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Electroanalytical Characterization of a Copper(II)-Rutin Complex

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A Cu(II)-rutin complex was prepared with a metal to ligand molar ratio of 3:2 by mixing stoichiometric amounts of rutin and $CuCl_2$. The isolated complex was characterized by IR and UV/Vis spectroscopy and incorporated into a plasticized PVC-membrane using o-nitrophenyloctylether as a plasticizer (66% plasticizer, 33% PVC and 1% Cu(II)-rutin complex). The sensing characteristics of the membrane against Cu(II) ion were investigated using direct potentiometry. The membrane showed a Nernstian response for Cu(II) ion with a slope of 28.5 mV/decade of activity between 5 x 10^{-4} and 4 x 10^{-2} M. The influence of several inorganic cations on the response of the Cu(II)-rutin complex based membrane was also investigated and the corresponding potentiometric selectivity coefficients were estimated and optimized with *Solver* (Excel).

Keywords: Rutin, Cu, metal complexation, sensor

1. INTRODUCTION

Flavonoids are a class of secondary plant phenols consisting of fifteen carbon atoms arranged in a C_6-C_3-C_6 configuration. They are mostly found in the leaves, seeds, bark and flowers of plants, where they have a protective role against ultraviolet radiation, pathogens and herbivores [1, 2]. Most of the beneficial health effects of flavonoids (e.g., anti-inflammatory, anti-cancer, cardio-protective) are attributed to their antioxidant and chelating abilities [3]. Some studies have shown that formation of complexes between flavonoids and transition metal ions kept metal ions from participating in the generation of free radicals [4]. Therefore, formation of metal-flavonoid complexes is very important for elimination of excess free radicals, which could have a negative effect on biomolecules [5].

The flavonoid rutin (quercetin-3-*O*-rutinose, Figure 1) is one of the most bioactive flavonoids and is usually found in buckwheat, black tea and apple peels. Rutin may have antioxidant, anti-inflammatory, anti-tumor, anti-thrombotic, cardio-protective and antibacterial activity [6]. It increases the strength of the walls of blood capillaries, regulates their permeability and can therefore reduce the symptoms of many other capillary diseases. In addition, the antioxidant activity of rutin can be enhanced by complexation with transition metal ions (Cu^{2+}, Fe^{2+}, Al^{3+}, Zn^{2+}) [7].

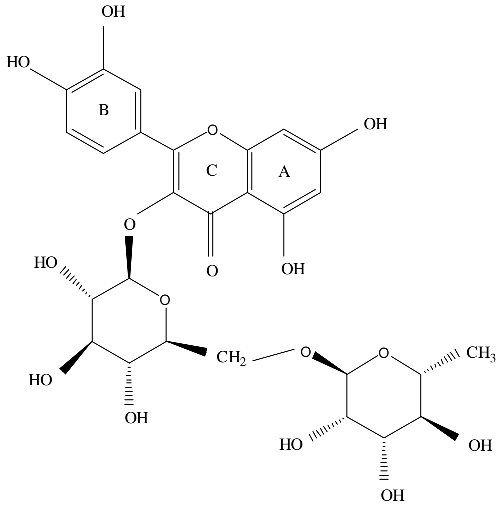

Figure 1. Chemical structure of rutin (3`,4`,5,7-tetrahydroxyflavone-3β-D-rutinoside).

Complexes of flavonoids with metal ions have been investigated using many different techniques. Some of the studies included electrochemical techniques (e.g., differential pulse anodic stripping voltammetry [8], cyclic voltammetry [9, 10] and differential pulse voltammetry [10]), theoretical methods (e.g., Density Functional Theory (DFT) methods [11]) and UV-Vis spectroscopy [12]. Their antioxidant properties have been studied by UV-Vis spectroscopy with DPPH [7, 13], cyclic voltammetry [7] and pulse radiolysis [14].

Characterization of these complexes was accomplished by Electrospray Ionization Tandem Mass Spectrometry (ESI-MS) [15, 16], IR [11, 13, 14, 17, 18] and UV-Vis [9, 11-14, 16, 17] spectroscopy, ^{1}H-NMR [13, 17], EPR [9, 17], Raman [14], elemental analysis [17, 18] and thermogravimetric analysis [14, 17]. This study describes the electroanalytical characterization of a Cu(II)-rutin complex.

