

Composition and stability constants of copper(II) complexes with succinic acid ions determined by capillary electrophoresis

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The advantage of usage of capillary electrophoresis technique was demonstrated for studying a complicated system owing to the dependence of direction and velocity of the electrophoretic movement on the charge of complex species. The stability constants of copper(II) complexes with ions of succinic acid were determined by capillary electrophoresis, including the 1:2 metal to ligand complexes which are rarely mentioned and taken into account in studies during recent years. The measurements were carried out at 25 °C and ionic strength of 0.1, obtained by mixing the solutions of succinic acid and lithium hydroxide up to pH 4.2 - 6.2. It was shown that while pH was more than 4.5 the zone of copper(II) complexes with succinate ions moves as an anion. It is impossible to treat this fact using only the complexes with a metal-ligand ratio of 1:1 (CuL^0 , CuHL^+). The following values of stability constants were obtained: $\log \beta(\text{CuL}) = 2.89 \pm 0.02$, $\log \beta(\text{CuHL}^+) = 5.4 \pm 0.5$, $\log \beta(\text{CuL}_2^{2-}) = 3.88 \pm 0.05$, $\log \beta(\text{CuHL}_2^-) = 7.2 \pm 0.3$.

Keywords: succinic acid, capillary electrophoresis, stability constant, Cu(II) complexes, charge of complex

1. Introduction

Copper is a biogenic metal that forms complexes with many ligands among which are ions of succinic acid (H_2L) [1-11]. In a number of studies, the only CuL^0 and sometimes CuHL^+ complexes are mentioned for the copper(II) - succinic acid system [12-15]. The well-known database based on the six-volume book by A.E. Martell and R.M. Smith, *NIST Critically selected stability constants of metal complexes* (Database 46 free downloaded from www.nist.gov/srd/nist46.cfm), contains only the stability constants for these complexes too. In addition, in a few recent papers [16-19], while complicated systems are studied including the ions of copper(II) and succinic acid, only these complexes are taken in consideration. At the same time, the *IUPAC Stability constants database* (Mini-SCDatabase free downloaded from <http://www.acadsoft.co.uk/scdbase/scdbase.htm>) contains the stability constant values for the 1:2 metal to ligand complexes, apart from those for the 1:1 complexes [20].

Such nature of the data can be related to methods used for the determination of composition and stability constants. In majority of the methods, the measuring properties are independent or slightly dependent on the charge of complex species. The electromigration

methods have advantage over other methods owing to the dependence of direction and velocity of the electrophoretic movement on the charge of complex species. Labile complexes produce one peak in electropherogram, the effective electrophoretic mobility μ_{eff} of which is the average weighted over mole fractions of species [21]:

$$\mu_{eff} = \sum_i^n \mu_i \cdot \alpha_i, \quad \alpha_i = \frac{[ML_i]}{C_M} = \frac{\beta_i [L]^i}{1 + \sum_i^n \beta_i [L]^i}, \quad i = 0, 1 \dots n \quad (1)$$

where μ_i is the ionic mobility of i^{th} species (for anions $\mu_i < 0$), α_i denotes the mole fraction of i^{th} species, $[ML_i]$ is the equilibrium concentration of the complex ML_i , C_M denotes the total concentration of the metal, $[L]$ means the ligand concentration into BGE, β_i means the stability constant of the complex ML_i . Similarly, the effective charge of the complex zone can be calculated as follows:

$$z_{eff} = \sum_i^n z_i \cdot \alpha_i \quad (2)$$

where z_i is the charge of i^{th} species.

