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Analysis of Copper(II) Ions in Human Blood Using a New Solid-Contact PVC Membrane Potentiometric Sensor Based on a N,N′-Bis(salicylidene)-1,3-diaminopropane Schiff Base

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The trace element copper is essential for vitality and promotes a healthy metabolism. Anemia, irregular heartbeat, bone illnesses, uremia, and hypertension are just a few of the major systemic toxic effects of copper ions. For the early detection and effective treatment of many important diseases, the assessment of copper(II) concentrations in biological fluids must be done quickly and accurately. A new copper(II) ion-selective solid contact poly(vinyl chloride) membrane electrode was developed in this study, with a neutral carrier of N,N′-bis(salicylidene)-1,3-diaminopropane Schiff base. The electrode displayed a Nernstian response to copper(II) ions over a broad concentration range (1.0×10^{-2}-1.0×10^{-5} M) with a slope of 30.4 ± 0.5 mV decade^{-1}. The membrane cocktail could be used for more than 6 months without any potential divergence, and it had a quick response time of 5-10 s. The electrode was employed for the estimation of Cu^{2+} ions in certified blood samples and the results obtained by potentiometry were in good agreement with those by the Inductively Coupled Plasma-Sector Field Mass Spectrometry (ICP-SFMS) method.

Keywords: Schiff base, PVC membrane, Copper, Biologic fluids, Blood

INTRODUCTION

Nowadays, quick and easy analysis techniques are needed in a variety of disciplines, from clinical to industrial applications. These demands have sped up the development of rapid analysis more than before. Many conventional methods now have the chance to be applied for analysis. Since their invention, electrochemical analysis techniques like ion selective electrodes (ISEs) have been widely employed and commercialized. These are just a few examples, ranging from basic pH meters to ion-selective methods recently employed in clinical laboratories to identify Li^+, Na^+, K^+, and Ca^{2+} ions in blood plasma.

In contrast to spectroscopic techniques, ISEs may identify the target analyte without consuming the sample. As a result, it is now possible to monitor analyte concentration online. They are easily portable and have basic operating systems, making it simple for novice users to utilize them. They are also inexpensive devices. The most important challenge in the design of an ion-selective electrode (ISE) is its sensing material, known as a "selectophore." Many selectophores for various ions have been developed over the years [1-13]. They are mostly organic compounds with several heteroatoms, a cavity or semi-cavity, or the ability to wrap around ions in cation-selective electrodes.

Copper is a trace element that is required for survival. It supports a healthy metabolism, promotes strong and healthy bones, and ensures that your nervous system functions properly. It is also found in all body tissues and aids in the production of red blood cells as well as the maintenance of

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nerve cells and the immune system. Adults typically have copper levels of 1.4 to 2.1 milligrams per kilogram of body weight. Although our bodies require such a small amount of copper, its contribution to human health cannot be overlooked and is as important as calcium, iron, and zinc. Anemia, irregular heartbeat, digestive system disorders, bone diseases, and other major systemic toxic consequences are all brought on by copper concentrations that are below or beyond the limits necessary for physiological processes [14]. Therefore, it is crucial to quickly and accurately determine the copper(II) concentrations in biological fluids in order to diagnose and treat a variety of dangerous disorders. Copper was determined using a number of different analytical methods. These include inductively coupled plasma optical emission spectroscopy (ICP-OES) [15], atomic absorption spectrometry (AAS) [16,17], graphite furnace atomic absorption spectrometry (GFAAS) [18], flame atomic absorption spectrometry (FAAS) [19,20], gravimetry [21,22], chromatography [23,24], and photometry [25-27].

Macrocyclic polyethers [28,29], copper chelates [30,31], non-cyclic neutral ionophores containing dithiocarbamate groups [32,33], calix[4]arenes [34,35], and Schiff bases [36-40] have been used to develop a number of Cu^{2+}selective sensors. However, the majority of the reported ion-selective electrodes have limited biological sample applications. As a result, improved copper sensors are still required.

A newly synthesized Schiff base was employed as a copper ion selectophore in light of this. A copper selective electrode with a PVC membrane was then made using the suggested selectophore. Using this electrochemical sensor, certified blood samples' copper content was examined. Finally, a statistical comparison was made between the outcomes and those of the Inductively Coupled Plasma-Sector Field Mass Spectrometry (ICP-SFMS) method.

