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A new electrochemical method for the determination of chondroitin sulfate based on its supramolecular interaction with the cupferron–lead(II) complex

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Abstract: In this paper, the interaction of cupferron (Cup) and its lead(II) complex [Cup–Pb(II)] with chondroitin sulfate (CS) was investigated by the linear sweep voltammetric method. In the selected medium of pH 5.5 (acetic acid––hexamine buffer solution), Cup can interact with Pb(II) to form a stable complex of [Cup–Pb(II)], which has a sensitive second order derivative polarographic reductive peak at –0.64 V *vs.* SCE. After the addition of CS into a solution of the Cup–Pb(II) complex, the reductive peak current decreased without any shift of the peak potential and no new peak appeared, which indicated that a non-electroactive supramolecular complex of CS with [Cup–Pb(II)] was formed. The binding reaction conditions were carefully investigated. The interaction mechanism under the optimal conditions was discussed. The decrease of reductive peak current, I_p'', was directly proportional to the CS concentration, thus a new quantitative determination method for CS was established with the linear regression equation as $\Delta I_p''$ / nA =36.97(c / mg L^{-1}) + + 12.45 (n = 10, γ = 0.995). The effects of other substances on the determination were carefully investigated and three synthetic samples were determined with satisfactory results. The binding constant (β_s) and the binding number (m) of CS with [Cup–Pb(II)] complex were calculated from the voltammetric data with the results $\beta_s = 1.89\times10^{10}$ and $m \approx 2.5$.

Keywords: cupferron; lead(II); chondroitin sulfate; linear sweep voltammetry; interaction.

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INTRODUCTION

Chondroitin sulfate (CS) is a glycosaminoglycan with a disaccharide unit of *β*-D-galactosamine and *β*-D-glucuronic acid.[1] CS was first extracted from cartelage in 1984 and it was reported to have many important biological and pharmacological activities, such as anticoagulant and anticancer properties, anti-inflammatory activity, lipid metabolism promotion, osteoporosis treatment, *etc*.[2,3] Thus, in clinical therapy CS has been used as an effective medicine for the treatment of chronic diseases such as rheumatism, degenerative arthritis, lumbago, gastric ulcer, cirrhosis and chronic photo damage.[4] Recently, more biomedical activities, including drug delivery, tissue engineering, regenerative medicine and surgery, have been reported.[5–7]

The determination of the content of polysaccharide is the most efficient way to inspect the effective components of a product in the quality control of polysaccharide drug, as well as the best stable basis for a study of drug stability. Therefore, it is significant to establish a sensitive method for the determination of CS. Nowadays, many different kinds of analytical methods have been proposed for CS determination, such as the Elson–Morgan method,[8,9] spectrophotometry,[10,11] HPLC[12–14] and electrophoretic methods.[15–17] However, these methods often suffer from some disadvantages, such as low sensitivity, poor selectivity or a complicated procedure. Owing to the advantages of higher sensitivity, wider liner range, faster response and cheaper instrumentation, electrochemical methods have drawn much attention and have been used to determine successfully different kinds of biomolecules, such as DNA,[18,19] RNA,[20] protein,[21,22] heparin[23,24] and chondroitin sulfate.[25] However, most of the papers were based on the interaction of the biomolecules with electroactive substances. To the best of our knowledge, they are seldom based on the interaction of biomolecules with metal complexes.

As a commonly used organic compound, cupferron (Cup) can form stable complexes with many metals, which has been widely applied for the determination of trace metal elements, such as Cr, Al, Pb, Zn, *etc*., by spectrophotometry and electrochemical methods.[26–29] The metal complexes can also form a polarographic adsorption wave on a mercury working electrode, which can be used for the sensitive detection of metal ions.[30] Sun *et al*. studied the interaction between Cup and Cd(II) and a new electrochemical method for determining proteins was established by using the complex of [Cup–Cd(II)] as a voltammetric probe.[31]

In this work, Pb(II) was selected to interact with Cup and a stable electrochemically active complex of [Cup–Pb(II)] was formed, which showed a sensitive and stable voltammetric reductive peak at –0.64 V (*vs*. SCE) on a dropping mercury working electrode. The polarographic behaviors of the [Cup–Pb(II)] complex was carefully investigated on a dropping mercury electrode.[32] Thus, the [Cup–Pb(II)] complex was selected as an electrochemical probe to study the

binding reaction with CS and its further use to determine CS. The addition of CS into a solution of the [Cup–Pb(II)] complex resulted in a decrease in the concentration of the free [Cup–Pb(II)] complex in solution and consequently a decrease in the electrochemical response without any change of the peak potential, which indicated that the [Cup–Pb(II)] complex could interact with CS to form a supramolecular complex. The decrease in the electrochemical response was directly proportional to the CS concentration; thus, a new sensitive analytical method for CS determination was established.