The capillary electrophoresis (CE) is most widespread among the electromigration methods. CE appeared in the 1980s and was rapidly developed due to transition to the silica capillaries with very small and regular diameters (tens of micrometers) and application of the straight detection in the fluid flow [22]. The CE and related electroseparation techniques are widely used for the estimation of stability constants because of rapidity, low cost of reagents, and high performance [23-31]. To study the interactions between a metal and ligand, several electropherograms are recorded with varying ligand content in background electrolytes (BGE) and metal solution injected as a sample. On basis of the effective electrophoretic mobilities of metal complexes and ligand concentrations in BGEs, the stability (formation, association or binding) constants are calculated. Sometimes, a metal solution is added to BGE and ligand is injected as a sample.

The CE and related electroseparation techniques were used for the determination of the stability constants of the copper(II) complexes with proteins [27, 32-33], nitrilotriacetate and cysteine [34], α -hydroxyisobutyric acid and sulfate ions [21]; constants of Cu(II) - alizarin complexone with amines [35], etc. The copper(II) complexes are also employed in CE for the metal determination [24] and in ligand-exchange CE for the separation of different organic analytes [36-40], including enantiomers [37]. But the CE technique has not been used early for studying the copper(II) complexes with succinic acid ions. The purpose of this study was to explore the copper(II)-succinic acid system and determine the stability constant values using CE.

2. Experimental

2.1. Instrumentation

The study was carried out using a capillary electrophoresis system with a diode-array detector Agilent ^{3D}CE G1600A (Agilent Technologies, Waldbronn, Germany) of the Krasnoyarsk Regional Center of Research Equipment, Siberian Branch of the Russian Academy of Sciences. An untreated fused silica capillary with 50 μ m id and the total and effective lengths of 48.5 and 40 cm, respectively, was used (Agilent Technologies). The capillary temperature was kept constant at 25.00 ± 0.04 °C. The data acquisition and processing were performed with the computer program ChemStation Rev.A.10.02. The separation was achieved by applying a voltage of +9-10 kV so that for each BGE the product of voltage and current was kept constant (Table 1). The detection wavelengths were 254 and 191 nm. The hydrodynamic pressure of 50 mbar was used to inject samples for 2 s and to reduce migration time during the separation [41-44].

A new capillary was first flushed with 1 M NaOH for 10 min, then with ultra pure water for 10 min. At the beginning of each day, the capillary was first flushed with 0.1 M NaOH for 5 min, twice with ultra pure water for 10 min and with running BGE for 15 min. Between the runs the capillary was flushed with BGE for 5 min.

All the pH measurements were made using a calibrated precise pH instrument «Expert-001-1» (Econix-Expert, Moscow, Russia) with a precision of 0.005 pH units.

2.2. Chemicals

Succinic acid ($\geq 99.5\%$), copper(II) chloride ($\geq 99.0\%$), lithium hydroxide monohydrate (99.95%), and dimethyl sulfoxide (DMSO) ($\geq 99.5\%$) were purchased from Sigma-Aldrich (Moscow, Russia). Deionized water with electrical conductivity less than $0.1 \cdot 10^{-6} \text{ S} \cdot \text{cm}^{-1}$ from a water purification system Direct-Q3 (Millipore, France) was used for solution preparation. DMSO was used as a marker of electroosmotic flow (EOF). A stock solution of copper(II) chloride was prepared with a concentration of 100 mM, a stock solution of DMSO was prepared with a concentration of 10% (v/v). Samples consisted of 1 mM copper(II) chloride and 0.05% (v/v) DMSO were daily prepared by diluting the above stock solutions with BGEs.

2.3. Measurements and data processing

The measurements were carried out using BGEs on basis of succinic acid (H_2L) with the addition of lithium hydroxide up to pH 4.2 - 6.2. For this pH range, succinic acid can be used as a single buffering constituent with acceptable buffer capacity because $\text{pK}_{\text{a}1}$, $\text{pK}_{\text{a}2}$ at $I = 0.1$ and $25\text{ }^\circ\text{C}$ are 3.99 ± 0.02 and 5.24 ± 0.04 from NIST and 3.99 and 5.20 from IUPAC databases (in this study, the data from IUPAC databases were used). The ligand concentration was varied by changing the pH values of BGEs. In order to fix the values of stability constants, the ionic strength was maintained at the constant value of 0.100 (Table 1). For BGE, the ionic strength was calculated by using equation 3 (concentration of OH^- ions was negligible in the studied pH range):

$$I = \frac{1}{2} \sum_i z_i^2 C_i = \frac{1}{2} ([\text{H}^+] + [\text{Li}^+] + [\text{HL}^-] + 4[\text{L}^{2-}]) \quad (3)$$

