Studying the copper(II)–ammonia complex formation equilibria in aqueous solutions

Theoretical background: P.W. Atkins: *Physical Chemistry*.
Type of practice: Individual.
Goal of practice: Determination of complex formation constants and distribution curves in a solution equilibrium system, in which the different species form simultaneously, that is complex formation processes overlap.

1 Introduction

Studies on solution equilibria aim to determine the stoichiometric composition of the forming species and their formation constants. The concentration of the different species can be calculated based on the total (analytical) concentrations and the formation constants. These formation constants can only seldom be found in the scientific literature. Furthermore, even if a parameter is available in the scientific literature, its value might not be precise enough for the current standards. Therefore it is necessary to develop and implement methods for the high precision determination of these formation constants. Knowing the formation constants is crucial in several fields, such as for understanding the effect of transition metal complexes in living organisms, in analytical methods or in liquid phase chemical reactions.

1.1 Fundamental equations

The mathematical description of a general complex equilibrium is very complicated, therefore the important equations will be derived here for a simple example. Although charged species might form in the process, the charges will be omitted from the equations for clarity. The following equilibrium processes must be considered in a system containing M metal ion, L ligand, H proton, HL, H_2L and ML complex species (associates):

$$H + L \overset{K_1^H}{\rightleftharpoons} HL, \qquad HL + H \overset{K_2^H}{\rightleftharpoons} H_2L, \qquad M + L \overset{K^M}{\rightleftharpoons} ML.$$

The stepwise formation constants in these processes are:

$$K_1^H = \frac{[HL]}{[H][L]}, \qquad K_2^H = \frac{[H_2L]}{[H][HL]}, \qquad K^M = \frac{[ML]}{[M][L]}.$$

Instead of the stepwise formation constants, the processes can also be described using the cumulative formation constants:[1]

$$\beta_1^H = K_1^H = \frac{[HL]}{[H][L]}, \qquad \beta_2^H = K_1^H \cdot K_2^H = \frac{[H_2L]}{[H]^2[L]}, \qquad \beta^M = K^M = \frac{[ML]}{[M][L]}.$$

Some general definitions must be discussed before we go into more detailed discussion of the complex equilibria systems. The basic building-blocks of all the associates are the components, these are the species from which the complex species can be built up. In an associate, the number of a given component is given by its stoichiometric number. These stoichiometric numbers are collected in the composition matrix that describes how the complex species are built up in the system. Each column of the matrix belongs to a component, and each row belongs to a species, therefore each component and associate have their own row. That is, the element in the i^{th} row and j^{th} column shows how many of the j^{th} component is necessary to build up the i^{th} species. The composition matrix of the above exemplary system is shown in Table 1.

[1] Note, that both the stepwise and the cumulative formation constant can be referred to as stability constant in general according to the IUPAC nomenclature.

The total concentration of each component is given by the sum of the equilibrium concentration of the components and associates weighted by their corresponding stoichiometric numbers. According to the principle of mass conservation, the total concentrations in the exemplary system can be described as:

Table 1: Composition matrix of the exemplary solution equilibrium system.

	H	M	L
H	1	0	0
M	0	1	0
L	0	0	1
HL	1	0	1
H_2L	2	0	1
ML	0	1	1

$$
\begin{aligned}
T_H &= [H] - [OH] + [HL] + 2[H_2L] = [H] - \frac{K_w}{[H]} + \beta_1^H[H][L] + 2\beta_2^H[H]^2[L] \\
T_L &= [L] + [HL] + [H_2L] + [ML] = [L] + \beta_1^H[H][L] + \beta_2^H[H]^2[L] + \beta^M[M][L] \\
T_M &= [M] + [ML] = [M] + \beta^M[M][L],
\end{aligned}
$$

where T_H and $[H]$ is the total and equilibrium concentration of hydrogen ion, respectively. Similarly, T_L and $[L]$ are the total and equilibrium concentrations of the ligand, and T_M and $[M]$ are the analogous parameters for the metal ion. T_{K_w} is the ionic product of water.

The total concentrations, T_H, T_M and T_L, are known from the solution preparation process (i.e., how much of the given chemical is weighted in the solutions). The equilibrium concentrations and cumulative formation constants are therefore the unknowns in the equations. The equilibrium concentrations ($[H]$, $[L]$, $[M]$) could be calculated easily from the total concentrations and the formation constants, if these latter are available.

1.2 Experimental methods for studying solution equilibria

The most frequently applied methods for determining the formation constant are *p*H-metric titration and spectrophotometry. Both methods require meticulous and precise experimental work. In the followings the application of these two methods for solution equilibria will be explained, together with the limitation of these techniques.