2. EXPERIMENTAL

2.1. Reagents and Materials

All chemicals were of the highest purity commercially available and were used without further purification. Rutin trihydrate ($C_{27}H_{30}O_{16}\cdot 3H_2O$), Cu(II) chloride dihydrate ($CuCl_2\cdot 2H_2O$) and methanol were obtained from Sigma-Aldrich (St. Louis, MO, USA). All other chemicals were obtained from Kemika (Zagreb, Croatia) and Alkaloid (Skopje, FYROM).

A stock solution of rutin (c = 0.01 M) was prepared in methanol and kept in the refrigerator (solution was stable for at least 1 month). Prior to use, it was diluted to the desired concentration with buffer supporting electrolyte and the ionic strength was adjusted with the addition of KCl (c = 1 M). Buffer supporting electrolyte solutions were prepared in high-purity water from a Millipore Milli-Q system (resistivity greater than or equal to 18 MΩ cm). Phosphate buffer solutions of pH 8.0 and 7.0, and acetate buffer solutions of pH 5.5, 4.5 and 3.5, were used in the experiments.

Cu(II) chloride solution (c = 0.01 M) was used as a chelating reagent. A rutin solution (c = 1.2 mM) was used to form the Cu(II)-rutin complex.

2.2. Preparation of the Cu(II)-rutin complex

The Cu(II)-rutin complex was prepared by mixing stoichiometric amounts of rutin (1 mmol) and $CuCl_2 \cdot 2H_2O$ (1.5 mmol) in methanol and heating to reflux for 5 h. A pale yellow precipitate was formed immediately when the cooled mixture was poured into water. The formed complex was left to stand overnight and then collected by vacuum filtration, washed 3 times with a 1:3 ethanol/water mixture, washed several times with water and dried under vacuum. The isolated complex was used for the preparation of the PVC-based membrane.

2.3. Electrode preparation

The electrode membrane was composed of o-nitrophenyloctylether (o-NPOE) as plasticizer (66%), high molecular weight poly(vinyl chloride) (PVC, 33%) (both the o-NPOE and PVC were purchased from Fluka, Switzerland) and Cu(II)-rutin complex as a sensing material (1.0%). A mass of 0.18 g of mixture was dissolved in 10 mL of tetrahydrofuran (the mixture was sonicated in an ultrasonic bath for 3 h). Then, the clear solution was carefully poured into a glass ring (i.d. 24 mm, lower side ground flat, Fluka) fixed tightly to a glass plate. After solvent evaporation and curing, small disks of 7 mm inner diameter were punched from the cast film and mounted in a Philips electrode body IS-561 (Glasblaeserei Moeller, Zurich, Switzerland). KCl, c = 1 mM) was used as the internal filling solution.

A silver/silver(I) chloride reference electrode (Metrohm, Switzerland) with a KCl electrolyte solution (c = 3 M) was used as reference. Between measurements, the electrode was kept in the Cu(II) chloride solution (c = 1 mM).

2.4. Apparatus and Procedure

A Dosimat 765 (Metrohm, Switzerland) with homemade software and combined with a Metrohm 780 pH meter (Metrohm, Switzerland) was used to dose the chelating reagent ($CuCl_2$). The solutions were magnetically stirred during titrations using a 728 Titration Stand (Metrohm, Switzerland). A platinum redox electrode (IJ64, Ionode, Australia) and the pH electrode (Metrohm) were used as potentiometric sensors to study the Cu(II)-rutin complexation. A Philips electrode with a PVC membrane containing the Cu(II)-rutin complex was used to measure the response characteristics of the Cu(II) ion. All measurements and titrations were performed at room temperature using a magnetic stirrer with adjusted ionic strength and pH. The Dosimat was programmed to work in MET Mode (Monotonic titration with fixed volume increments). The waiting time before each increment addition was 30 s.

UV/Vis spectra in methanol were recorded on a UV-1700 Pharma Spec spectrophotometer (Shimadzu, Japan) using standard 1.00 cm quartz cells. The IR spectra were recorded on an FTIR-8400 spectrophotometer (Shimadzu, Japan) using KBr pellets.