The electroneutrality equation was expressed as follows:

$$[\text{H}^+] + [\text{Li}^+] = [\text{HL}^-] + 2[\text{L}^{2-}] \quad (4)$$

Combination of equations 3 and 4 gave $I = [\text{HL}^-] + 3[\text{L}^{2-}]$. Taking into account

$$[\text{HL}^-] = C_L \cdot \alpha_{\text{HL}^-} \quad \text{and} \quad [\text{L}^{2-}] = C_L \cdot \alpha_{\text{L}^{2-}} \quad (5)$$

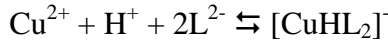
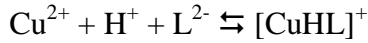
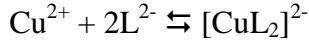
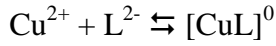
where C_L is the total concentration of succinic acid in BGE, the ionic strength was calculated as follows:

$$I = C_L \cdot \frac{[H^+]K_{a1} + 3K_{a1}K_{a2}}{K_{a1}K_{a2} + [H^+]K_{a1} + [H^+]^2} \quad (6)$$

where K_{a1} , K_{a2} are the acidity constants of succinic acid of 1st and 2nd steps, respectively, $[H^+] = 10^{-pH} / \gamma_{H^+}$. The activity coefficients γ_{H^+} were calculated using the extended Debye–Hückel expression [45] (equation 29) with the effective diameter of the hydrated H^+ (H_3O^+) of 9 Å. Thus, the total concentration of succinic acid in BGE C_L was calculated as follows (Table 1):

$$C_L = I \cdot \frac{K_{a1}K_{a2} + [H^+]K_{a1} + [H^+]^2}{[H^+]K_{a1} + 3K_{a1}K_{a2}} \quad (7)$$

For the system under study, the following equilibria were considered:



$$\beta_{CuH_xL_y} = \frac{[CuH_xL_y]}{[Cu^{2+}][H^+]^x[L^{2-}]^y}, \quad x = 0 \text{ or } 1, y = 1 \text{ or } 2 \quad (8)$$

The mole fractions of the hydroxo complexes $Cu(OH)_i$ under the studied conditions were negligible. The stability constants of hydroxo complexes $Cu(OH)_i$ were taken from *IUPAC Stability constants database* at 25 °C and $I = 0$: $\log \beta_1 = 6.3$, $\log \beta_2 = 10.7$, $\log \beta_3 = 14.2$, $\log \beta_4 = 16.4$. The values were recalculated by means of the extended Debye–Hückel expression for the ionic strength of 0.1 [45]. Taking into account that the ionic mobility of $[CuL]^0$ equaled to zero, equation 1 for the studying system was expressed as follows:

$$\mu_{eff} = \frac{\mu_{Cu^{2+}} + \mu_{CuL_2^{2-}} \beta_{CuL_2^{2-}} [L^{2-}]^2 + \mu_{CuHL^+} \beta_{CuHL^+} [H^+][L^{2-}] + \mu_{CuHL_2^-} \beta_{CuHL_2^-} [H^+][L^{2-}]^2}{1 + \beta_{CuL^0} [L^{2-}] + \beta_{CuL_2^{2-}} [L^{2-}]^2 + \beta_{CuHL^+} [H^+][L^{2-}] + \beta_{CuHL_2^-} [H^+][L^{2-}]^2} \quad (9)$$

$$[L^{2-}] = C_L \cdot \alpha_{L^{2-}} = C_L \cdot \frac{K_{a1}K_{a2}}{K_{a1}K_{a2} + [H^+]K_{a1} + [H^+]^2} \quad (10)$$