EXPERIMENTAL

Apparatus

The second order derivative linear sweep voltammetric measurements were performed on a model JP-303 polarographic analyzer (Changed Apparatus Factory, China) with a conventional three-electrode system, composed of a dropping mercury electrode as the working electrode (DME), saturated calomel as the reference electrode (SCE) and a platinum wire as the auxiliary electrode. A pHS-25 acidimeter (Shanghai Leici Instrument Factory, China) was used for the pH measurement. All the experiments were performed at 25±1 °C. All potentials in the paper are expressed against SCE.

Reagents

Chondroitin sulfate (CS, 99 %, Shandong Linyitianli Biochemical Company, China) was used as received without further purification. A 1.0 mg mL^{-1} stock solution of CS was prepared by directly dissolving 0.1000 g of CS in water, then diluting to the mark in a 100-mL volumetric flask and stored at 4 °C. The working solutions were obtained by directly diluting the stock solution with water. A 1.0×10^{-3} mol L^{-1} solution of cupferron (Cup, Shanghai Far Navigation Chemical Reagent Factory, China) was prepared by dissolving 0.0155 g Cup in water and diluting to 100 mL. A 1.0×10^{-3} mol L^{-1} solution of lead nitrate (Shanghai Jinshan Chemical Plant, China) was prepared by dissolving 0.0333 g of the substance in water and diluting to 100 mL. A 1.0 mol L^{-1} acetic acid–hexamine buffer solution was used to control the acidity of reaction solutions. The buffer solution was prepared by mixing 34.05 g of hexamine diluted to 250 mL and adjusting the pH to 5.5 by diluting with 1.0 mol L^{-1} acetic acid (HAc). All of other employed reagents were of analytical reagent grade and doubly distilled water was used throughout.

Procedure

Into a dry 10 mL calibrated tube, the following reaction solutions were added in the following order: 1.5 mL of pH 5.5 HAc–hexamine buffer, 0.5 mL of 1.0×10^{-3} mol L^{-1} Cup, 0.25 mL 1.0×10^{-3} mol L^{-1} Pb (II) and an appropriate amount of CS or sample solution. The mixtures were diluted to the mark with water, mixed thoroughly and allowed to stand for 20 min at 25 °C. A blank solution was also prepared by the same procedure but without the addition of CS. Then the solution was transferred to a 10 mL electrochemical cell and the second order derivative linear sweep polarographic curve was recorded over the potential range from –0.3 to –0.8 V *vs.* SCE. The values of peak current of the [Cup–Pb(II)] complex in the presence (I_p'') and absence ($I_{p,0}''$ of CS at –0.64 V were measured, and the difference in the peak currents ($\Delta I_p'' = I_{p,0}'' - I_p''$) was used for CS determination.

RESULTS AND DISCUSSION

Second order derivative linear sweep polarogram

The second order derivative linear sweep polarograms of HAc–hexamine, Cup, Pb(II) and their mixture with CS are shown in Fig. 1. Curve 1 is the polarogram of the HAc–hexamine buffer solution and no polarographic peak appeared, which indicated that no electroactive substances existed in the HAc––hexamine buffer. Curve 2 is the polarogram of the Cup solution; a reductive peak at –0.54 V was obtained, resulting from the reduction of Cup on the mercury electrode. Curve 3 is the polarogram of Pb(II); no peak appeared in the scanned potential range. Curve 4 is the polarogram of the Cup–Pb(II) solution; a new well-defined polarographic reductive peak at –0.64 V was obtained, which was due to the interaction between Cup and Pb(II) and the deoxidization of the newly formed complex [Cup–Pb(II)] on the mercury electrode. Curve 5 and 6 are the polarograms of the mixture of different amount of CS with [Cup–Pb(II)]. Owing to the interaction of CS with [Cup–Pb(II)], the concentration of free [Cup–Pb(II)] in solution decreased, resulting in decreases in the reductive peak current. The decrease in the peak current was proportional to the concentration of CS, which could be further utilized for the determination of CS.