EXPERIMENTAL

Chemicals

N,N′-bis(salicylidene)-1,3-diaminopropane Schiff Base (SB) used as an ionophore was synthesized in Dr. Atakol's research lab. High-molecular-weight poly(vinyl chloride) (PVC), tris(2-ethylhexyl) phosphate (TEHP), *o*-nitrophenyl octyl ether (*o*-NPOE), dibutyl phthalate (DBP), o-ethyhexyl) adipate (BEHA), dibutyl sebacate (DBS), bis(1-butylpentyl) adipate (BBPA), and tetrahydrofuran (THF) in selectophores were obtained from Fluka. The lipophilic anionic additives potassium tetrakis(4-chlorophenyl)borate (KTpClPB), cesium tetrakis(3-methylphenyl)borate, tetrabutylammonium tetraphenylborate, tetraheptylammonium tetraphenylborate, tetradodecylammonium tetrakis(4-chlorophenyl)borate, tetraphenylphosphonium tetraphenylborate, tridodecylmethylammonium chloride were obtained from Aldrich. NiO nanoparticles were purchased from Nanograph. Choline chloride, ethylene glycol, sulphuric acid, nitric acid, acetic acid, and phosphoric acid were purchased from Sigma-Aldrich, Germany. Boric acid was from JT Baker, Netherlands. $NaH_2PO_4.H_2O$ was obtained from Riedel-de-Haën, Germany, and $Na_2HPO_4.2H_2O$ from Fluka, Switzerland. Ethalin was prepared in our laboratory from the interaction between the choline chloride (ChCl) and the ethylene glycol (EG) corresponding to a molar ratio of 1:2. Copper (II) nitrate (Merck) and nitrate salts of the metal cations (Fluka) were used without further purification. A certified reference blood sample (Seronorm™ L3 (LOT 1112691) obtained from the Ministry of Health, Ankara Provincial Health Directorate, was used in the real sample application of the electrode.

Instruments

The potential measurements were taken with a pH-ion meter with the model number Orion 720A. A modified brass wire electrode (BWE) was used as an indicator electrode and a saturated Ag/AgCl double junction reference electrode (Orion 900200) served as the external reference electrode in a two-electrode system for the potentiometric measurements. Electrochemical impedance spectroscopy (EIS) measurements were performed with CHi 660D Model potentiostat/galvanostat combined with BASi C3 cell stand. Pt wire counter electrode (BASi MW-1032), Ag/AgCl/KCl (3.0 M) reference electrode (BASi MF-2052), and PVC membrane-modified brass wire working electrode (BWE) were used in a conventional three-electrode setup for this purpose. Solutions were deoxygenated with high-quality nitrogen for three minutes prior to each experiment. All measurements were carried out at ambient temperature. The impedance spectra were then matched to an equivalent

circuit using the Autolab impedance analysis tool.

The pH of the prepared buffer solutions was adjusted using the Ingold (10.402.3311) glass-pH combination electrode. In order to weigh the compounds, a precise balance was used (Mettler Toledo). A vortex mixer (ISOLAB Laborgeräte GmbH, Eschau, Germany) and an ultrasonic bath (Bandeln Sonorex) were used to prepare the homogenized solution. The surface morphology of SB/PVC/BWE was examined by scanning electron microscopy (SEM) using the FEI Inspect F50 scanning electron microscope at an accelerating voltage of 1.0 kV and 1600 magnification.

Synthesis of Schiff Base

The N,N′-bis(salicylidene)-1,3-diaminopropane Schiff base (Fig. 1) that was used as a selectophore was synthesized as follows:

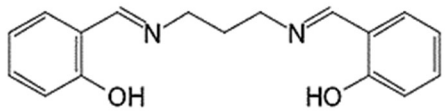

Fig. 1. N,N′-bis(salicylidene)-1,3-diaminopropane

A 100 ml double-mouth flask with a refluxer was filled with 0.05 mol salicylaldehyde in 25 ml of ethanol solution. Within a minute, 0.025 mol of 1,3-diamino propane in 25 ml of ethanol was added in two parts to this solution. The mixture was heated to the boiling point and then allowed to cool. The product was then allowed to settle overnight before being filtered. The resulting yellow Schiff base crystals were recrystallized in ethanol, washed several times with hot deionized water (DI), ethyl alcohol, and diethyl ether, and dried in a vacuum oven.

Construction of the SB/PVC Membrane Electrode

Brass wire with a diameter of 3 mm was purchased from the market and cut into lengths of 70.0 mm. It was cleaned using slurries made from 0.01 μm aluminium oxide on a smooth polishing pad before the electrode construction process. It was then rinsed with deionized water, followed by five minutes of sonication in ethanol and DI water.

In order to prepare the PVC membrane, 1 ml of tetrahydrofuran was thoroughly mixed with 2.0 mg of Schiff base and 68.0 mg of o-NPOE as a plasticizer. To this combination, 29.75 mg of powdered PVC was gradually added. While maintaining these ratios, 0.25 mg of a lipophilic ingredient was added to the mixture. Then, 10 μl of membrane cocktail was dropped onto the surface of a brass wire electrode and left at room temperature for 24 h to allow the tetrahydrofuran to evaporate. The membrane cocktail was stored at +4 °C under dry conditions when not in use.

Measurements of emf

The potentiometric measurements were performed within the range of 1.0×10^{-8}-1.0×10^{-2} M copper solutions at pH 5.0. While stirring the experimental solution, the potentials were recorded after the equilibrium potentials were reached. The measured potential value was plotted against the negative logarithm of the ion concentration to create the calibration graph.