3. RESULTS AND DISCUSSION

3.1. UV-VIS and IR spectra

Rutin exhibited two major absorption bands in the ultraviolet/visible region. The absorptions in the 320 to 385 nm range corresponded to the B ring of the rutin molecule, and the absorptions in the 240 to 280 nm range corresponded to the A ring. The spectra were related to the $\pi \rightarrow \pi^*$ transitions in the ligand. These bands were shifted to higher wavelengths in the Cu(II)-rutin complex spectra (Figure 2) and a new d-d band at 419 nm appeared.

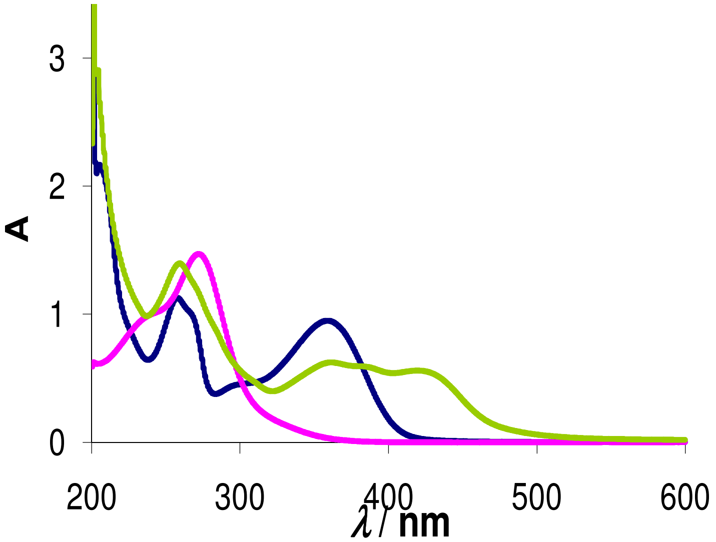

Figure 2. UV/Vis spectra of $CuCl_2$ (▬), rutin (▬) and the Cu(II)-rutin complex (▬) in methanol.

IR spectra of rutin (Figure 3) and the Cu(II)-rutin complex (Figure 4) presented evidence of the coordination between Cu and rutin. The results are summarized in Table 1. Worthy of note was the ν

(M-O) = 623 cm^{-1} frequency, which appeared in the IR spectrum of the Cu(II)-rutin complex (Figure 4), but not in the IR spectrum of rutin (Figure 3). In addition, no shift of the carbonyl ν(C=O) bond was observed, probably because the 3-OH group of the C-ring in rutin was blocked by rutinose (the sugar moiety). Finally, the ν(O-H) = 3600 to 2800 cm^{-1} frequencies appeared as medium broad bands in the Cu(II)-rutin spectrum, indicating the presence of water (Figure 4).

Table 1. IR data for rutin and the Cu(II)-rutin complex.

Compound	ν(M-O)/ cm^{-1}	ν(C=O)/ cm^{-1}	ν(C-O-C)/ cm^{-1}	ν(C=C)/ cm^{-1}	ν(O-H)/ cm^{-1}
Rutin	-	1655 s	1296 s	1599 s	3600...3000 b,m
Cu (II)-rutin complex	623 w	1630 s	1286 s	1600 s	3600...2800 b,m

