing the formation of a 1:1 complex between EBSI and Cu(II) ion in the organic phase, the response mechanism of this optical sensor can be described by the following ion-exchange mechanism:

$$H_2 L_{org} + C u_{aq}^{2+} \rightleftharpoons C u L_{org} + 2 H_{aq}^+$$
(1)

Thus, based on the ion-exchange mechanism for the optical sensor, its measuring principle can be explained as follows. Upon the establishment of the complexation equilibrium between Cu^{2+} ion in aqueous sample solution and the ionophore (H₂L) in the organic membrane phase (Eq. (1)), CuL complex can be formed

$$K_{\text{exch}} = [\text{CuL}]_{\text{org}} [\text{H}^+]_{\text{aq}}^2 / [\text{H}_2 \text{L}]_{\text{org}} [\text{Cu}^{2+}]_{\text{aq}}$$
(2)

The relative absorption intensity, α , is defined as the ratio of complexed ionophore in the membrane phase, [CuL]_{org}, to its total uncomplexed form [H₂L]_{org}, so

$$\alpha = [CuL]_{org} / [H_2L]_{org} = (A - A_0) / (A_1 - A_0)$$
(3)

 A_0 and A_1 are the limiting absorbance intensities of the optical sensor at $\alpha = 0$ (i.e. uncomplexed H₂L) and $\alpha = 1$ (i.e. totally complexed



Fig. 3. Absorption spectra for Cu–EBSI complex in the membrane containing 2 mg EBSI, 61 mg DOS, 4 mg OA and 30 mg PVC. Inset: Absorption spectra for Cu–EBSI complex in the solution containing 1.0×10^{-5} M EBSI and 6.4μ g mL⁻¹ Cu²⁺ at pH = 4.0.



Fig. 4. Absorption spectra for the ionophore EBSI incorporated into membrane and varying amounts of Cu(II) ions (a–g: 0.0, 1.3, 3.4, 5.4, 6.3, 8.7, 13.4 μ g mL⁻¹) in aqueous solution with pH = 4.0; Membrane composition: 2 mg of EBSI, 61 mg of DOS, 4 mg of OA, and 30 mg PVC.

H₂L), respectively. The relationship between α values and the concentration of Cu²⁺ in aqueous sample solution $[Cu^{2+}]_{aq}$ can be obtained from Eqs. (2) and (3):

$$\alpha/(1-\alpha) = (K_{\text{exch}}/[\text{H}^+]^2) [\text{Cu}^{2+}]_{aa}$$
(4)

Eq. (4) could be used as a basis for the quantitative determination of copper ions using the proposed optical membrane.

Effect of pH on the membrane response

Sensitivity to Cu(II) depends the pH in the aqueous solution. This implies that the binding curves for Cu(II) should be measured with pH-buffered solutions. Fig. 5 shows the effect of pH values on the dynamic range of the optical membrane in the presence of Cu(II) ions. The response curve data were obtained by measuring the absorbance values for Cu(II) at different pH values. At the pH value in the range of 1.0–7.0, the working dynamic range reached the widest value, $0.01-32.0 \ \mu g \ m L^{-1}$. As it is seen, the best result was obtained at pH 4.0. This is in agreement with proposed proton/copper exchange mechanism. However, in pH lower than 4.0 the response of sensor in test solution, decreases. It is probably, due to chemical decomposition of ionophore under acidic condition that is a result of the acidic hydrolysis of EBSI or electrostatic repulsion between Cu²⁺ and protonated ligand in highly acidic media. Hence the dynamic range would of course also decrease. At pH values higher than 4.0 also the response of membrane decreased that may be due to the hydrolysis of metal ion.

Effect of membrane composition

Choice of solvent mediator (plasticizer)

It is obvious that the selectivity and sensitivity obtained for a given ionophore depends significantly on the membrane composition and the nature of solvent mediator and additives used [57]. Thus, the influence of membrane composition on the response behavior and leaching of sensor was investigated.

The membrane composition and the nature of the plasticizer largely influence the response characteristics and the working concentration range of the optical sensors [57]. Optode films with a high amount of plasticizer have optimum physical properties and ensure relatively high mobility of their constituents. In order to give a homogeneous organic phase, the membrane solvent must be physically compatible with the polymer. Five plasticizers such



Fig. 5. Effect of pH on the response of membrane in the presence of $3.3-6.8 \ \mu g \ mL^{-1} \ Cu(II)$ at 400 nm; Membrane composition: 2 mg of EBSI, 61 mg of DOS, 4 mg of OA, and 30 mg PVC.

as DBS, DOS, DBP, NB and AP were tested as potential plasticizers for preparing the membrane. The membranes were prepared from a mixture of PVC (30 mg), plasticizer (61 mg), additive (4 mg) and ionophore (2 mg) in THF (1.5 mL). The membranes containing DOS revealed the best physical properties with maximum sensitivities and wide concentration range (Fig. 6c). Membranes obtained from AP and DBS as plasticizers did not show any absorption change against Cu(II) ion.