Application of the KTpClPB/SB/PVC/BWE Membrane Electrode for Analytical Determination of Cu(II)

In order to create a blood sample solution, 1.5 ml of concentrated nitric acid and 0.5 ml of 30% hydrogen peroxide were heated and mixed with the lyophilized powder of Seronorm™ L3 (LOT 1112691). The residual portion of the sample was then dissolved in the smallest volume of water. An acetic acid/sodium acetate buffer solution was used to quantitatively transfer the clear solution into a glass beaker and adjust the pH to 5.0. The constructed electrode was used in a direct potentiometric measurement of copper(II) in the prepared solution. The linear range was determined by creating calibration curves for Cu(II) added with an increasing concentration between 5 mg l^{-1} and 15 mg l^{-1} by the standard addition method to the commercial human blood sample. Using calibration curves, the concentration of Cu(II) in the sample was determined.

RESULTS AND DISCUSSION

Characterization of the Electrode Membrane

Scanning electron microscopy (SEM) analysis. SEM analysis of the surface morphologies of PVC/BWE,

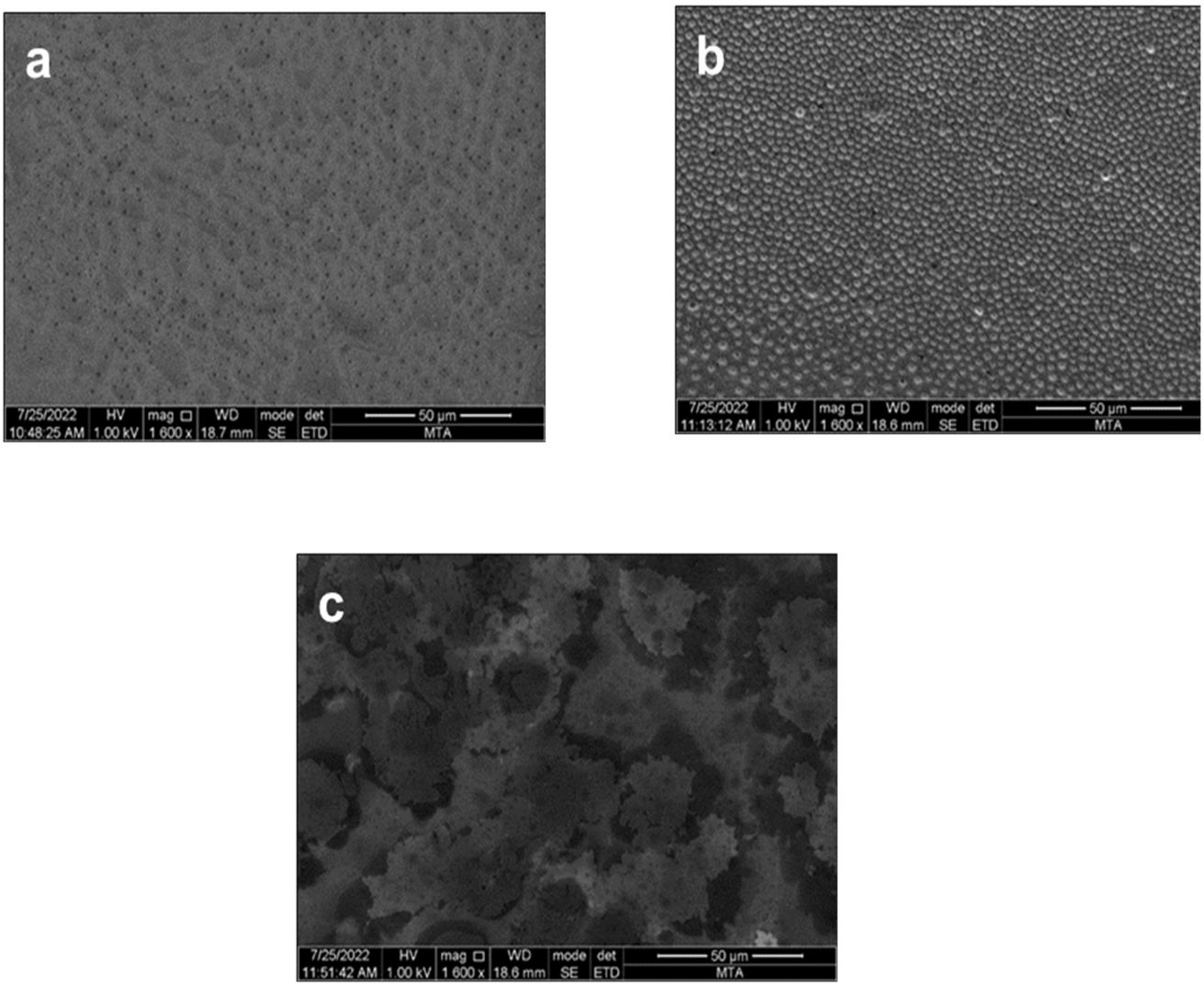

Fig. 2. The SEM image of the PVC/BWE (a), SB/PVC/BWE (b) and KTpClPB/SB/PVC/BWE (c).

