Full Paper

Determination of Copper Content of Human Blood Plasma by an Ion Selective Electrode based on a New Copper-Selectophore

Farnoush Faridbod, and **Mina Bahman**

Center of Excellence in Electrochemistry, School of Chemistry, University of Tehran, Tehran, Iran

*Corresponding Author, Tel.: +98-21-61113813

E-Mail: faridbodf@ut.ac.ir

Abstract- A new selectophore was introduced for Cu^{2+} ions. Spectroscopic studies showed a selectivity of a new organic compound (L) toward copper ions and several transitional metal ions. Hence, L was utilized in designing several ion selective electrodes for these cations. In practice, Cu^{2+} ion selective electrode behaved Nernstian (slope of 27.95 ± 0.3 mV decade^{-1}) over a wide linear range of 1.0×10^{-6}-1.0×10^{-2} mol L^{-1}. Optimum performance of the indicator electrode was observed by a membrane containing 7% selectophore, 2% sodium tetraphenyl borate (NaTPB) as an ionic additive, 61% nitrobenzene (NB) as solvent mediator and 30% polyvinyl chloride (PVC). The sensor showed a short response time of about 20 s and an acceptable life time, the potentiometric response is independent on the pH of the solution in the range of 3.5-7. The optimized copper sensor was used for analysis of copper content of some blood plasma samples. The obtained results were in good agreements with ICP-OES method.

Keywords- Copper; Selectophore; Sensor; Potentiometry; Ion Selective Electrode

1. INTRODUCTION

Now a days, simple and rapid analysis methods are demanded in many fields range from clinical to industrial applications. These requests has caused rapid analyses grow fast more than

ever. Many classical techniques got a chance again to be used for analyses. Among them, ion selective electrodes are one of the electrochemical analysis method which have been widely commercialized and utilized since their findings. From old simple pH-meters, ion selective electrodes utilized in environmental portable determination kits, to ion selective devices recently used in clinical laboratories for determination of Li^+, Na^+, K^+, and Ca^{2+} ions in blood plasma, all are some examples.

Compare to atomic spectroscopic methods, they are able to determined target analyte without consuming the sample. Thus, online monitoring of analyte concentration becomes possible. They can easily be portable and possess a simple operatory systems which makes determination easy for non-expert users. Furthermore, they are inexpensive devices.

The important issue in designing an ion selective electrode (ISE) is the sensing material inside. It is called "selectophore". During the years, many selectophore have been introduced for variety of ions [1-20]. In case of cation selective electrodes, selectophores are mostly organic compounds having several heteroatoms, cavity or semi-cavity, or ability to wrap around the ions (so-called ligand). In most case, the ligand forms a 1:1 complex with target cation and produce a chemical signal which later convert to an electrochemical ones by a transducer (here, internal reference electrode). In fact, an ISE is a chemical sensor which translates the activity of a target ion in a solution into an electrical potential. Based on Nernst equation, the produced voltage is theoretically corresponds to logarithm of the ionic activity.

Copper is one of the essential trace elements in human body. Its level affects functioning of some organs and metabolic processes [21]. It is a co-factor of many redox enzymes, Ceruloplasmin being the most abundant Cu-dependent ferroxidase enzyme with a copper-dependent oxidation activity [22]. Its deficiency and excess level can be harmful for human health. Hence, in the fields of microbiology, toxicology, nutrition, and health risk assessments, it is important to define the precise copper levels to prevent deficient or excess copper intakes. Many analytical techniques determined copper through various analytical methods [23-29]. Among them, inductively coupled plasma optical emission spectroscopy (ICP-OES), is a standard method for trace level analyses of copper. This technique determine total amount of copper while ISEs in comparison can also measure ionic species of copper (Cu^{2+}) in addition of total content.

In this work, a new organic ligand was introduced as a selectophore for copper ions. To find out the selectivity trends of the synthesized selectophore, UV-Vis spectroscopic method was used. The proposed selectophore was then utilized in designing a copper selective electrode in a polymeric matrix. The copper content of some blood plasma samples were analysed using this electrochemical sensor. The results was finally compared statistically to ICP-OES method.