b = broad, s = strong, m = medium, w = weak

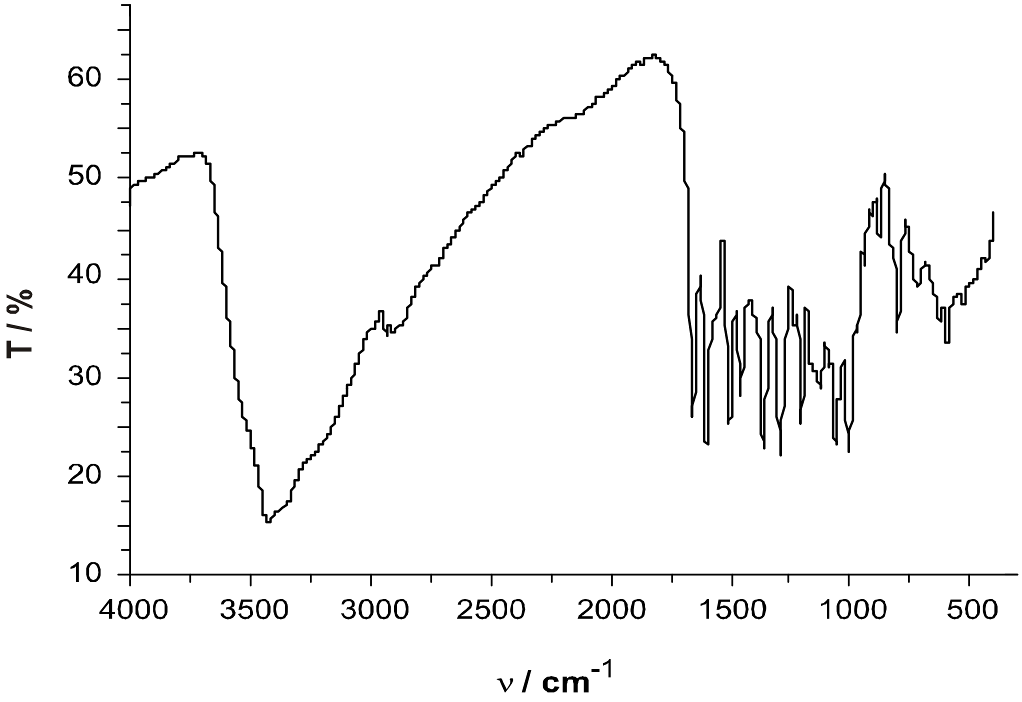

Figure 3. IR spectrum of rutin.

3.2. Sensing properties of the Cu(II)-rutin complex

3.2.1. Response characteristics of the Cu(II)-rutin complex Response to Cu(II) ion

The electromotive force of the membrane electrode assembly when dipped in a solution of Cu(II) ion is given by the Nernst equation:

$$E = E^0 + S \cdot \log a_{Cu^{2+}} \quad (1)$$

where E^0 = a constant potential term, S = the electrode slope, and $a_{Cu^{2+}}$ = the activity of the Cu(II) ion. The response characteristics of a Cu(II)-rutin complex-based electrode in solutions of Cu(II) are shown in Figure 6.