SB/PVC/BWE, and KTpClPB/SB/PVC/BWE is depicted in Fig. 2. The featureless morphology of the PVC-modified BWE is seen in the SEM image (image a). Image B shows that the Schiff bases were distributed uniformly throughout the PVC matrix, with a small spherical structure. As seen in image c, the KTpClPB was also diffused within the SB/PVC membrane without any visible agglomeration. The porous granular surface of the SB/PVC membrane was perfect for copper ion exchange. The KTpClPB/SB/PVC/BWE electrode's porous structure can enhance the electrode's effective surface area, allowing copper ions to diffuse into the membrane.

Electrochemical Impedance Spectroscopy (EIS) Analysis

Electrochemical impedance spectroscopy was used to assess the brass wire PVC membrane electrochemical performance and rate of electron transfer. The EIS spectra of the suggested copper(II) sensing membrane and the same membrane without ionophore (N,N′-bis(salicylidene)-1,3-diaminopropane) immersed into 1.0×10^{-3} M Cu(II) solution (pH 5.0) are shown in Fig. 3A. Ionophore-containing membrane (KTpClPB/SB/PVC/BWE) had substantially lower electrical resistance than the ionophore-free membrane (PVC/BWE) exhibited. The charge transfer resistance (Rct)

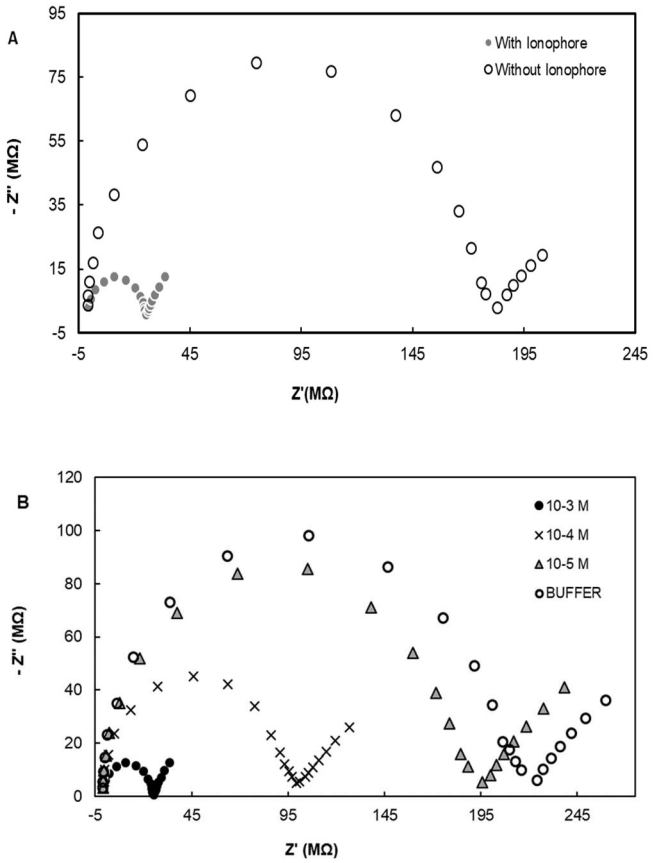

Fig. 3. A) Nyquist plots of (o) PVC/BWE (without ionophore), (•) KTpClPB/SB/PVC/BWE (with ionophore) B) KTpClPB/SB/PVC/BWE in different Cu(II) concentrations (•) 1.0×10^{-3} M; (×) 1.0×10^{-4} M; (▲) 1.0×10^{-5} M; (o) Acetic acid/acetate buffer (all solutions were prepared in acetic acid/acetate buffer solution (pH 5) containing 0.1 M $NaNO_3$).

value of 185 MΩ was obtained from the Nyquist plot of PVC/BWE (curve o). The SB/PVC/BWE matrix behaves as an effective electron conducting composite, as shown by the Rct (32 MΩ) of KTpClPB/SB/PVC/BWE (curve •).

The Nyquist plots of the proposed KTpClPB/SB/PVC/BWE were assessed in three different Cu(II) concentrations (1.0×10^{-3} M, 1.0×10^{-4} M and 1.0×10^{-5} M) containing 0.1 M acetic acid/acetate buffer at pH 5.0. The impedance graphs are presented in Figure 3B. This kind of illustration shows how the membrane of the electrode behaves. The signal exhibits a constrictive semicircle between 0.1 and 200 kHz and is primarily controlled by resistance and geometric capacitance parallel to the membrane at high frequencies. On the other hand, the diffusional component (45° line), which is associated with the diffusion of the analyte from the solution into the membrane, is only visible at low frequencies.

The largest semi-curve was obtained for buffer solution and relatively narrower curves were obtained for 1.0×10^{-5} M, 1.0×10^{-4} M, and 1.0×10^{-3} M Cu(II) concentrations, respectively.