2. EXPERIMENTAL SECTION

2.1. Chemicals

Chemicals used in this work are all from Merck Chemical Company. The used selectophore L, named as (1Z, 4Z,7Z,11Z)-diethyl7,14-bis(4-nitrophenyl)-1,4,8,11-tetraazazcyclotetradeca-4,7, 11, 14-tetraene-5, 12- dicarboxylate, L, (Scheme 1) was synthesized as follow.
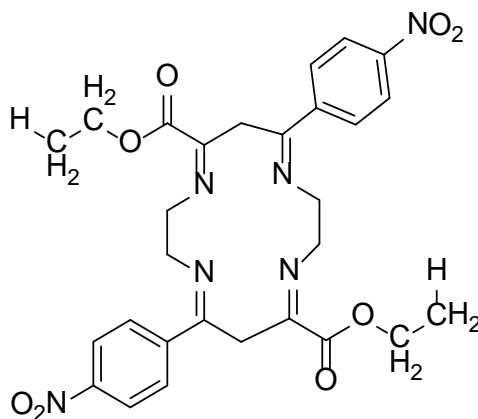
Scheme 1. Chemical structure of the used selectophore

A mixture of ethylene diamine (1 mmol) and ethyl-2,4-dioxo-4-nitrophenol butanoate (1 mmol) in ethanol was refluxed for 5 hours. The reaction progress was controlled through thin-layer chromatography using a 1:2 n-hexane and ethyl acetate ratio. After the reaction completed, it was cooled to room temperature and the resulting precipitate was filtered, washed with 20 ml of ethanol and crystallized to produce a pure product L.

2.2. Instruments

The proposed ISE was used as an indicator electrode in a potentiometric cell consisting of an Ag/AgCl double junction reference electrodes (Azar-Electrode Co., Iran) as the external reference electrode. The following cell assembly was use for potentiometric measurements:

Ag-AgCl, KC1 (satd.) internal reference electrode ǁ $CuCl_2$ solution 10^{-3} mol L^{-1}| Ion selective PVC membrane | Copper sample solution ǁ Ag-AgCl, KC1 (satd.)

An ion analyzer with a 250 mV-meter with ±0.1 mV precision was utilized to connect two electrodes in the measurements. A Metrohm pH-meter with a double junction glass electrode was used for pH adjustment.

UV–Vis spectra were recorded on a PerkinElmer Lambd-2 UV–vis spectrophotometer. For spectroscopic studies, 3 mL of L solution in acetonitrile (1.0×10^{-5} mol L^{-1}) are poured in a quartz cell (1 cm-pass length). For complexation study, a known amount of a metal cation solution (1.0×10^{-3} mol L^{-1}) is added to L solution, in a step-wise manner, using a calibrated micropipette. The absorbance of the final solution is recorded after each addition.

2.3. Preparation of the sensors

Generally, PVC membrane was prepared by mixing variety amounts of selectophore along with proper amounts of PVC, a plasticizer and an ionic additive were dissolved in tetrahydrofuran (THF), and the solution was mixed well into a glass dish of 2 cm diameter. Next, THF was gradually evaporated while waiting for an oily concentrated residue. After this step, a plastic tube (about 3 mm o.d.) was dipped into the mixture for about 10 s while rotating the tube, a thick transparent membrane was formed at the end of tube (Fig. 1). The membrane was left in room temperature to dry for about 3 to 4 hours. Finally, the tube was filled with an internal filling solution of 1.0×10^{-3} mol L^{-1} of CuCl$_2$. The electrode should be soaked in 1.0×10^{-3} mol L^{-1} of CuCl$_2$ solution before first use for 24 h [12-19].

Fig. 1. Image of plastic tube with the PVC membrane

2.4. Plasma sample preparations

Blood plasma sample was obtained from Iranian Blood Transfusion Organization. To precipitate the plasma proteins, a 10 mL sample was treated with 20 μL nitric acid 0.1 M and be centrifuged at 5000 rpm for 10 min. Then, the upper solution, which was a clear solution, removed and transferred to a new vial diluted 10-fold.