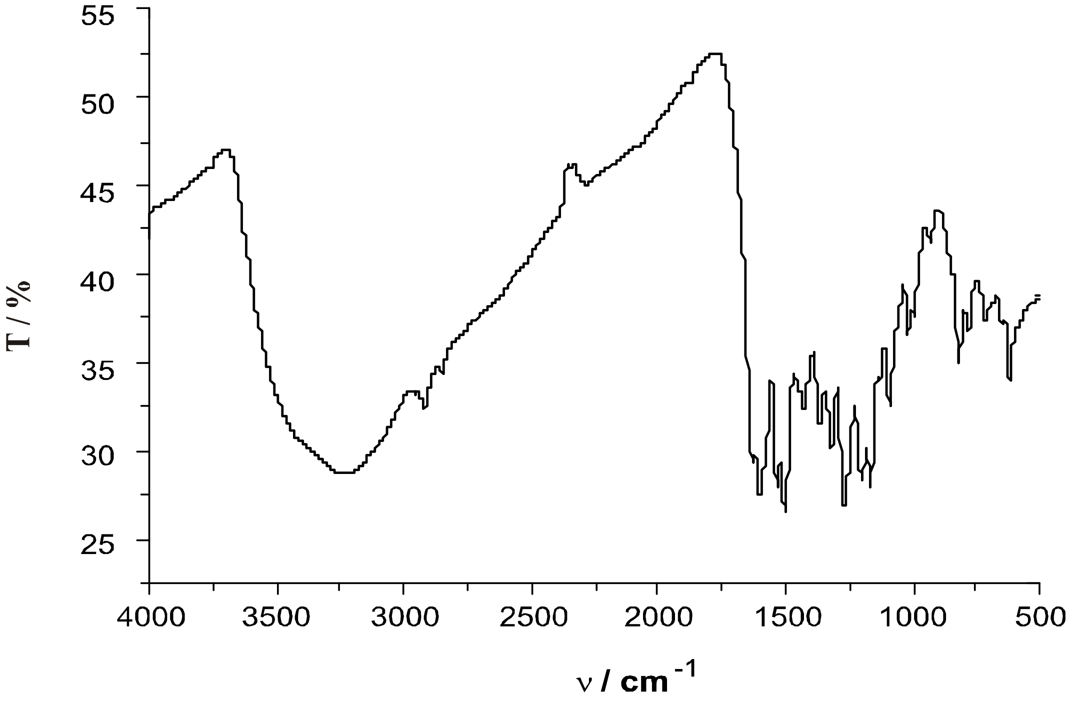

Figure 4. IR spectrum of the Cu(II)-rutin complex.

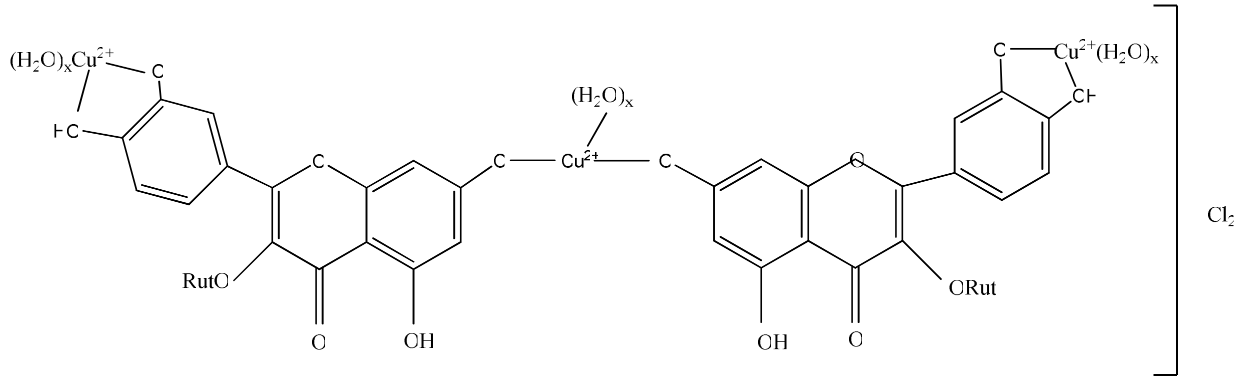

Figure 5. The proposed structure of the Cu(II)-rutin complex

Based on similar spectroscopic results, a structure for the investigated complex has been proposed (Figure 5) (the structure was taken from reference 11).