Potentiometric Detection Optimization of Analytical Conditions

Assessment of membrane composition. It is well known that the composition of the electrode membrane, the presence of a plasticizer, and the additives utilized have a significant impact on the performance characteristics of the ionophore-incorporated polymer membrane [41-44]. As a result, by altering the ratios of the membrane components, the effect of membrane composition on the potentiometric response of the electrodes was investigated. 30 mg of PVC was used in each case. Some of the studied membrane compositions are shown in Table 1.

According to the literature, the performance of the electrode is significantly impacted by the complex stoichiometry between the analyte ion and macrocyclic molecule [45]. As a result, the ionophore content of the membrane increased from 1% (E1) to 3% (E3). The best Nernstian response was displayed by the electrode constructed with a polymeric membrane containing 2% ionophore (E2) over the broadest linear working range of 1.0×10^{-2}-1.0×10^{-5} M with a slope of 30.4 ± 0.5 mV/pCu. Many PVC membrane electrodes with a plasticizer/PVC ratio (m/m) of nearly 2 have been reported in the literature to have very suitable performance characteristics [46]. Our findings support this behavior as well.

The nature of the plasticizer is another important factor that influences the response characteristics of an ion-selective electrode [47]. As is well known, there are four basic requirements for an appropriate plasticizer. The plasticizer must have sufficient lipophilicity, no crystallization in the membrane, and no oxidation. Furthermore, it must have selectivity properties [48,49]. As a result, various types of plasticizers were used in the preparation of membrane

Table 1. Membrane Compositions and their Potentiometric Response Characteristics of Cu-ISE Based on a N,N′-Bis(salicylidene)-1,3-diaminopropane, Measured in Acetic Acid/Acetate Buffer Solution at pH 5.0

Electrode No	Membrane composition (%)				Slope (mV/pCu)	Linear working range (M)	R^2
	Ionophore (%)	PVC	Plasticizer	Liphophilic additive			
E0	0	30	70 *o*-NPOE	−	2.1	1.0×10^{-3}–1.0×10^{-6}	0.6319
E1	1	30	69 *o*-NPOE	−	9.7	1.0×10^{-6}–1.0×10^{-9}	0.9887
E2	2	30	68 *o*-NPOE	−	30.3	1.0×10^{-2}–1.0×10^{-5}	0.9930
E3	3	30	67 *o*-NPOE	−	26.5	1.0×10^{-2}–1.0×10^{-4}	0.9894

Table 2. Effect of the Nature of the Plasticizer on the Analytical Performance of Cu-ISE (2% Ionophore + 68% Plasticizer + 30% PVC)

Electrode No	Plasticizer	Slope (mV/pCu)	Linear working range (M)	R^2
E2-1	*o*-NPOE	29.7	1.0×10^{-2}–1.0×10^{-5}	0.9424
E2-2	*o*-NPPE	35.2	1.0×10^{-2}–1.0×10^{-6}	0.8925
E2-3	DBP	28.9	1.0×10^{-3}–1.0×10^{-6}	0.8781
E2-4	BBPA	30.5	1.0×10^{-3}–1.0×10^{-6}	0.9432
E2-5	BEHA	39.1	1.0×10^{-2}–1.0×10^{-4}	0.9080
E2-6	DBS	23.9	1.0×10^{-1}–1.0×10^{-4}	0.9754
E2-7	TEHF	18.1	1.0×10^{-7}–1.0×10^{-9}	0.9650

o-NPOE: o-Nitrophenyloctyl ether, NPPE: Nitrophenylpentyl ether, DBP: Dibutyl phthalate, BEHA: Bis(2-ethylhexyl) adipate, BBPA: Bis(1-butylpentyl) adipate DBS: Dibutyl sebecate, TEHF: Tris(2-ethylhexyl) phosphate.

electrodes. From the data in Table 2, the linear part of the calibration curve of the electrode with o-NPOE is longer and the detection limit is lower compared to values for the electrode containing the other plasticizers used. So, this plasticizer has been chosen for further studies.

It is generally known that the presence of lipophilic salts has a significant impact on both charged carrier- and neutral carrier-based ISEs [31,50]. Neutral carrier-based cation selective electrodes require lipophilic anionic sites, but for the charged carrier-based ISEs, the ionic sites must bear the same charge as the analyte ion. The electrodes' selectivity and response behavior are improved by these ionic sites, which also reduce membrane resistance and interference from sample ions in the solution [51].

Therefore, to evaluate the effect of the added ionic sites on the performance of Cu-ISE, we investigated eight different lipophilic additives. Table 3 summarizes the performance of the electrodes. As can be seen from Table 3, the potentiometric response of the electrode prepared with the potassium tetrakis(4-chlorophenyl)borate (KTpClPB) significantly improved the Nernstian response when compared to other lipophilic salts. These assessments allow us to conclude that SB behaves as a neutral carrier.

The electrode (E2-1-1) constructed with the membrane containing 2% SB, 68% o-NPOE, 29.75% PVC, and 0.25 mg KTpClPB demonstrated the best copper response when considering both the slopes and the linear working range of the electrodes.

