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DOI

[10.1016/0003-2670\(94\)80190-8](https://doi.org/10.1016/0003-2670(94)80190-8)

Publication date

1994

Published in

Analytica Chimica Acta

[Link to publication](#)

Citation for published version (APA):

Erim, F. B., Boelens, H. F. M., & Kraak, J. C. (1994). Applicability of capillary zone electrophoresis to study metal complexation in solution. *Analytica Chimica Acta*, 294, 155. [https://doi.org/10.1016/0003-2670\(94\)80190-8](https://doi.org/10.1016/0003-2670(94)80190-8)

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Applicability of capillary zone electrophoresis to study metal complexation in solution

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Received 20th January 1994

Abstract

The suitability of capillary zone electrophoresis for the determination of stability constants of metal complexes was investigated. Three variants were tested viz., the frontal analysis, the Hummel-Dreyer method and the vacancy peak method, in the step-wise complexation between copper(II) and 1,10-phenanthroline as well as 2,2'-bipyridyl. The investigation reveals that CZE has attractive features for the quantitative study of complex formation with neutral ligands. However, the full exploitation requires more sensitive detection systems and application of an automated CZE system.

Key words: Capillary zone electrophoresis; Metal complexes; Stability constants; Neutral ligands

1. Introduction

Metal complexation in solution plays an important role in various areas. In order to understand its role in these areas information about the formation constants of the complexes in solution is indispensable. During the last century numerous analytical techniques have been developed to study metal complexation [1]. Of all these techniques potentiometry and spectrophotometry are by far most applied. Generally the stability constants are calculated from direct or indirect measurement of the concentration of the free or bound ligand in the mixture. For inert, i.e., slowly

decomposing complexes, in principle separation techniques like liquid chromatography can be applied to distinguish the free ligand from the complex without significantly influencing the equilibrium [2]. Size exclusion chromatography is frequently applied to determine protein–drug binding constants [3] but not for studying metal complexation in aqueous solutions. This is due to fact that the ligand and complex cannot be separated on basis of their difference in size. In that respect capillary zone electrophoresis (CZE) offers better perspectives [4]. With this recently rediscovered separation technique charged species can be separated, under the influence of an electrical field, according to the differences in the electrophoretic mobilities. The separation is performed in a fused silica capillary and can be considered as a one phase separation system.

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Since CZE has been successfully used by us for studying protein–drug binding [5], we found it worthwhile to investigate the applicability of this technique for the determination of stability constants of metal complexes. For that purpose three different methods to determine the free or bound ligand concentration without disturbing the equilibrium were tested e.g., the Hummel-Dreyer method, the vacancy peak method and the frontal analysis. As model system the stepwise complexation of copper(II) with 1,10-phenanthroline and 2,2'-bipyridyl was selected. The determined stability constants will be compared with values reported in literature and the perspectives of the CZE technique will be discussed.

2. Theory

The extent of complexation is described by the average number of ligands bound to the metal, $\bar{n}$, which is the ratio of the concentration of the bound ligand and total metal concentration. The relationship between $\bar{n}$ and the free ligand concentration according to the theory of successive complex formation is:

$$\bar{n} = \frac{\sum_{j=1}^N j\beta_j[L]^j}{1 + \sum_{j=1}^N \beta_j[L]^j}$$

All methods to determine stability constants are based on the direct or indirect measurement of the free ligand concentration, calculation of $\bar{n}$, and extraction of the β values from Eq. 1.

The present CZE method exploits the difference in electrophoretic mobility of the ligand and complex to determine the total free and/or bound ligand. The total free ligand is the sum of the protonated ligand, $[HL^+]$ and free ligand $[L]$. From the total free ligand concentration and the pK_a of the ligand, the free ligand concentration can be calculated.

From computer simulations it appeared that the three applied CZE methods are only applicable when the electrophoretic mobilities of the

metal and metal complex are close together and differ from that of the ligand. If this is not the case than the equilibrium during the electrophoresis is not preserved. This restricts the application of the described methods to complexes with neutral ligands because the charge of the metal and complex are the same and their mobilities will differ only slightly.