The effective electrophoretic mobility from experimental data was calculated as follows:

$$\mu_{eff} = \frac{l \cdot l_{eff}}{U} \left(\frac{1}{t} - \frac{1}{t_{eof}} \right) \quad (11)$$

where l and l_{eff} are the total and effective capillary lengths, respectively, U is the voltage, t is the migration time, t_{eof} is the migration time of the EOF marker.

The stability constants and confidence intervals were determined from equation 9 using non-linear regression fitting of the program MS Excel as follows. For each BGE composition, the formulas to calculate the mole fractions of all species and theoretical values of effective electrophoretic mobilities were typed in Excel cells. The formulas contained references to the cells with the values of the stability constants. Thus, changing the stability constants resulted in the change of the mole fractions and electrophoretic mobilities. Then, the formulas for calculation of differences in the theoretical and experimentally measured values of the effective electrophoretic mobilities were typed and the formula for the sum of squares of the differences was composed. Thereafter, the values of the constants were fitted by a method of successive approximations using Excel Solver Add-in so that the sum of squares was as small as possible.

The ionic mobilities of the species were calculated using the theory of Onsager and Fuoss (OF) for the mixtures of electrolytes [46] by the computational tool set ElphoSeparation [47] (Table 2):

$$\mu_i = \left(\left| \mu_i^0 \right| - \left(B_1 z_i \left| \mu_i^0 \right| \sum_{n=0}^5 C_n R_i^{(n)} + B_2 |z_i| \right) \frac{\sqrt{\Gamma}}{1 + \frac{Ba}{\sqrt{2}} \sqrt{\Gamma}} \right) \cdot \text{sgn}(\mu_i) \quad (12)$$

$$B_1 = \frac{e^3}{12\pi} \sqrt{\frac{N_A}{(\varepsilon kT)^3}}, \quad B_2 = \frac{e^2}{6\pi\eta} \sqrt{\frac{N_A}{\varepsilon kT}}, \quad \Gamma = \sum_{i=1}^s c_i z_i^2 = 2I$$

μ_i^0 is the ionic mobility of the i -th ion at $I = 0$, $R_i^{(n)}$ is the n -th component of the vector R_i , C_n is the n -th member of a series [46], $Ba / \sqrt{2} = 1.5$, e is the elementary charge, N_A is the Avogadro constant, ε is the permittivity of the solution (for diluted solution, ε equals to the product of the dielectric constant of water 78.36 at 25 °C and permittivity of a vacuum $8.854 \cdot 10^{-12}$

¹² F·m⁻¹[48]), k is the Boltzmann constant, T is the absolute temperature, η is the solution viscosity, and s is the overall number of all the ionic species in the solution. Because the mobility is in proportion to the charge/radius ratio [49] and radiuses of hydrated ions have close values, the assumption was used that μ° of the singly charged ions equaled to $\mu^\circ(\text{CuOH}^+)$ and μ° of the double-charged ions equaled to $\mu^\circ(\text{Cu}^{2+})$, assuming the possible inaccuracy of the evaluation through $\mu_i \pm 2 \cdot 10^{-9} \text{ m}^2 \text{ V}^{-1} \text{ s}^{-1}$ [47].