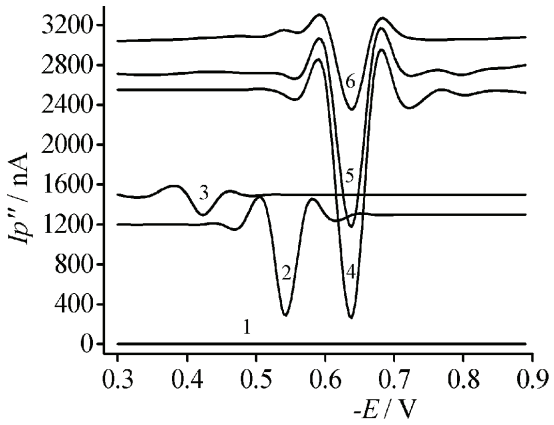

Fig. 1. Second order derivative linear sweep voltammograms of the Cup–Pb(II)–CS interaction system. 1. pH 5.5, HAc–hexamine; 2. 1 + 5.0×10^{-5} mol L^{-1} Cup; 3. 1 + 2.5×10^{-5} mol L^{-1} Pb(II); 4. 1 + 5.0×10^{-5} mol L^{-1} Cup + 2.5×10^{-5} mol L^{-1} Pb(II); 5. 4 + 10.0 mg L^{-1} CS; 6. 4+20.0 mg L^{-1} CS. The potentials are expressed *vs.* SCE.

Optimal reaction conditions

The influence of the reaction conditions, such as the acidity of the buffer solution, the ratio of Cup and Pb(II) concentrations, reaction time, reaction temperature, the instrumental conditions, the ion strength, *etc*., were carefully investigated.

The effect of buffer acidity on the difference of peak current ($\Delta I_p''$) was investigated by keeping the Cup, Pb(II) and CS concentration constant and changing the buffer pH in the range from 3.5 to 6.5. The results are shown in Fig. 2, from which, it can be seen that the value of $\Delta I_p''$ reached its maximum at pH 5.5; hence, pH 5.5 was selected for this assay. The volume of HAc–hexamine buffer solution added into the solution was investigated in the volume range of 1.0–5.0 mL and 1.5 mL was found suitable for the following procedures.

The effect of the ratio of Cup and Pb(II) on the value of $\Delta I_p''$ was studied by keeping CS concentration at 20.0 mg L^{-1}, the Cup concentration at 5.0×10^{-5} mol L^{-1} and changing the Pb(II) concentration. The results showed that the value of $\Delta I_p''$ reached its maximum when the Pb(II) concentration was 2.5×10^{-5} mol L^{-1}. Hence, the ratio of Cup to Pb(II) was kept constant at 2:1 in the following experiments.

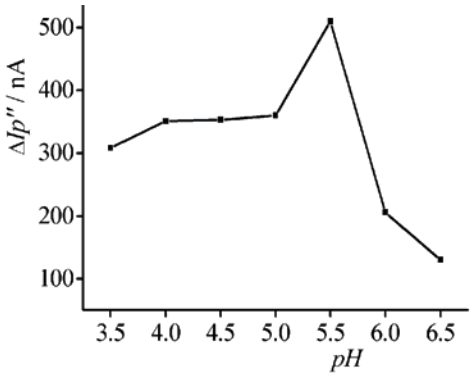

Fig. 2. The influence of pH on the binding interaction; 5.0×10^{-5} mol L^{-1} Cup + 2.5×10^{-5} mol L^{-1} Pb(II) +15.0 mg L^{-1} CS in different pH (HAc–hexamine buffer) solutions.

After mixing Cup, Pb(II) with CS, the value of $\Delta I_p''$ reached the maximum within 20 min and remained constant for about 2 h. Thus, the system gave sufficient time for routine experiments.

The influence of adding order of Cup, Pb(II), CS and buffer on the value of $\Delta I_p''$ was also studied. The order Cup, Pb(II), buffer and CS was selected as the optimal adding order, which indicated that the formation of the [Cup–Pb(II)] complex was the key for the binding reaction.

The effect of the reaction temperature on the interaction was also tested in the range of 10–40 °C. The results showed that there were no obviously differences on $\Delta I_p''$ in the selected temperature range. Therefore, the reaction temperature had little influence on the interaction and 25 °C was used throughout.

The effect of instrumental conditions, such as the scan rate and the dropping mercury standing time (the lifetime of the mercury drop) were tested. As shown in Fig. 3, the results indicated that the value of $\Delta I_p''$ increased with increasing

potential scan rate in the range of 300 to 1000 mV s^{-1} and mercury drop time. However, the mercury drop would fall down naturally when the dropping mercury standing time exceeded 24 s. Hence, the scan rate and the standing time were selected as 1000 mV s^{-1} and 22 s, respectively.