3. RESULTS AND DISCUSSION

3.1. Spectroscopic study

To have an ideal ISE, choosing a suitable sensing material to interact with a target ion is of great importance. Complexation studies before designing an ISE can be a facilitative way to finding a selectophore for an ion. Interaction between a ligand (called donor) and an ion (called acceptor) can be generally studied by electrochemical (such as conductometry) or spectroscopic techniques. Here, interaction of the synthesized L with metal cations was studied by UV-vis spectroscopy. Fig. 1 shows absorption spectra of L and cation ions in acetonitrile-water solution 70:30.
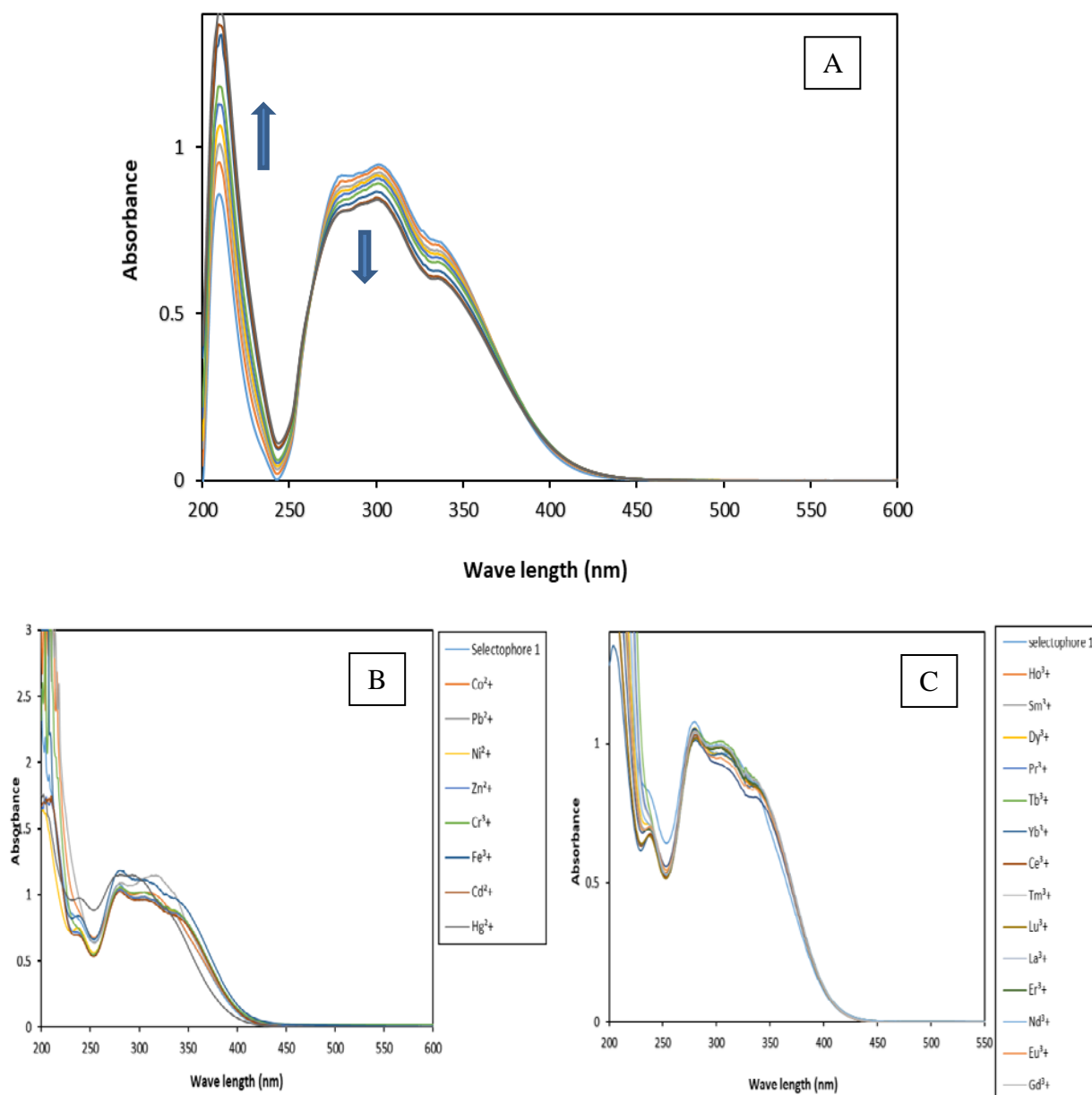