The values of the stability constant of CuHL^+ defining according to equation 8 were calculated from the values of the NIST database and articles [13, 14] as follows:

$$\beta_{\text{CuHL}^+} = \frac{[\text{CuHL}^+]}{[\text{Cu}^{2+}][\text{H}^+][\text{L}^{2-}]} = \frac{[\text{CuHL}^+]}{[\text{Cu}^{2+}][\text{HL}^-]} \frac{1}{K_{a2}} = \frac{K_{\text{CuHL}^+}^{\text{NIST}}}{K_{a2}} \quad (13)$$

$$\log \beta_{\text{CuHL}^+} = \log K_{\text{CuHL}^+}^{\text{NIST}} + pK_{a2} \quad (14)$$

where $K_{\text{CuHL}^+}^{\text{NIST}}$ is the equilibrium constant for the following reaction from NIST database or articles [13, 14]: $\text{Cu}^{2+} + \text{HL}^- \rightleftharpoons [\text{CuHL}]^+$.

3. Results and discussion

The electropherograms of 1 mM copper(II) solution with the addition of 0.05 % DMSO (as a neutral substances, EOF marker) were recorded using the succinic buffers as BGEs with the pH range of 4.2-6.2 at 25 °C and ionic strength of 0.1 (Figure 1). The measurements were carried out at positive voltage polarity using two detection wavelengths, 254 and 191 nm. These wavelengths corresponded to the maxima of the UV spectra of copper(II) solution and DMSO recorded by the diode-array detector of the used CE system. Two wavelengths were used because DMSO did not give a peak in the electropherogram at 254 nm and the peak of copper(II) complexes was low-intensity at 191 nm.

For the positive voltage polarity, in electropherograms cations are first recorded, then the EOF marker is recorded and thereafter anions appear. As shown in Figures 1 and 2, the zone of copper complexes was cationic if pH was less than 4.5, and the zone was anionic if pH was greater than 4.5. The appearance of the anionic complexes cannot be explained in the network of

the existence of only the CuL^0 , CuHL^+ species. On basis of equation 9 using non-linear regression fitting, the stability constants were calculated (Table 3). Figure 2 shows the experimental points and theoretical curve of the effective mobility versus BGE pH. Figure 3 shows the fraction diagrams for the system of copper(II) - succinic acid as a function of pH.

In Table 3, the stability constants available in literature for the studied system are also shown. The constants were substantially determined by potentiometric titration, but calorimetric titration and polarography were used too. As can be seen in Table 3, the stability constant for $[\text{CuL}]^0$ obtained by CE is in the range of scattering the literature data, but for the other complexes the obtained values are distinguished from literature data. It is not surprising, in principle, that the data obtained on basis of considering two equilibria are distinguished from the data obtained on basis of considering four equilibria because ignoring a number of equilibria would distort the calculated values of stability constants. But this is far from it for the constants from UIPAC mini-SCDatabase (for $[\text{CuL}]^0$ and $[\text{CuHL}]^+$ species), they accurately agree with the data obtained on basis of two equilibria. It is probable that the authors of paper [20] during the calculation used the literature data of these complexes as the basis that is not quite correct because of the above-mentioned. The results obtained by CE seem to be more reliable because in CE the measured property is not only dependent on mole fractions of complex species, but, in contrast to potentiometric titration and other methods, on the charge of complex species too.

4. Conclusion

The electrophoretic mobilities of copper(II) complexes with succinate acid ions were measured at 25 °C and ionic strength of 0.1 within the pH range of 4.2 - 6.2. The zone of copper(II) complexes has been shown to move as a cation or an anion subject to the experimental conditions. These experiments allow one to accurately calculate the stability constants of copper(II) complexes using the set of electrophoretic mobilities, including constants for the 1:2 metal to ligand complexes for which the literature data are contradictory and which are not often taken into consideration in a number of recent studies.

Disclosure statement

No potential conflict of interest was reported by the authors.