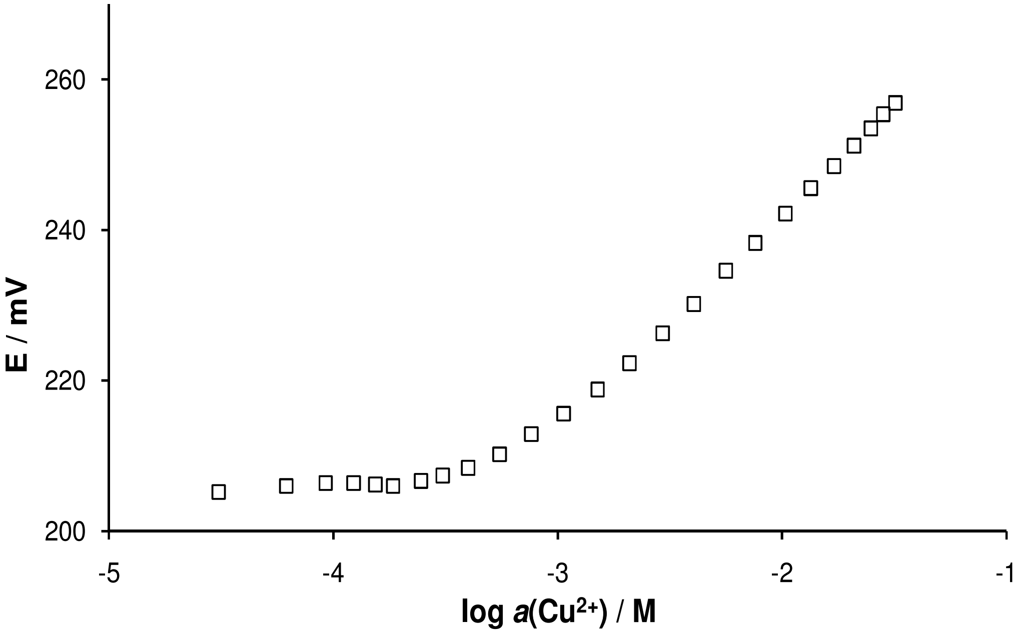

Figure 6. Response characteristics of a Cu(II)-rutin complex-based electrode toward Cu(II) ion.

The slope values and correlation coefficients were calculated from the linear region of the calibration graphs on the five series of measurements using linear regression analysis. The detection limits were estimated according to the IUPAC recommendations [19]. The deviations from linearity at lower concentrations were caused by gradual dissolution of the Cu(II)-rutin complex from the membrane. Agreement between several electrode coating procedures was satisfactory concerning analytical performance of the electrode. Detailed regression statistics of the electrode characteristics are given in Table 2.

Table 2. Regression statistics of the Cu(II)-rutin complex-based electrode toward Cu(II) ion.

Parameters	Values
Slope (S)	28.49
SE_S	0.30
CI_S (p=0.05)	0.66
Intercept (I)	299.02
SE_I	0.65
CI_I (p=0.05)	1.43
Correl. Coef. R^2	0.9987
Limit of detection (mmol/L)	0.54

SE = standard error
CI = confidence interval

The electrode exhibited a Nernstian slope in the linear response range, as shown in Figure 6, with a satisfactory linear correlation between the measured electromotive force of the electrode and the logarithm of the Cu(II) ion activity.

3.2.2. Interferences

3.2.2.1. Determination of selectivity coefficients

The influence of interferents on the response of the described Cu(II)-rutin complex-based electrode is defined by the Nikolskii-Eisenman equation:

$$E = E^0_{M^{n+}_{\mathrm{det}}} + \frac{RT}{nF}\cdot\ln\left[a_{M^{n+}_{\mathrm{det}}} + K^{pot}_{M^{n+}_{\mathrm{det}}M^{m+}_{int}}\cdot a^{n/m}_{M^{m+}_{\mathrm{int}}}\right] \qquad (2)$$

where $K^{pot}_{M^{n+}_{\mathrm{det}}M^{m+}_{int}}$ = a selectivity coefficient, $a_{M^{n+}_{\mathrm{det}}}$ and $a_{M^{m+}_{int}}$ are the activities of analyte (determined) ion (det) and interfering ion (int), respectively.

The mixed solution/fixed interference method [20] was used for measurement of selectivity coefficients because it yields more realistic data than the separate solution method for the systems investigated. The electrode response was measured in a series of solutions of varying primary (determined) ion activity, $a_{M^{n+}_{\mathrm{det}}}$, and fixed interfering ion activity, $a_{M^{m+}_{int}}$.