Table 3. Effect of Nature of Lipophilic Additives on the Analytical Performance of Cu-ISE (2% Ionophore + 68% o-NPOE + 30% PVC + Lipophilic Additives 70% Mole Ratio to Ionophore)

Electrode No	Lipophilic additive	Slope (mV/pCu)	Linear working range (M)	R^2
E2-1-1	Potassium tetrakis(4-chlorophenyl)borate	29.7	1.0×10^{-2}–1.0×10^{-5}	0.9954
E2-1-2	Cesium tetrakis(3-methylphenyl)borate	26.3	1.0×10^{-2}–1.0×10^{-4}	0.9961
E2-1-3	Tetrabutylammonium tetraphenylborate	15.2	1.0×10^{-2}–1.0×10^{-5}	0.8730
E2-1-4	Tetraheptylammonium tetraphenylborate	12.6	1.0×10^{-2}–1.0×10^{-4}	0.9403
E2-1-5	Tetradodecylammonium tetrakis(4-chlorophenyl)bor	13.1	1.0×10^{-2}–1.0×10^{-4}	0.9331
E2-1-6	Tetraphenylphosphonium tetraphenylborate	16.9	1.0×10^{-2}–1.0×10^{-4}	0.9425
E2-1-7	Tridodecylmethylammonium chloride	7.2	1.0×10^{-7}–1.0×10^{-9}	0.9999
E2-1-8	NiO nano particles	17.7	1.0×10^{-6}–1.0×10^{-8}	0.9995
E2-1-9	Ethalin DES	25.7	1.0×10^{-2}–1.0×10^{-4}	0.9848

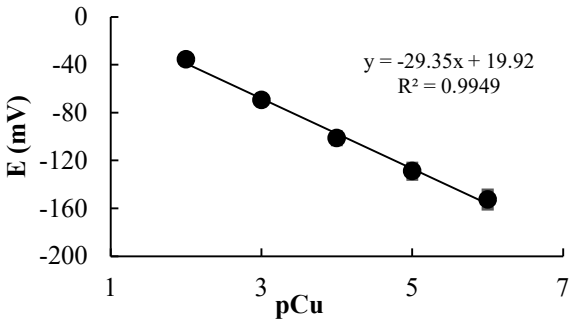

Fig. 4. Calibration graph of KTpClPB/SB/PVC/BWE (Error bars indicate the SD of three repeated measurements).

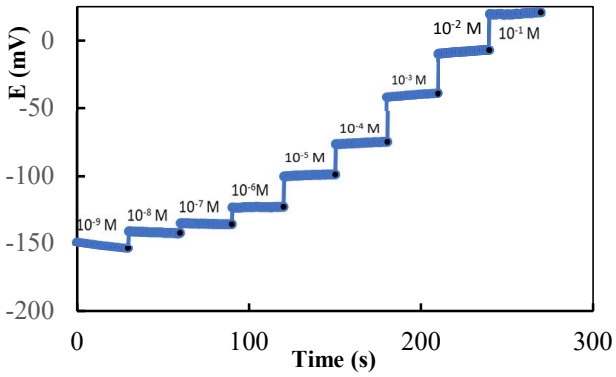

Fig. 5. The emf values for different solutions displaying increasing copper(II) concentrations recorded over time.

Working Concentration Range and Slope

The analytical performance of the Schiff base functionalized PVC membrane electrode was evaluated by performing potential measurements as a function of copper(II) concentration at room temperature (25-30 °C). Herein, the calibration graph of the KTpClPB/SB/PVC/BWE was obtained by plotting copper(II) concentration *versus* emf values (Fig. 4). The linearity range was determined as 1.0×10^{-2} M and 1.0×10^{-5} M. The linear regression equation and correlation coefficient were found to be emf (mV) = -29.35 mV/pCu + 20.78, $R^2 = 0.9956$, respectively.

Response Time

An ion-selective electrode must have a sufficiently fast response time to be used in analytical applications. Figure 5 shows the change of potential over time for different

copper(II) concentrations ranging from 1.0×10^{-2} to 1.0×10^{-9} M. As expected, the lowest concentration of Cu(II) gives the longest response times because of the longer equilibration time. Thus, concentrations between $1.0 \times 10^{-7} - 1.0 \times 10^{-9}$ M have response times of about 10-15 s, whereas concentrations higher than 1×10^{-6} M have response times of less than 10 s. This is most likely because of the fast exchange kinetics of Cu(II) ions with ionophore at the test solution-membrane interface. The response time is similar to, or even shorter than, those described in the literature that were shown to give linear responses in the same or narrower working ranges [52].

pH

The pH of the working solution can influence the potential response of the ISE's. Therefore, the impact of the pH on the copper response of the KTpClPB/SB/PVC/BWE was investigated over the pH range between 2.0 and 8.0 in Britton Robinson (BR) buffer solutions containing 1.0×10^{-3} M Cu(II) solution. As is shown in Fig. 6, the electrode demonstrated H^+-sensitivity in the pH range of 2-8.