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Tables

Table 1. BGE concentration ($I = 0.100 \text{ M}$), equilibrium ligand concentration $[\text{L}^{2-}]$, voltage U , current, and product of voltage and current ($190 \pm 5 \text{ kV} \cdot \mu\text{A}$).

pH	C_L (mM)	$[\text{L}^{2-}]$ (mM)	U (kV)	current (μA)	$U \cdot \text{current}$ (V·A)
4.200	146.5	6.62	9.2	20.5	189
4.403	114.6	9.44	9.2	20.2	186
4.700	83.9	14.6	9.4	20.2	190
4.900	69.7	18.5	9.6	20.2	194
5.200	54.6	23.7	9.7	19.6	190
5.397	47.9	26.5	9.8	19.4	190
5.600	43.0	28.7	10.0	19.5	194
6.005	37.4	31.4	10.0	19.1	190
6.200	35.9	32.0	10.0	19.0	190

Table 2. Calculated values of the ionic mobilities μ_i ($10^{-9} \text{ m}^2\text{V}^{-1}\text{s}^{-1}$) for copper(II) ions and complexes with ions of succinic acid for the studied conditions ($25 \text{ }^\circ\text{C}$, $I=0.1$).

pH	Cu^{2+}	$[\text{CuL}_2]^{2-}$	$[\text{CuHL}]^+$	$[\text{CuHL}_2]^-$
4.2	33.8	-35.3	24.4	-24.8
4.403	33.3	-35.7	24.3	-24.9
4.7	32.4	-36.4	24.0	-25.1
4.9	31.8	-36.9	23.9	-25.2
5.2	30.9	-37.7	23.6	-25.4
5.397	30.5	-38.0	23.5	-25.6
5.6	30.1	-38.3	23.4	-25.6
6.005	29.7	-38.7	23.3	-25.7
6.2	29.5	-38.8	23.3	-25.8

Table 3. Values of $\log \beta$ for the copper(II) complexes with succinic acid ions ($25 \text{ }^\circ\text{C}$, $I=0.1$).

PT - potentiometric titration, CT - calorimetric titration, P - polarography.

$[\text{CuL}]^0$	$[\text{CuHL}]^+$	$[\text{CuL}_2]^{2-}$	$[\text{CuHL}_2]^-$	Method, reference
2.89 ± 0.02	5.4 ± 0.5	3.88 ± 0.05	7.2 ± 0.3	CE (this study)
2.7 ± 0.1	7.04 ± 0.36	-	-	NIST Database
2.59	7.03	4.30	9.59	Mini-SCDatabase (IUPAC), PT [20]
2.5 ± 0.1	-	-	-	CT [14]
2.6	-	-	-	PT [15]
2.608 ± 0.006	7.01 ± 0.3	-	-	PT [14]
2.85	7.08	-	-	cited in [14]
2.98 ± 0.02	7.04 ± 0.4	-	-	PT [13]
3.20	-	-	-	cited in [13]
3.22 ± 0.02	-	a	-	PT [12]
4.00	-	6.57	-	P, cited in [20]

^a Could not be determined because of precipitation

Figure captions

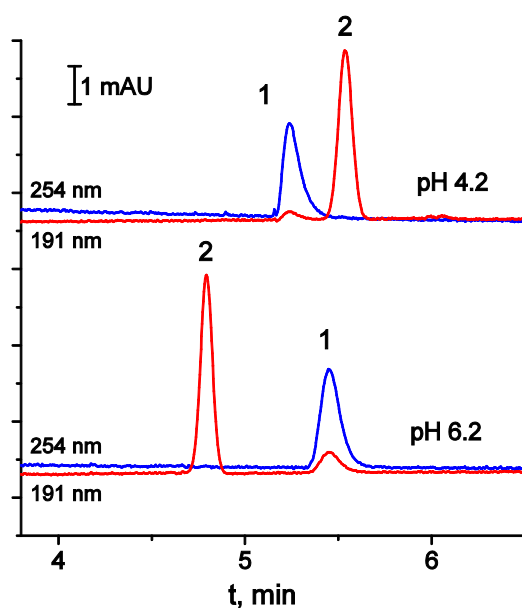


Figure 1. Electropherograms of 1 mM copper(II) solution (1) with 0.05 % DMSO (2) as an EOF marker for BGEs with pH 4.2 and 6.2 (recording at 254 and 191 nm).