1.2.1 *p*H-metric titration

Equilibrium processes in water are typically affected (either directly or indirectly) by the *p*H change. In case of direct effect, protons or hydroxide ions participate in the equilibrium process. Typical examples are the protonation/deprotonation of basic ligands, or the hydrolysis of the metal ions. In case of the indirect effect H^+- (or OH^-) ions are not involved in the association process, but at least one of the components participates in a protonation equilibrium process as well.

Most of the typical ligands are compounds of which conjugate acid is a weak acid (e.g., carboxylates, amines, amino acids, peptides), therefore there is always a competition between the protonation of the ligand and the complex formation with the metal ion. Here, we show a derivation for the minimum number of different solutions to be measured in order to be able to quantify the formation constants. Take a system, where n components are present, a p different complex species might form. The total concentration of each component can be described with and equation, that means n equations. As we measure *p*H, the concentration of H^+, as one of the components, is known in every solution. This leaves us with $n-1$ unknown concentrations and p unknown formation constants in every equation. If we measure pH in q different solutions, we get $q \cdot n$ equations (as the total concentrations are different in every solutions) As *p*H is measured in q solutions, the number of unknowns is $p + q \cdot n - q$. This means that at least this many equation is necessary for the quantification of all unknowns: $q \cdot n \geq p + q \cdot n - q$, which can be simplified to $q \geq p$, hence the minimum number of the studied solutions must be equal or more than the number of the different associates in the system.

1.2.2 Spectrophotometry

The Beer-Lambert law gives the relation between the absorbance and the concentration of colored species. The absorbance is additive within the validity region of the Beer-Lambert law, hence if there are more than one species present that absorbs at the same wavelength (λ_i), the total absorbance will be the sum of the individual absorbances,

$$\frac{A_{\lambda_i}}{\ell} = (\varepsilon^{M}_{\lambda_i}[M] + \varepsilon^{ML}_{\lambda_i}\beta^{M}_{1}[M][L]) \ ,$$

where A is the absorbance, ε is the molar absorption coefficient and ℓ is the length of the light path in the cuvette (1 cm in most cases). Keeping the notations introduced regarding *p*H-metric titrations, and performing absorbance measurements at m different wavelengths gives us $n+m$ equations for each solutions: n equation for the total concentrations, and m Beer-Lambert equation for each studied wavelength. The number of unknowns in this case is p formation constant and $m \cdot (n+p)$ molar absorption coefficients and the equilibrium concentrations of n components. Note, that the number of unknowns could be lower if not all species present absorb light at all studied wavelengths. Having q different solutions gives us a maximum of $p+m \cdot (n+p)+n \cdot q$ unknowns, as the number of molar absorption coefficients and formation constants is not increasing with the number of the studied solutions. The number of equations must be at least the number of unknowns, hence: $q \cdot (n+m) \geq p+m \cdot (n+p)+n \cdot q$, that can be simplified to $q \geq n+p \cdot (1+1/m)$. Here the number of solutions and wavelengths both affect the number of necessary equations.

The equation system becomes overdetermined if more solutions or wavelengths are studied than the minimum. This allows to account for the experimental errors, therefore it is recommended to gather significantly more (even orders of magnitudes more) experimental data than what is absolutely necessary. Experimental errors might occur for example during solution preparations, due to the drift in the baseline during the absorbance measurements in case of very low absorbances.

1.3 How to plan the composition of the studied solutions?

Here we shortly describe the most commonly applied experimental methods to study solution equilibrium processes. One of these methods will be used during the Experiment.

1.3.1 Job's method

In the studied solutions the sum of the total concentration of the metal ion and the ligand is kept constant, while the mole fraction of the ligand is varied from 0 to 1. Some intensive property of the solution is measured, and this is plotted as function of the mole fraction of the ligand. In case of a simple system this results in a maximum type curve. From the mole fraction at the maximum (x_{max}) we can determine the composition of the complex, as:

$$mM + nL = M_mL_n, \quad \frac{n}{m} = \frac{x_{max}}{1-x_{max}} \ .$$

This simple method can only be applied if the system is not too complicated, only one (or two) different complexes are formed. This method is widely used, as it can be realized with small volumes, but the complex formation is still studied on a wide range of mole fractions. Note, that these measurements can be analyzed for complex systems (with several different complexes forming) using modern computational methods.

1.3.2 Mole fraction method

Here the total concentration of one component is kept constant, and an intensive parameter of the system (e.g., light absorption) is investigated as function of the mole fraction of the other component. In case of a stable complex formation a clear breakpoint can be seen on the curve. The evaluation of this method is similar to Job's method, but the experiments are more easy to plan and implement. However, that changes measured by this method are smaller than in case of Job's method.