For determination of the effect of plasticizer amount on the optode response, membranes that contain different amounts solvent mediator DOS (30, 61, 72 mg) were prepared. Absorption measurements showed that membrane containing 61 mg DOS revealed the best physical properties and wide concentration range (Fig. 7A).

Effect of the PVC amount

It is noteworthy that the best membrane characteristic is reported to usually obtain at a plasticizer/PVC ratio of 1.6–2.2. In general, the thickness and hardness of the membrane depend on the amount of PVC used. At lower PVC contents, the membrane becomes mechanically weak and swells up easily in aqueous solution,



Fig. 6. Effect of the type of plasticizer on the response of the optical sensing film in aqueous solution with pH = 4.0; Membrane composition: 2 mg of EBSI, 61 mg of plasticizer, 4 mg of OA, and 30 mg PVC.



Fig. 7. Effect of the amount of plasticizer (A), ionophore (B) and additive (C) on the response of the optical sensing film in aqueous solution with pH = 4.0.

and leaching of the membrane is significant. By increasing the amount of PVC, the membrane becomes dense, preventing the leaching of ionophore from membrane into aqueous solution. In this work, we examined the various amounts of PVC on the response characteristic of membrane. The results illustrated that increasing the amount of PVC (more than 35 mg) did not improve the sensitivity and were not suitable because of the decreasing of ion diffusion into the bulk of membrane.

Effect of amount of the ionophore

lonophores play a key role in the selectivity of ion-selective optodes. The rational design of synthetic carriers takes advantages of the different elements of molecular recognition. The creation of suitable binding sites and proper topology in the ionophore that are complementary to the size and charge of a particular ion can lead to very selective interaction.

The optical sensing film proposed is based on the incorporation of EBSI with two hydroxyl functional groups in its structure as an ion exchanger. This ionophore possesses a double binding property towards Cu(II) ion in sensing membrane. This ionophore is insoluble in water in its neutral form, while it is quite soluble in organic solvents. The effect of amount of the ionophore on the response of the membrane is illustrated in Fig. 7B. The blank membrane (membrane without ionophore in presence of Cu(II) ion with pH = 4.0) was taken as the reference. As it is seen from Fig. 7B, the presence of 2 mg of ionophore in the PVC membrane resulted in the best optical response of the Cu ion-sensing film. Higher amounts of ionophore (more than 2 mg) did not improve the sensitivity and were not suitable due to the leaching of ionophore.

Effect of the additive

In order to facilitate the establishment of a thermodynamic equilibrium between the bulk liquid membrane optodes and the sample solution of interest, a rather fast mass transfer of analyte from the sample into the membrane is required [58]. Thus, in this proposed Cu optical sensing film containing EBSI as an ionophore, the incorporation of a suitable additive was necessary to ensure the fast establishment of the corresponding ion-exchange equilibrium. OA and NaTPB were tested as additive for preparing the membrane. The membranes were prepared from a mixture of PVC (30 mg), plasticizer (61 mg), additive (4 mg) and ionophore (2 mg) in THF (1.5 mL). The results showed that the membrane containing OA revealed good response and transparency. The enhanced sensitivity may be attributed to the OA existing in the PVC membrane, changing the structure of the membrane and improving the passing efficiency of Cu into the organic membrane. It favorably makes much more Cu(II) extract into the membrane, and leads to a higher sensitivity. For study of the effect of additive amount on the optode response, membranes that contain different amounts OA (2, 4, 6 mg) were prepared. Absorption measurements showed that membrane containing 4 mg OA revealed best response (Fig. 7C).

Calibration range and detection limit

The optical response of the proposed Cu(II) sensitive membrane under optimum experimental conditions was obtained. The plot of normalized absorbance value against *p*Cu can be used as a calibration plot for determination of Cu ions in the concentration range $(0.01-32.0 \ \mu g \ m L^{-1})$ with equation of Y = -0.2686X + 1.8683($R^2 = 0.9888$), where *Y* is α and *X* is negative logarithm of Cu ion concentration in molar. Detection limit of the sensing film, which is defined as the concentration of the sample yielding a signal equal to the blank signal three times of its SD, was found to be $0.008 \ \mu g \ m L^{-1}$. The relative standard deviations for the copper determinations were 0.41% (n = 10) at 2.0 $\mu g \ m L^{-1}$ Cu(II).