The effect of NaCl concentration on this assay was also examined and the results are shown in Fig. 4, from which it could be seen that the presence of NaCl had a significant influence on the interaction. The value of $\Delta I_p''$ decreased with increasing salt concentration in the range 0.01–0.2 mol L^{-1}, which proved that the interaction of [Cup–Pb(II)] with CS was mainly caused by electrostatic attraction. The electrostatic shielding effect of the charges on binding reaction with the increasing Na^+ concentration was detrimental to the formation of the CS–Cup–Pb(II) complex.

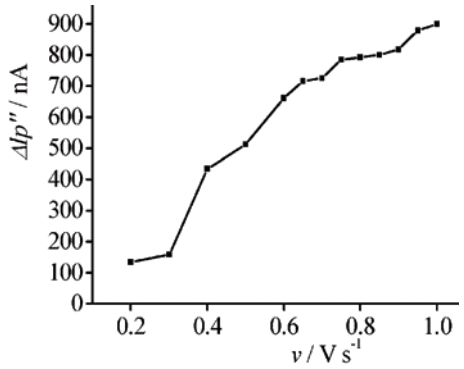

Fig. 3. The influence of scan rate on the binding interaction. 5.0×10^{-5} mol L^{-1} Cup + 2.5×10^{-5} mol L^{-1} Pb(II) + 15.0 mg L^{-1} CS in pH 5.5 HAc–hexamine buffer.

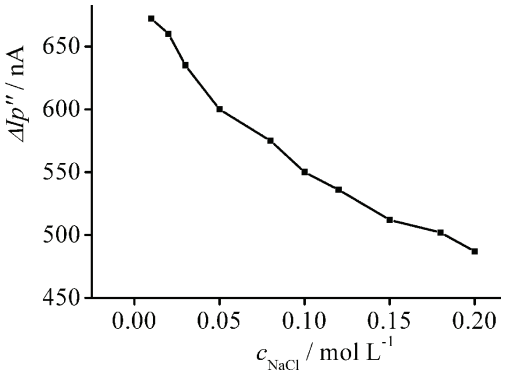

Fig. 4. The effect of NaCl concentration on the binding interaction. 5.0×10^{-5} mol L^{-1} Cup + 2.5×10^{-5} mol L^{-1} Pb(II) + 15.0 mg L^{-1} CS and different amount of NaCl in pH 5.5 HAc–hexamine buffer.

Influences of co-existing substances

The influences of co-existing substances on the determination of 15.0 mg mL^{-1} CS were also tested and the results are listed in Table I. They show that the commonly present substances such as metal ions, amino acids and glucose, had little effects on the determination.

TABLE I. Influence of co-existing substances on the determination of 15.0 mg L^{-1} CS

Co-existing substance	Concentration mg L^{-1}	Relative error, %	Co-existing substance	Concentration mg L^{-1}	Relative error, %
Ni^{2+}	2.0×10^{-6} mol L^{-1}	0.62	L-Glutamate	1.0	–1.55
Zn^{2+}	2.0×10^{-6} mol L^{-1}	–7.57	L-Glutamine	1.0	3.40
SDS	1.0	10.51	L-Valine	1.0	4.02
β-CD	1.0	–10.05	L-Arginine	1.0	1.22
RNA	1.0	–8.04	L-Cysteine	1.0	4.82
HSA	1.0	2.94	L-Leucine	1.0	0.93
DNA	1.0	4.79	Glycin	1.0	–2.78
Glucose	1.0	1.24	L-Tyrosine	1.0	4.50

Calibration curve

Under the optimal conditions, a calibration curve for CS determination, Fig. 5, was obtained in the concentration range of 1.0–25.0 mg L^{-1} with the linear regression equation: $\Delta I_p''$ / nA = 36.97(c / mg L^{-1}) + 12.45 (n = 10, γ = 0.995). The relative standard deviation (*RSD*) for eleven parallel determinations of 15.0 mg L^{-1} CS was 2.59 % and the detection limit was calculated as 0.69 mg L^{-1} (3σ).