1.3.3 Competition method with constant metal ion and ligand concentration

The measurements can be performed at constant ligand and metal ion concentration, by dosing acid or base solution into the studied solution. As most of the ligands are weak (or mildly strong) bases, the formation of a complex is always a competition between the metal ion and the proton for the ligand. In such systems the free ligand concentration is determined by its protonation, that can be varied in a very wide range (even 10 orders of magnitude). This method provides the most information on the system, but at the same time, this is the technically most challenging. The measurements can be performed by making individual solutions, or by a continuous titration. This latter means that the analyte contains metal ions, ligand and acid too. The *p*H of this solution is gradually increased by dosing an alkaline solution. In case of individual solutions, we create solutions in that the metal and ligand concentrations are the same, but the *p*H is different. We record the *p*H or the absorbance spectra of these solutions.

1.3.4 Planning of the experiments and solution preparation

When choosing the experimental methods we aim to gather as detailed information as possible on all the different species present in the solution. The mole fraction and Job's methods are performed at constant *p*H, hence these provide limited information on the system. These rapid and hence widely applied methods are suitable in simple systems, where only one complex forms, that can be studied at a single *p*H and/or at a single wavelength. In this case a so-called effective formation constant can be determined, which is valid only for the defined experimental conditions. When planning the solution compositions the only important aspect is to be able to characterize the forming complex(es), but all other considerations depend on the studied system and the chosen method and instrumentation. Some specific considerations could be:

- For spectrophotometric detection, the wavelength and the concentrations should be chosen so that the maximum absorbance is below 1.3.
- Conditions must be such that the complexes are formed to the highest possible extent. In some cases this requires to apply excess amount (sometimes several orders of magnitudes higher than what is dictated by stoichiometry) of the ligand.
- During competition measurements, the initial acid concentration in the analyte should be enough to fully protonate the ligand, hence the complexation of the metal ion is negligible. If necessary, strong acid must be added to the solution.

2 Spectrophotometric study of the Cu(II)-tetramine complex formation

Before starting the experiments, the Instructor should define the following parameters:

- the temperature of the measurements,
- the wavelength range of the measurements,
- the Cu(II) concentration in the stock solutions (R), in the a 0.006–0.013 M range,
- the ionic strength of the solutions (I) in the range of 0.6–1.0 M,
- the deprotonation level (P) of ammonium ions between 18–42 %.

2.1 Stock solutions

The volume of the solutions should be planned by the students, based on the available equipment and the volume needs of the experiments.

A: $\sim$0.5 M HNO_3-solution. The concentration of this solution should be determined by titration with bicarbonate solutions and methyl red indicator. *B*e careful when diluting concentrated acid solution, and pay attention to the general rules of this method! Use colorless concentrated nitric acid!

B: $\sim$0.5 M KOH solution ($\sim$1.0 M, if $P > 30\,\%$). The concentration of this solution must be determined by titration using the **A** solution and methyl red indicator. Special attention should be paid to avoid carbonation due to the reaction between KOH and carbon dioxide! Weigh at least twice the amount of KOH in a beaker than what is calculated for the solution, and rinse it with a few droplets of water to remove to carbonated surface layers. Repeat this 5–6 times, until roughly half of the material is dissolved (and removed). Dissolve the remaining material, and prepare solution **B** in a volumetric flask. If available, use previously boiled (to remove carbon dioxide) and cooled deionized water to prepare the solution.

C: R M $CuSO_4$, 0.05 M HNO_3 and $(I - 4 \cdot R - 0.05)$ M NH_4NO_3.

D: R M $CuSO_4$ and $(I - 4 \cdot R)$ M NH_4NO_3.

E: R M $CuSO_4$, $(I - 4 \cdot R)$ M NH_4NO_3, and precisely the amount of **B** solution to achieve the deprotonation of $P\,\%$ of the ammonium ions.

Table 2: Text file containing the measurement conditions and the source files of the measurements.

1:	Johnny Precise	
2:	1997.01.18.	
3:	40.0	
4:	0.008	
5:	0.8	
6:	25.0	
7:	51	
8:	400	
9:	410	
⋮	⋮	
58:	900	
59:	p002.csv	0.0
60:	p003.csv	0.5
⋮	⋮	⋮
81:	p019.csv	25.0
82:	p020.csv	10000.0
83:	p001.csv	