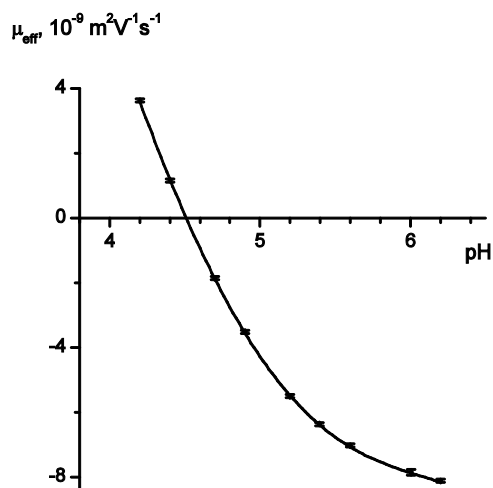


Figure 2. Experimental points and theoretical dependence of the effective mobility of copper(II) on BGE pH ($I = 0.1$). The values used for the calculation of the theoretical curve were as follows: $pK_{a1}(H_2L) = 3.99$, $pK_{a2}(H_2L) = 5.20$; $\log \beta(CuL) = 2.89 \pm 0.02$, $\log \beta(CuHL^+) = 5.4 \pm 0.5$, $\log \beta(CuL_2^{2-}) = 3.88 \pm 0.05$, $\log \beta(CuHL_2^-) = 7.2 \pm 0.3$.

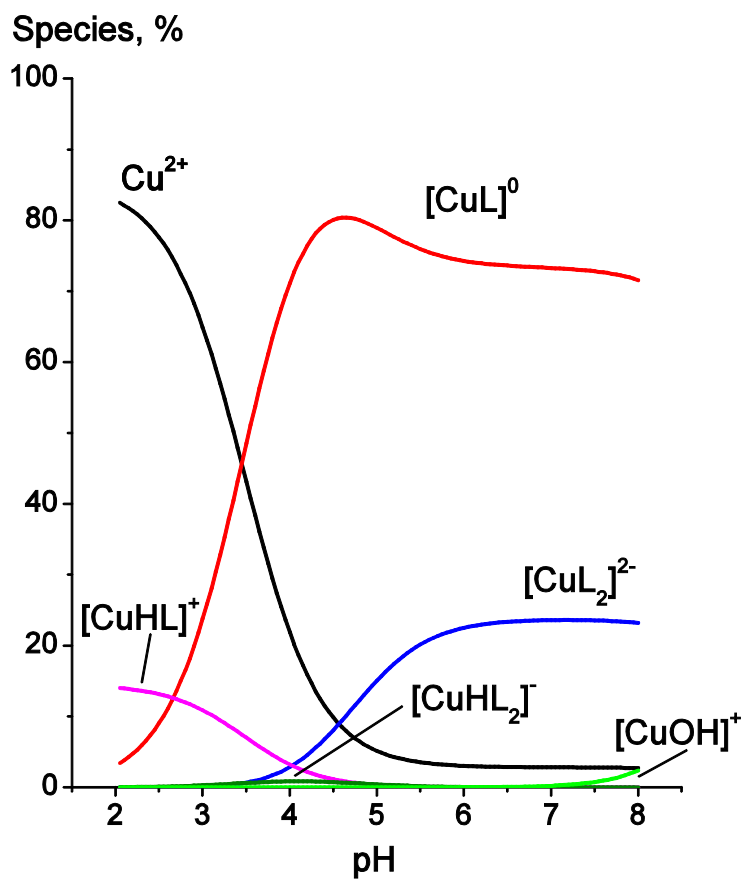


Figure 3. Fraction diagrams for the system of copper(II) - succinic acid as a pH function (I = 0.1).