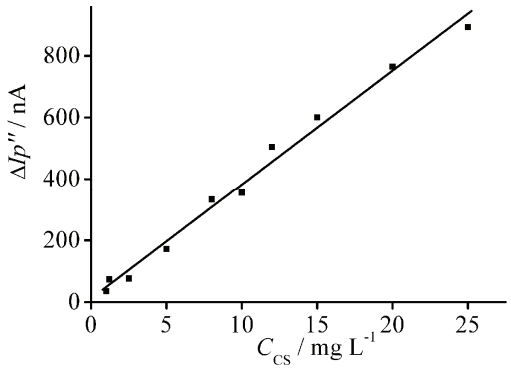

Fig. 5. Relationship between the difference in the peak current and the concentration of CS. 5.0×10^{-5} mol L^{-1} Cup + 2.5×10^{-5} mol L^{-1} Pb(II) and different amount of CS in pH 5.5 HAc–hexamine buffer.

Sample determinations

Three synthetic samples containing CS, metal ions, amino acids, *etc*. were analyzed by the proposed method with the results listed in Table II. It can be seen

that this new method was practical and reliable in the determination of CS in synthetic samples with a recovery in the range of 94.3–105.8 %.

Stoichiometry of CS–Cup–Pb (II) complex

According to a method given in the literature,[33] the binding number and the equilibrium constant of the supramolecular complex were determined. It was presumed that Cup–Pb(II) interacting with CS formed only a single complex of CS–*m*Cup–Pb(II). The binding number (*m*) and the equilibrium constant (β_s) of the binding reaction could be calculated from the following equations:

$$\text{CS}+m\text{Cup–Pb(II)} \rightarrow \text{CS}–m\text{Cup–Pb(II)} \quad (1)$$

The equilibrium constant was deduced as follows:

$$\beta_s = \frac{[\text{CS-}m\text{Cup-Pb}]}{[\text{CS}][\text{Cup-Pb}]^m} \quad (2)$$

Since:

$$\Delta I_{max} = kc_{CS} \quad (3)$$

$$\Delta I = k[\text{CS}–m\text{Cup–Pb(II)}] \quad (4)$$

$$[\text{CS}] + [\text{CS}–m\text{Cup–Pb(II)}] = c_{CS} \quad (5)$$

Therefore:

$$\Delta I_{max}–\Delta I = k(c_{CS}–[\text{CS}–m\text{Cup–Pb(II)}]) = [\text{CS}] \quad (6)$$

Combination of Eqs. (2), (4) and (6) gives:

$$\log [\Delta I / (\Delta I_{max} – \Delta I)] = \log \beta_s + m\log [\text{Cup–Pb(II)}] \quad (7)$$

where ΔI is the difference of the peak current in the presence and absence of CS and ΔI_{max} corresponds to the obtained value when the concentration of [Cup––Pb(II)] is much higher than that of CS. CCS, [CS], [CS–*m*Cup–Pb(II)] correspond to the total, free and bound concentration of CS in the solution, respectively.

TABLE II. Determination results of CS in synthetic samples (n = 5); conditions: L-glutamine, L-leucine, glucose, L-arginine and glutamic acid, 1.0 mg L^{-1}; Ni^{2+}, Mg^{2+} and Ni^{2+}, 2.0×10^{-6} mol L^{-1}

Sample	Foreign co-existing substances	Added mg L^{-1}	Found mg L^{-1}	Recovery %	*RSD* %
1	L-Glutamine, L-leucine, glucose, Mg^{2+}	10.0	9.43	94.3	3.84
2	L-Arginine, glutamic acid, Mg^{2+}, Ni^{2+}	10.0	9.68	96.8	0.51
3	L-Arginine, glutamic acid, Mg^{2+}, Ni^{2+}	20.0	21.16	105.8	1.12

The relationships between I_p'', $\Delta I_p''$ ($I_p1'' – I_p2''$) and the concentration of CS are shown in Fig. 6. The relationship of log [ΔI / ($\Delta I_{max} – \Delta I$)] with log [Cup–

–Pb(II)] is shown in Fig. 7. From the intercept and the slope $m \approx 2.5$ and β_s = = 1.89×10^{10}, respectively, were deduced. The results indicate that a 2:5 complex of 2CS–5Cup–Pb(II) was formed under the selected conditions.