Fig. 1. UV-vis spectra of the interaction of L in acetonitrile-water solution (70:30) with Cu^{2+} ions (A) and variety of cations (B) and lanthanide ions (C)

Acetonitrile was normally selected as solvent for complexation study because it has a low donocity number. Solvents with low donocity number are not able to compete with ligand in interaction with cation. The important practical point in choosing a selectophore is selecting a ligand which can form selective complex with K_f between 10^3 and 10^7. If K_f is too high, stable complexation causes blocking the active sensing sites on the membrane and prevents signalling. A good selectophore should have a selective, and recyclable interaction with target ions. Primarily complexation study showed that among all cations tested (alkali, alkaline earth, transition and lanthanide cations) L had a selective behaviour to Cu^{2+}, Fe^{3+}, and Hg^{2+} and Pb^{2+} ions. Since suitable selectophore in ISE should also master the hydration energy of cations solvated in aqueous solution and extract the cations to organic layer of the polymeric membrane through complexation mechanism, in next study ion selective electrodes were prepared for these ions. In practice only copper selective electrode showed a Nernstian response in comparison with other ones.

3.2. Copper selective sensor

After finding a suitable sensing elements, the amounts of selectophore along with other membrane ingredients should be optimized. The amount of PVC was selected 30 mg in all cases. The selectophore amount was optimized to 7 mg. Table 1 lists some of the membrane composition tested. Effects of each ingredients have been previously discussed several times [30-43].

Table 1. Some membrane composition tested in designing copper selective electrode

No	Plasticizer (%wt)	Selectophore L (%wt)	NaTPB (%wt)	Slope (mV/decade)
1	63(BA)	7	-	5.0±0.3
2	63(DBP)	7	-	4.5±0.2
3	63(o-NPOE)	7	-	7.6±0.3
4	63(NB)	7	-	8.3±0.4
5	61(BA)	7	2	14.2±0.5
6	61(DBP)	7	2	18.7±0.3
7	61(o-NPOE)	7	2	20.9±0.4
8	61(NB)	7	2	27.9±0.3
9	60(NB)	7	3	20.9±0.5
10	63(NB)	5	2	21.6±0.4
11	68(NB)	-	2	2.7±0.3

The optimized amount of membrane ingredients was 7% selectophore L, 2% sodium tetraphenyl borate (NaTPB) as an ionic additive, 61% nitrobenzene (NB) as solvent mediator and 30% polyvinyl chloride (PVC) which showed the best Nernstian response, slope of 27.95 ± 0.3 mV decade^{-1} over a wide linear range of 1.0×10^{-6}-1.0×10^{-2} mol L^{-1} as shown in Fig. 2.
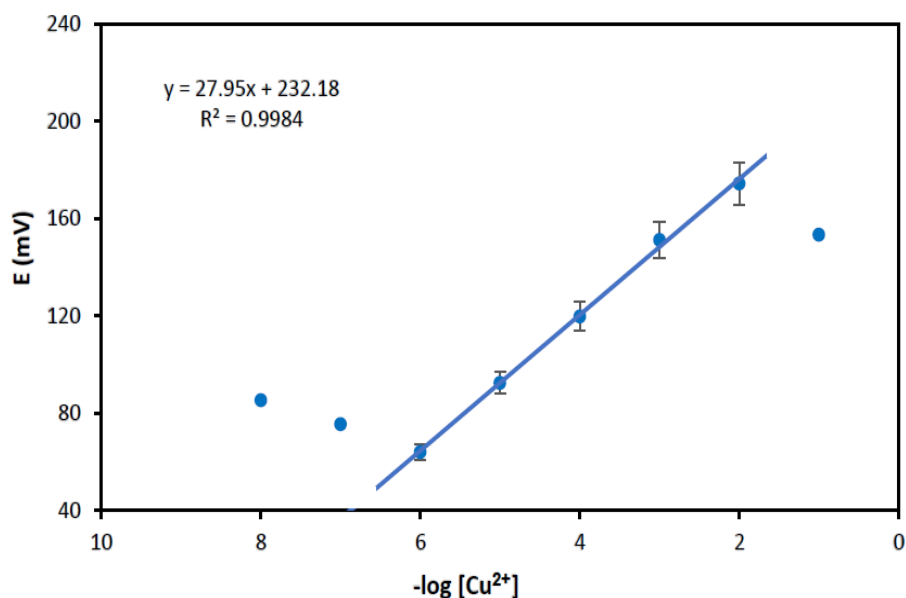
Fig. 2. Nernstian response of copper selective sensor with membrane composition no. 8