2.2 Execution of the experiment

1. Prepare the **A**–**E** solutions. *Note that the volumes should be planned considering the needs for rinsing the equipment and performing the titration! The calculations must be checked and approved by the Instructor before preparing the solutions. The solutions must be prepared with high precision to maintain constant ionic strength!*
2. Record the spectra of the **C** and **D** solutions and the following solution compositions: 40 cm^3 D + 0.5; 1.0; 1.5; 2.0; 2.5; 3.2; 4.0; 4.8; 5.6; 6.5; 7.5; 8.7; 10.0; 12.0; 14.0; 17.0; 25.0 cm^3 **E** solution. To form these compositions, titrate 40 cm^3 of solution **D** with solution **E** from a 25 cm^3 burette, and record the spectrum of the solution at the given titration points in the 400–900 nm wavelength range, with 10 nm resolution. The measured sample portion must be poured back in the solution without any loss. Hence only 40 cm^3 D solution, and 25 cm^3 E solution is needed for preparing all the samples.
3. Record the spectrum of solution **E**

2.3 Analysis of the experimental results

1. The recorded spectra should be exported in separate ASCII files with two columns. The first column contains the wavelength, and the second the absorbances. The values should be space or tab separated.
2. Create a file that contains the measurement conditions and the name of the data files containing the spectra. The file structure is shown in Table 2.:

- The first 6 rows contain the name of the student, the date of the measurement, the volume of solution **D** that was titrated to form the samples, and the value of *R*, *I* és *P*, accordingly.
- The 7th row shows how many wavelengths should be used in the data fitting. Rows nr. 8–58 contain the selected wavelengths.
- Rows nr. 59–81 contain the files in which the spectra are stored, one row for each file name. After the file name the volume of solution **E** added to solution **D** during the titration is indicated.
- The 82nd row contains the name of the file containing the spectrum of solution **E**. 10000.0 must be written after the file name!
- The 83rd row contains the name of the file containing the spectrum of solution **C**.

Based on the provided example this data file should be created by the student performing the experiment,

3. Ask the help of the Instructor to further evaluate the results using the PSEQUAD software.
4. Create two figures based on the stability constants (formation constants) and the other results of the fitting:
   - One showing the molar absorption coefficient spectra of Cu(II) ion and the different C(II)-amine complexes in one figure,
   - The measured and the calculated distribution diagram of Cu(II) ion and the different Cu(II)-amine complexes as function of the amine concentration (α_i vs. -lg[NH_3]), where α_i is defined as (*n* is the maximum coordination number gathered from the evaluation of the spectra with the PSEQUAD software, and i = 0, 1,...*n*):

$$\alpha_i = \frac{[Cu(NH_3)_i]^{2+}}{T_{Cu^{2+}}} = \frac{\beta_i[Cu^{2+}][NH_3]^i}{[Cu^{2+}] + \sum_{j=1}^{n} \beta_j[Cu^{2+}][NH_3]^j} = \frac{\beta_i[NH_3]^i}{1 + \sum_{j=1}^{n} \beta_j[NH_3]^j}.$$

     This figure should be prepared in the $[NH_3] = 1.0 \cdot 10^{-7} - 1.0\,M$ concentration range, at 50–100 different concentrations (equidistant points should be chosen on the logarithmic axis). Also indicate the measurement results at the studied lg[NH_3] values on the same figure .

5. Provide a detailed evaluation and discussion of the measurements, the calculations and the figures.

Control question

Week 1.: Preparations for the measurements

1. What is the equilibrium, analytical and total concentration of a given compound? What is the difference between these measures?
2. What is a component and a compound?
3. What is the stepwise and the cumulative formation constant?
4. What is the composition matrix?
5. Give the composition matrix for a phosphoric acid solution!
6. What is the ionic strength?
7. What is the ionic strength in a solution containing M^{2+}, A^- and MA^+, if $T_M = 0.02\,M$, $T_A = 0.04\,M$ and the formation constant is 100?
8. Derive the minimum number of data points that must be measured in a *p*H-metric titration to describe a system composing of *n* components and *p* associates?

9. The question is the same as the previous, but for spectrophotometric measurements.
10. What is the ionic product of water? What process does it describe?

2nd week: Measurement and evaluation

1. What is the Beer–Lambert law? Define all the symbols!
2. Which experimental methods can be used for the determination of equilibrium constants in solution equilibrium processes? Describe each method shortly (e.g., 2 sentences)!
3. What is Job's method?
4. What is the mole fraction method?
5. What is the underlying principle for determining formation constants at constant metal ion and ligand concentrations?
6. Describe the correlation between the total and equilibrium concentrations in a solution containing H, M, A, HA, H_2A, MA and MAH!
7. Why is it important to maintain a constant ionic strength during the determination of formation constants?
8. Why is it necessary to collect more data than the necessary minimum?
9. Give the formula for the calculation of the total concentration of the metal ion (M) and the ligand (L) when we dose L solution to an M solution, with an initial volume of V_0!
10. What is a distribution diagram?