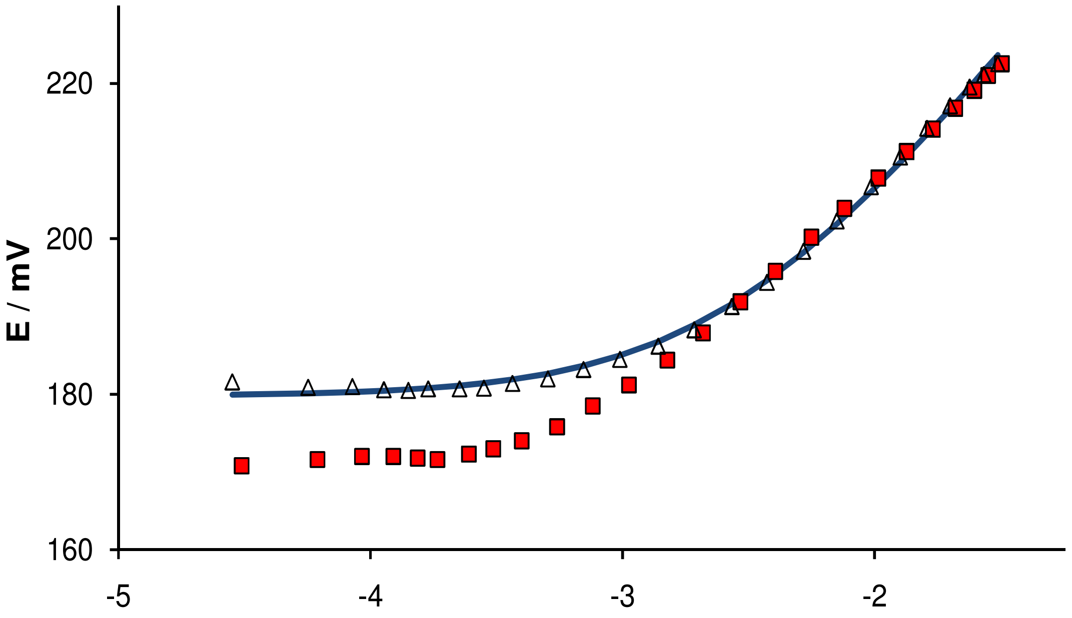

Figure 7. Estimation of parameters by fitting of the Nikolskii-Eisenman equation to experimental data for the Cu(II)-rutin complex-based electrode from the fixed interference method using Cu(II) chloride as analyte and cadmium sulfate (c = 0.1 M) as the interfering ion. (■) Cu(II); (Δ) Cu(II) + Cd (*c* = 0.1 M); (▬) model given by the Nikolskii-Eisenman equation.

The selectivity coefficients were then estimated graphically, which is a very subjective and rough method. The more reliable method involved fitting the Nikolskii-Eisenman equation (used as a model) to the experimental data obtained by the mixed solution/fixed interference method. Using Solver, an analysis tool incorporated into Microsoft Excel, the minimal sum of squared residuals was calculated by varying the values of E^0, S and $K^{pot}_{M^{n+}_{\det}M^{m+}_{int}}$ (Figure 7).

The selectivity coefficients of some potentially interfering metallic cations were determined for the Cu(II)-rutin complex-based electrode (Table 3). For all selectivity measurements, Cu(II) chloride was used as the primary ion in the range of 40 µM to 44 mM, while the concentration of the interfering ion was held constant at 0.1 M.

Table 3. Potentiometric selectivity coefficients for different metallic cations measured with the Cu(II)-rutin complex-based sensor. Cu(II) ion was used as the primary (analyte) ion and the concentration of the interfering cation was 0.1 M.

Interference, M_{int}	$K^{pot}_{M^{n+}_{\det}M^{m+}_{int}}$
Ammonium	1
Sodium	2.1×10^{-1}
Potassium	9.1×10^{-1}
Magnesium	1.4×10^{-2}
Calcium	8.7×10^{-6}
Zinc	7.3×10^{-3}
Cobalt(II)	1.8×10^{-2}
Nickel(II)	9.4×10^{-3}
Cadmium	3.9×10^{-2}
Barium	9.1×10^{-3}
Lithium	6.4×10^{-2}
Lead	7.3×10^{-1}

The Cu(II)-rutin complex-based electrode exhibited satisfactory selectivity performances for Cu(II) ion for most of the cations investigated. However, ions of alkali metals (i.e., lithium, sodium, potassium), lead and ammonium ion exhibited strong interfering effects due to the formation of complexes with Cu(II).

4. CONCLUSIONS

The Cu(II)-rutin complex was synthesized and spectroscopically characterized. The complex was incorporated into a plasticized PVC-membrane using o-nitrophenyloctylether as plasticizer. The membrane response characteristics toward Cu(II) ion were investigated using direct potentiometry and the membrane exhibited a Nernstian behavior.

The influence of several inorganic cations on the response of the Cu(II)-rutin complex-based electrode was investigated. The corresponding potentiometric selectivity coefficients were estimated and optimized with *Solver* (Excel) by fitting the Nikolskii-Eisenman equation to the experimental data obtained from the mixed solution/fixed interference method using least-squares criterion.

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