Other important parameters of an ion selective electrode are applicable pH range, response time, lifetime, and selectivity.
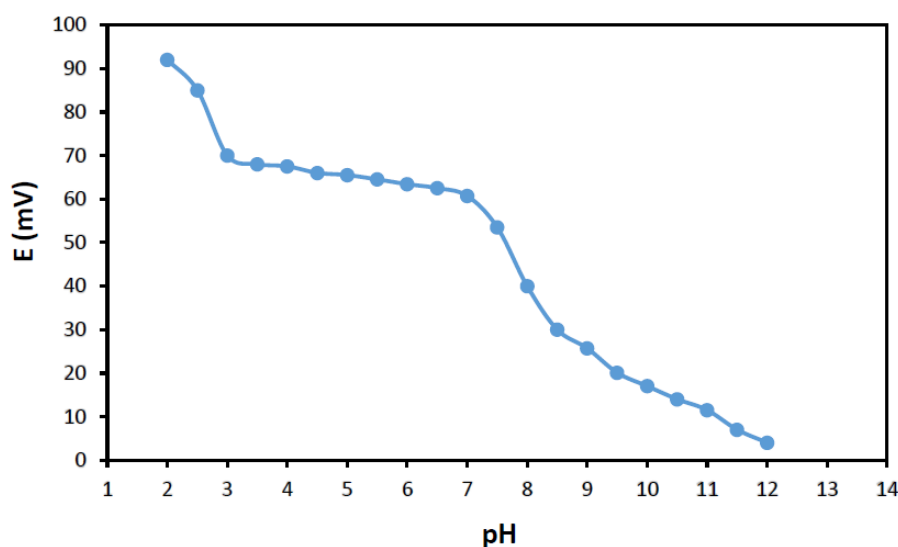

Fig. 3. Applicable pH range of the proposed copper selective sensor in test solution of

These parameters was also optimized. The applicable pH range of the sensor was 3 to 7 as shown in Fig. 3. This range was obtained through pH changes of a solution of 10^{-6} mol L^{-1} Cu^{2+} ion from 2 to 12 and recording the resulting potential. Below pH 3, heteroatoms of the L are protonated and above pH 7, copper cations gradually OH^- compete with L in interaction with copper cations.
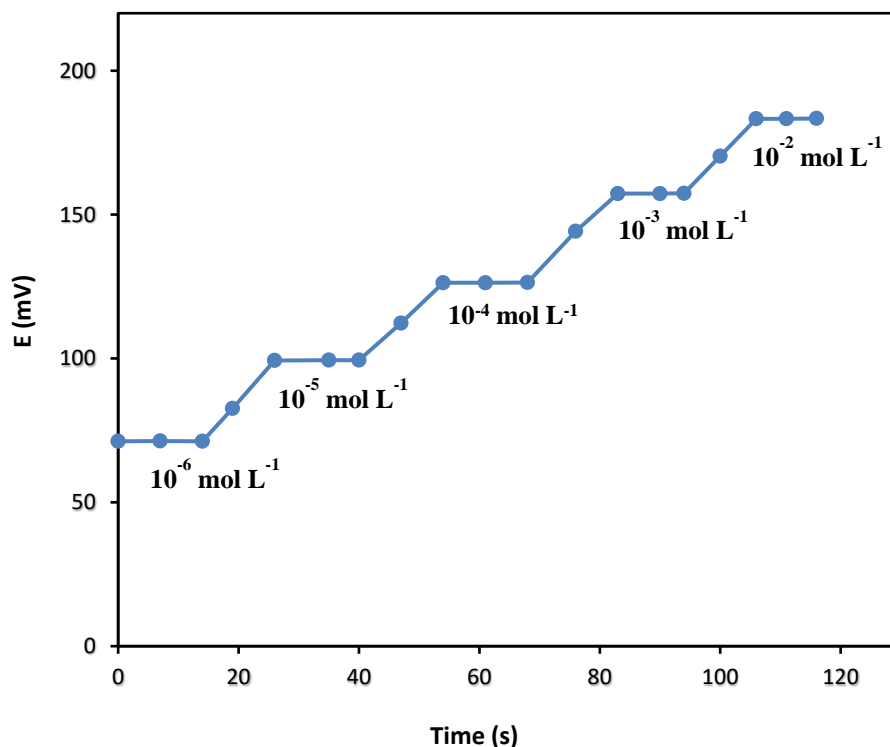