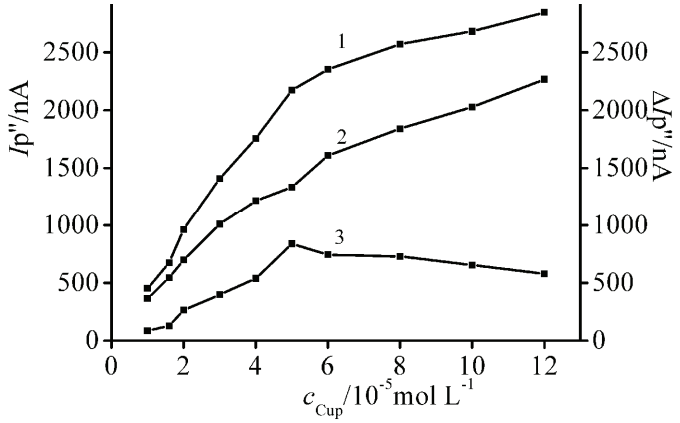

Fig. 6. Relationship between I_p'' and $c_{complex}$ (1 and 2) and $\Delta I_p''$ and $c_{complex}$ (3). 1) $c_{CS} = 0$, 2) $c_{CS} = 15$ mg L^{-1}, 3) $\Delta I_p'' = I_{p1}'' - I_{p2}''$.

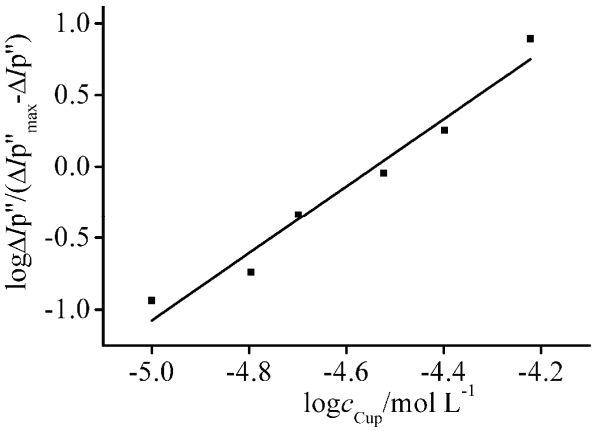

Fig. 7. A plot of log ($\Delta I/(\Delta I_{max} - \Delta I)$) against log [Pb(II)].

CONCLUSIONS

This paper described a new electroanalytical method for the determination of CS by using Cup–Pb(II) as an electrochemical probe. The interaction of Cup with Pb(II) in the solution formed a stable complex, which had a sensitive linear sweep voltammetric peak at –0.64 V *vs.* SCE. The addition of CS into the Cup––Pb(II) solution caused a decrease of the reductive peak current of Cup–Pb(II) without a change in the peak potential, indicating a new supramolecular complex

had been formed. The binding interaction of CS with [Cup–Pb(II)] could be further applied to the determination of micro-amounts of CS with satisfactory results.

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ИЗВОД

НОВА ЕЛЕКРОХЕМИЈСКА МЕТОДА ЗА ОДРЕЂИВАЊЕ ХОНДРИТИН-СУЛФАТА БАЗИРАНА НА СУПРАМОЛЕКУЛАРНОЈ ИНТЕРАКЦИЈИ СА [КУПФЕРОН– Pb(II)] КОМПЛЕКСОМ

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Испитивана је интеракција купферона (Cup) и Pb (II) комплекса [Cup–Pb(II)] са хондритин-сулфатом (CS) помоћу волтаметрије са линеарном променом потенцијала. У медијуму pH 5,5 (пуфер сирћетна киселина–хексамин), Cup реагује са Pb(II) формирајући стабилан комплекс [Cup–Pb(II)], који даје осетљив поларографски редукциони пик другог реда на –0.64 V према ЗКЕ. После додатка CS у раствор комплекса [Cup––Pb(II)], струја редукционог пика се смањује без померања и без појаве новог пика, што указује на формирање електрохемијски неактивног супрамолекуларног комплекса CS са [Cup–Pb(II)]. Услови ове реакције су детаљно испитивани. Дискутован је механизам интеракције под оптималним условима. Смањење струје редукционог пика је директно пропорционално концентрацији CS, па је постављена нова квантитативна метода за одређивање CS, са линеарном регресионом једначином $\Delta Ip''$ / nA = 36,97(c / mg L^{-1}) + + 12,45 (n = 10, γ = 0,995). Испитиван је ефекат утицаја других супстанци на одређивање и анализирана су три синтетичка узорка са задовољавајућим резултатима. Из волтаметријских података израчунати су константа формирања (β_s) и број везаних CS (m) са комплексом [Cup–Pb(II)] и износе $\beta_s = 1{,}89\times10^{10}$ и $m \approx 2{,}5$.

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