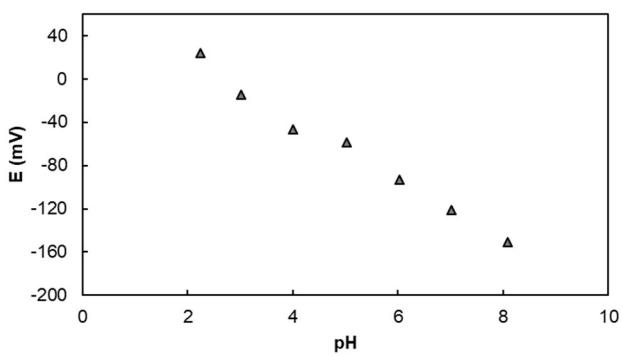

Fig. 6. The effect of buffer pH on the potential response of KTpClPB/SB/PVC/BWE.

From the related calibration graphs, the potentiometric response was decreased by pH until 4.0, almost remained constant at approximately 1 pH unit, and then was diminished by a slope of about 28 mV. The protonation of the nitrogen atoms in the ionophore can be used to explain this variation in the behavior and sensitivity of the electrode to H^+. As a result, it was decided that the pH should be kept constant in a controlled manner. The Cu(II) ion would precipitate as copper hydroxide at high pH values, so it was studied at pH 5.00.

The pH of the calibration solutions was adjusted to pH 5.0 using both BR and acetic/sodium acetate buffer solution to determine the best buffer type. The proposed electrode showed relatively best Nernstian response and wider linear range with acetic acid/acetate buffer. So, acetic acid/acetate buffer at pH 5.0 was chosen as the working pH in subsequent experiments.

Potentiometric Selectivity Coefficient

The effect of interferences on the potential response of KTpClPB/SB/PVC/BWE was also investigated. The mixed solution method was used to calculate the selectivity coefficients of the electrode using potential measurements of solutions made with a constant concentration of copper (1.0×10^{-3} M) and a variable concentration of interfering [53,54]. Studies on interference were done for the cations Na^+, K^+, NH_4^+, Ca^{2+}, Sr^{2+}, Mg^{2+}, Zn^{2+}, Ba^{2+}, Al^{3+}, and Fe^{3+} because they were present in the tested certified blood sample. The selectivity coefficients for these ions were given in Table 4.

According to the data given in Table 4, the interfering effect of the cations is in the following order:

Table 4. Selectivity Coefficients of KTpClPB/SB/PVC/BWE Using Mixed Solution Method (MSM)

Interfering ion	$k_{i,j}^{pot.}$	Interfering ion	$k_{i,j}^{pot.}$
Na^+	1.39×10^{-6}	Mg^{2+}	5.59×10^{-5}
K^+	1.97×10^{-3}	Zn^{2+}	2.78×10^{-4}
NH_4^+	2.07×10^{-5}	Ba^{2+}	9.53×10^{-3}
Ca^{2+}	9.98×10^{-5}	Al^{3+}	9.36×10^{-5}
Sr^{2+}	4.97×10^{-4}	Fe^{3+}	9.66×10^{-5}

$$Na^+ < NH_4^+ < Mg^{2+} < Al^{3+} \sim Fe^{3+} \sim Ca^{2+} < Zn^{2+} < Sr^{2+} < K^+ < Ba^{2+}$$

The assessment of the data in Table 4, it is evident that the electrode is selective for copper(II), and all cations whose interfering effect was studied, except K^+, had no effect on the electrode's response. This result showed that when measuring the copper(II) ion in the presence of the other ions studied, the suggested electrode did not create any interference.

N,N'-bis(salicylidene)-1,3-diaminopropane is an ONNO type ligand. In a study by Drew *et al.* (1985), the molecular structures of the Cu(II) and Ni(II) ion complexes with this ligand were published [55]. With this ligand, copper forms a distorted tetrahedral or square planar coordination while nickel forms a complex with a square planar structure. The bond angle between the C atoms in the methylene groups was found to be 133.9º in the nickel(II) complex, whereas it was 131.2º in the copper(II) complex. It should be noted that the bond angle in the copper complex is more similar to that of the tetrahedron. As a result, the chelate ring is tighter and ONNO donors can more easily coordinate copper than nickel, cobalt, or other first-order transition metals. It can be concluded that, when measuring the copper(II) ion in the presence of other ions under investigation, the response of the suggested electrode is unaffected by these ions.

On the o ther hand, the electrode's selectivity coefficients exhibit anti-Hofmeister behavior and don't conform to the Hofmeister series. This is likely due to the different stability constants of the new complexes formed between the cations and the Schiff base.

Method Validation

Limit of detection (LOD) and limit of quantification (LOQ). The limit of detection (LOD) and limit of quantification (LOQ) values were calculated according to the equations of LOD = $3 \times s_b/m$ and LOQ = $10 \times s_b/m$ [56]. In the equations, m represents the slope of the calibration plot and s_b represents the standard deviation (n = 10) of the blank solution. LOD and LOQ of the developed electrode were found to be 6.31×10^{-6} M and 2.10×10^{-5} M, respectively.