Fig. 4. Response time of the copper sensors

Response time of the sensor, as seen in Fig. 4, was 20 s in all concentration range. This time was obtained by dipping the sensor in 10^{-6} mol L^{-1} solution of copper cation, recording the correspond voltage, then changing the concentration of the solution by micro-pipetting of a concentrated solution of copper ions on the primary solution, and recording the voltages.

Potentiometric selectivity coefficients of the copper sensor were studied by matched potential method (MPM) [44-49] as listed in Table 2. As can be seen, there are negligible interferences in the performance of the sensor.

Sensor lifetime was estimated through considering the calibration curve slope, detection limit and response time of three sensors during 10 weeks. A periodical test of a sensor in a standard solution series of copper cation and calculation of their responses was done for this purpose. After 6 weeks utilization of the sensors, a slight gradual decrease in the slope (from 27.9 to 23.7 mV/decade) and an increase in the detection limit (from 1.0×10^{-6} to 8.3×10^{-4} mol L^{-1}) and increase in response time (from 20 to 1 min) was observed.

Table 2. Selectivity coefficients of various interfering cations for the proposed sensor

Ion	Log K_{MPM}
Co^{2+}	−3/8
Ni^{2+}	−3/7
Fe^{3+}	−4/1
Cd^{2+}	−3/2
Hg^{2+}	−2/3
Zn^{2+}	−3/8
Pb^{2+}	−2/6
Alkali and alkaline earth cations	**Less than 5.3**
Lanthanide cations	**Less than 4.7**

Repeatability of the sensor were measured using 3 replicate standard samples of copper ions. The RSD values were 3.6, 4.1, and 3.9%. Reproducibility of the sensor was also tested. 5 sensors which were prepared at the same time and with same conditions, were analyzed three same standard solutions of copper ions. RSD values in this case were 4.3, 4.5, and 4.6%.

3.3. Copper content measurement of human blood plasma

The copper ion selective sensor can also be used to determine the amount of copper in some plasma samples. The copper content of blood plasma is normally 80-155 micrograms per dL for women and 70-140 micrograms per dL for men [50]. In this study, some human blood plasma samples were analysed by the proposed copper selective sensors and ICP-OES method. The sample were prepared as described above.

Table 3. Results of copper analysed by copper ISE and ICP-OES methods

Sample	Found by ISE* (μg/dL)	Found by ICP-OES* (μg/dL)	t-test $t_{experimenal}$ ($t_{theoritical=2.44}$, 95%)
1-female	87.6±0.4	91.0±2.8	2.08
2-female	120.3±0.6	117.8±1.7	2.40
3-male	132.4±1.1	129.5±2.3	1.97
4-male	94.8±0.8	97.2±1.6	2.32

*the results of 3 replicate measurements

The standard addition method was used to measure the amount of copper in the plasma samples. Table 3 shows the values obtained for this measurement. As a result of t-test, there is no significant difference between two data and there is a good agreement.

4. CONCLUSION

In this study, an organic compound was introduced as a new selectophore for Cu^{2+} ions. Selectivity of the selectophore was investigated in a potentiometric ion selective electrode. The designed membrane sensors showed advanced performances, a fast response time, a lower detection limit of 1.0×10^{-6} mol L^{-1} and lifetime of 6 weeks. Reproducibility and repeatability of the portable designed sensor gives an opportunity to have a precise analysis method. The sensors respond based on complexation mechanism. Because of a selective trend of selectophore toward copper ions, selectivity coefficients of the sensor was acceptable. The sensor enabled the copper determination in some blood plasma samples accurately. Due to the importance of determination of copper level in blood for human health, ISEs can be a suitable replacement for time-consumed and expensive atomic spectroscopic techniques.

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