Selectivity

The selectivity of optical sensor, which reflects the relative response of the sensor for primary ion over divers ions present in solution, is perhaps the most important characteristics of an optical sensor. In order to access the possible analytical application of this sensing method, the effects of some alkali, alkaline earth and heavy metal ions were investigated. The experiment was carried out with a fixed concentration of Cu(II) at 0.64 μ g mL⁻¹ and then measuring the changes in absorbance intensity before and after adding different foreign interferents in the Cu²⁺ solution buffered at pH = 4.0. The tolerance limit was set as the amount of foreign ion causing ±5% error in determination of Cu. The results are summarized in Table 1. As can be seen from Table 1, several metal ions do not interfere even at high concentrations. In addition to Cu, the sensing film also produces a response to some other metal ions (Hg^{2+}, Ni^{2+}) that form complex with EBSI at pH = 4.0. The results obtained may be important in terms of application of the sensing film to determination of other ions.

Reversibility, reproducibility, short-term stability and lifetime

Some reagents including HCl, HNO₃, H_3PO_4 , H_2SO_4 , EDTA and $(NH_2)_2CS$ were studied as regenerating and masking reagents. It was found that none of the above reagents or their mixtures could regenerate optode membrane completely and thus the membrane could be used as a probe for Cu ion determination.

The short-term stability of the optical sensing film was determined by its absorbance intensity measurements in contact with a buffer solution (pH = 4) in a cuvette. The signal was recorded every 30 min at wavelength of 330 nm (λ_{max} of ionophore) over a period of about 5 h. It was found that no significant loss of the ionophore occurs during this time. The membrane was stable over a period of 3 months when not in use (membrane was kept in air) and the signal value of the membrane did not change. The difference in the response of individual membranes was evaluated by

Table 1

Effect of some diverse ions on the optode response for the determination of 0.64 $\mu g\,m L^{-1}$ Cu²⁺. The reasonably less than ±5% relative error was tolerated.

Foreign ions	Tolerance limit $(W_{ion}/W_{Cu(II)})$
Mg ²⁺ , Ca ²⁺ , Cd ²⁺ , Co ²⁺ , Fe ²⁺ , Pb ²⁺ , Na ⁺ , Mn ²⁺ , Sr ²⁺ , Bi ³⁺ ,	1000
Zn ²⁺ , Li ⁺ , Cl ⁻ , I ⁻ , SO ₄ ²⁻ , NO ₃ ⁻	
Cr ³⁺ , Al ³⁺ , Sn ²⁺ , Ag ⁺	100
Hg ²⁺ , Ni ²⁺	10

preparing five membranes from the same mixture and the reproducibility was obtained by determining the signal of the membrane to $6.4 \,\mu g \, m L^{-1}$ solution of Cu(II). The mean absorbance was found to be 0.357 with RSD = 1.94%. The results show that the reproducibility is satisfactory.

Response time

From the literature and preliminary experiments, the amounts of PVC and plasticizer can influence the response time of the membrane. The response time of the present optical sensing film is controlled by the time of required for the analyte to diffuse from the bulk of the solution toward the membrane interface to associate with the ligand. The results revealed that the membranes containing 30 mg PVC and 61 mg plasticizer gave the shortest response time. The data show that the response time of membrane can reach to 98% of its final (steady state) value in a time of less than 3 min.

Analytical application

The proposed sensor was also applied to the direct determination of copper in various real samples. The samples were prepared as described in section 2.5 and determined with both the proposed sensor and AAS. From the results of three replicate measurements given in Tables 2 and 3, it is immediately obvious that there is satisfactory agreement between the results obtained by the Cu(II)selective optode and AAS. The results were also statistically compared by the paired *t*-test. This test revealed the absence of significant differences between the results obtained by both methods (at 95% confidence level). There was thus no evidence of the presence of systematic error in the results. Table 4 presents a comparison between the proposed method and other spectrophotometric methods for the determination of Cu [30–38]. The results show that proposed method is comparable to the existing spectrophotometric methods.

Conclusions

On the basis of the results presented in this work, the proposed Cu(II) ion-selective optode has many advantages including: easy preparation, low cost, fast response time, wide dynamic range,

Table 2Determination of copper in standard alloys.