Reproducibility, Repeatability, and Stability of the Electrode

By plotting calibration graphs with five equivalently fabricated ion-selective electrodes, the electrode-to-electrode reproducibility of the KTpClPB/SB/PVC/BWE was evaluated, and the relative standard deviation (RSD) of the potentiometric response was found to be 7.6%. The RSD obtained with one KTpClPB/SB/PVC/BWE for five sequential calibration graphs was 2.9%, indicating that the measurements were repeatable.

The long-term stability of the KTpClPB/SB/PVC/BWE was also tested for 1, 3, 10, and 20 days. After 3 days, the potential response decreased to 91.9%, while 54.1% of the initial response was preserved after 10 days. The developed sensor was stable for 10 days. When compared to other copper-selective electrodes described in the literature, the electrode appears to have a short lifetime [31]. The most important advantage, however, is that the cocktail can be stored for a long time without spoiling and that daily electrode preparation (approximately 1 h) is very simple.

Application in Human Blood Sample

It is crucial to detect copper ions in biological samples with great sensitivity, especially before it reaches harmful concentrations. When working on standard solutions and according to the obtained validation parameters, results encourage the use of the suggested electrode for the determination of copper in biological samples. The lyophilized blood powder sample named Seronorm™ L3 (LOT 1112691) was provided by Ankara Provincial Health Directorate.

Without employing sample extraction, evaporation, or filtration techniques, the direct potentiometric method was used to measure the copper content in the blood sample. The results show that the proposed electrode was successfully applied for the assay of copper in human blood (Table 5).

Table 5 shows the results in terms of A1, A2, P (%), and z-score. In the equations, X_{ref} is the certified value, X_{exp} is the experimental value, and SD_{ref} *and* SD_{exp} are the standard deviation of reference material and the experimental value, respectively.

The results are considered "Acceptable" for trueness evaluation if A1 > A2. The letter "*P*" indicates a precision assessment of the results. The concentration data obtained for this study is evaluated using the z-scores [57]. The validity of the proposed method was considered acceptable if the z-scores were less than or equal to 2. A z-score of two to three

Table 5. The Results of the Cu(II) Analysis on Standard Reference Human Blood

Sample	Certified (mg l^{-1})	Found (mg l^{-1})	A1	A2	z-value
SERONORM™ L3 (LOT 1112691)	2.47 ± 0.25	2.46 ± 0.19	0.01	0.815	0.024

$A1 = |X_{ref} - X_{exp}|$; $A2 = 2.58\sqrt{SD_{ref}^2 + SD_{exp}^2}$; $P = \sqrt{\left(\frac{SD_{ref}}{X_{ref}}\right)^2 + \left(\frac{SD_{exp}}{X_{exp}}\right)^2} \times 100\%$; $Z - score = \frac{|X_{ref} - X_{exp}|}{\sqrt{SD_{ref}^2 + SD_{exp}^2}}$

Table 6. Recovery Studies for Copper(II) from Spiked Human Blood Sample

Added Cu (mg l^{-1})	Found Cu (mg l^{-1})	Average recovery (%)	Bias (%)
5.07	5.37	105.9	5.58
7.57	7.60	100.3	0.39
10.07	10.36	102.8	2.80
15.07	14.43	95.7	-4.43
20.07	18.65	92.9	-7.61

denotes questionable outcomes. If |Z| is bigger than 3, the analysis is regarded as inappropriate. The findings for the detection of copper in a blood sample are presented in Table 5, which shows the good accuracy and validity of the suggested method.

The accuracy of the methods was also determined by their recovery during spiked experiments. The recovery studies were carried out after the addition of known amounts of the standard copper solution (1000 mg l^{-1}) to the analyzed blood sample. The applicability of the KTpClPB/SB/PVC/BWE electrode was supported by the obtained good recovery values (Table 6).

The method was found to be suitable for use in the routine measurement of copper ions in blood samples after achieving satisfactory findings from the certified reference material and good recoveries from spiked samples.

CONCLUSIONS

Based on the results of this study, the (N,N′-bis(salicylidene)-1,3-diaminopropane) Schiff base can be successfully used as an ionophore to construct the copper(II) ion-selective solid state PVC membrane electrode. The 3.00 mm diameter of the electrode's tip enables the analysis of a small volume of the samples. In addition, the use of brass wire makes the electrode much cheaper compared the other conductive materials like glassy carbon, platin, and gold.

It was found that the composition of 2% SB, 68% o-NPOE, 29.75% PVC, and 0.25 mg KTpClPB exhibits the best response slope with a detection limit of 6.31×10^{-6} M. The detection of copper(II) ion is unaffected by common metal ions. The suggested electrode was also used to direct the detection of copper(II) ions in human blood with the outcomes showing good agreement with the Inductively Coupled Plasma-Sector Field Mass Spectrometry (ICP-SFMS) method. Most importantly, novel solid-state Cu-ISE has a great potential to detect copper(II) ions in human blood without complicated sample preparation or separation procedures.

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