Sample	Composition (%)	Concentra	centration (%)	
		Certified value	Found ^a	
JSS 653-7 stainless steel	C, 0.068; Si, 0.63; Cr, 22.53; Co, 0.35; Mn, 1.72; Ni, 13.91; N, 0.0276	0.030	0.027 ± 0.003	
NKK No. 920 aluminum alloy	Si, 0.78; Fe, 0.72; Mg, 0.46; Cr, 0.27; Zn, 0.80; Ti, 0.15; Sn, 0.20; Pb, 0.10; Sb, 0.10; Bi, 0.06; Ga, 0.05; Ca, 0.03; Co, 0.10; Mn, 0.20; Ni, 0.29; V, 0.15	0.71	0.74 ± 0.04	
NKK No. 916 aluminum alloy	Si, 0.41; Fe, 0.54; Mg, 0.10; Cr, 0.05; Zn, 0.30; Ti, 0.10; Sn, 0.05; Pb, 0.04; Sb, 0.01; B, 0.0006; Zr, 0.05; Bi, 0.03; Co, 0.03; Mn, 0.11; Ni, 0.06; V, 0.02	0.27	0.28 ± 0.02	
NKK No. 1021 Al, Si, Cu, Zn, Cr, alloy	Si, 5.56; Fe, 0.99; Mg, 0.29; Zn, 1.76; Ti, 0.04; Sn, 0.10; Pb, 0.18; Sb, 0.01; Zr, 0.01; Bi, 0.01; V, 0.007; Ca, 0.004; Mn, 0.11; Ni, 0.14	2.72	2.68 ± 0.08	

^a Average of five determination ± standard deviation.

Table	3
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Sample	Concentration $(\mu g g^{-1})^{a}$		<i>t</i> -test ^b	F-test
	Proposed method	AAS method		
Hair	11.32 ± 0.15	11.21 ± 0.12	0.99	1.56
Rice	3.02 ± 0.13	2.81 ± 0.08	2.38	2.64
Tea	4.3 ± 0.4	4.8 ± 0.2	1.94	4.0
Tomato sauce	0.50 ± 0.03	0.48 ± 0.02	0.96	2.25
Celery	0.76 ± 0.08	0.89 ± 0.09	1.87	1.26
Cabbage	0.20 ± 0.07	0.23 ± 0.05	0.60	1.96
Multivitamin capsule	0.43 ± 0.03	0.50 ± 0.05	2.08	2.78
Mineral water ^c	$0.97 \pm 0.11 ~(\mu g m L^{-1})$	$0.94 \pm 0.08 \; (\mu g \; m L^{-1})$	0.38	1.89

^a Average of three determination ± standard deviation.

^b The theoretical values of *t* and *F* at P = 0.05 are 2.57 and 19.00, respectively.

^c The water obtained from Vata spring (Ardebil, Iran).

 Table 4

 Comparisons of the proposed method with the existing spectrophotometric methods mentioned in the literature.

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_	Method	$LDR \ (\mu g \ m L^{-1})$	$LLD \;(\mu g \; m L^{-1})$	RSD (%)	Type of samples	Ref.
	Spectrophotometry	0.25-6.35	0.021	0.19 (<i>n</i> = 9)	Natural waters, certified vitamin and simulated samples	[30]
	Spectrophotometry	0.04-5.0	0.03	1.4(n = 10)	Standard alloys	[31]
	Spectrophotometry	0.1-10.0	0.01	2.0(n = 10)	Natural waters and pharmaceutical samples	[32]
	Spectrophotometry	0.5-4.0	-	0.057 (<i>n</i> = 10)	-	[33]
	Spectrophotometry	1.015-8.122	$3.19 imes10^{-2}$	0.67 (n = 5)	Synthetic mixture, sea and well water samples	[34]
	Spectrophotometry	0.1–5.0 (µg L ⁻¹)	$0.025 (\mu g L^{-1})$	0.45 (<i>n</i> = 5)	Certified standard material and water samples	[35]
	Spectrophotometry	0.48-12.8	0.05	5.0(n=6)	Water and alloy samples	[36]
	Spectrophotometry	0-1.024	$7.03 imes 10^{-4}$	4.68 (<i>n</i> = 5)	Vegetables and tea	[37]
	Spectrophotometry	2.5–75 (μg L ⁻¹)	0.85 (μg L ⁻¹)	0.5(n = 7)	Tap water, drinking water and wastewater	[38]
	Spectrophotometry	0.01-32.0	0.008	0.41 (n = 10)	Standard alloys, mineral water, human hair, foods and pharmaceuticals	This work

LDR: linear dynamic range; LDL: lower limit of detection.

low detection limit, and good reproducibility. It was applied to determine the concentration of copper (II) ions in real samples. This sensing membrane has potential application for determination of Cu in a variety of real samples, and alloys. The sensing film can also respond quantitatively to other metal ions (Hg, Ni). Thus, the system could be optimized for detection of other metals, or adopted to the detection of several metals in real sample.

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