Therefore in the present study we selected the complexation of copper with 1,10-phenanthroline and 2,2'-bipyridyl. The selection was also done on basis of the following considerations: (i) the complexation of copper and these ligands have been extensively studied and values of stability constants are available in literature; (ii) the pK_a values of the ligands are known; (iii) the molar absorptivities of both ligands are high enough to detect them by on-column UV detection; (iv) both are neutral ligands and protonated forms dissolve in aqueous solutions.

3. Principle of the CZE methods

The various species in the mixture are separated in a fused silica capillary, under the influence of a high electrical field, and detected by using on-column UV detection. Fig. 1 depicts schematically the experimental set-up for the measurements. Two different sample introduction techniques were tested: pressure and electromigration. No significant differences were found between the two techniques but electromigration was selected in this study because of its simplic-

Fig. 1. Schematic representation of the experimental CZE set-up.

ity. With the experimental set-up three different variants to measure the total free or bound ligand were investigated, e.g., the frontal analysis, the Hummel-Dreyer method and the vacancy peak method. These are now briefly discussed, where for the sake of simplicity only a 1:1 complex is considered, although in the actual research higher complexes were also included. A more extensive description of the methods can be found in Ref. 5.

3.1. Frontal analysis

In the frontal analysis, the buffer vials and the capillary are filled with the buffer. Then a large sample plug (60 s, 10 kV), containing buffer + metal + ligand, is injected into the capillary and the voltage applied. Due to the difference in electrophoretic mobilities of the species (in our case $\mu_M \approx \mu_{ML} > \mu_L$) at the front edge of the sample zone free metal is leaving the sample plug while at the rear edge of the sample zone the free ligand leaks out of the sample zone. This finally results in three plateaus in the electropherogram: the first is related to the free metal, the second to the metal complex and the third to the total free ligand. Due to the selected wavelength, the free metal plateau is not visible in the electropherogram and only two plateaus will be found (see Fig. 2A). The height of the third plateau reflects the total free ligand concentration and thus the free ligand concentration can be calculated from the known pK_a value of the ligand and pH of the buffer solution.

3.2. Hummel-Dreyer method

In the Hummel-Dreyer method, the buffer vials and the capillary are filled with a solution containing buffer + ligand. The presence of the ligand in this solution causes a large background detector signal. Then a small amount of a solution of buffer + metal is injected (2.4 s, 10 kV) and the voltage applied. Due to the difference in mobility of the metal and ligand, both species are mixed in the beginning of the capillary and complexation occurs. When the ligand is present in excess the equilibrium is reached after a short

distance. However, when the metal is present in excess then the distance will be significantly larger. In the injected plug the total ligand concentration is similar to the ligand concentration in the buffer but part of the ligand is bound to the metal. When the migration proceeds the complex migrates from the sample zone and leaves a local deficiency in ligand concentration. This deficiency causes a negative peak which moves with the mobility of the ligand (see Fig. 2B). The area

Fig. 2. Typical electropherograms of the copper(II)-1,10-phenanthroline complex obtained with the CZE methods. (A) Frontal analysis method, 20 kV; inj. 60 s, 10 kV, plateau 1: complex; plateau 2: total free ligand. (B) Hummel-Dreyer method, 12 kV; inj. 2.4 s, 10 kV, peak 1: complex; peak 2: deficiency in ligand. (C) Vacancy peak method, 12 kV; inj. 2.4 s, 10 kV, peak 1: deficiency in complex; peak 2: deficiency in ligand.

reflects the amount of ligand bound to metal. The complex moves in the buffer + ligand and is thus always in equilibrium with the ligand. The presence of the complex causes a positive peak on the background.

3.3. Vacancy peak method

The vacancy peak method shows some similarities to the frontal analysis. The buffer vials and the capillary are filled with a solution containing buffer + ligand + metal. This results in a large background detector signal. Then a small volume of only buffer is injected (2.4 s, 10 kV) and the voltage applied.

As a whole the vacancy will move to the cathode since all mobilities are of the same sign. However, at the front edge, ligand is penetrating the vacancy, as its mobility is lowest. At the rear edge metal and complex moves into the vacancy, as its mobility is higher. After some time, dependent on the width of the injected zone, these disturbances will meet, and the complex will be reconstituted according to the equilibrium. From then on the equilibrium is restored but the introduced deficiencies in the total free ligand and complex will be intact during further migration and this will cause two negative peaks in the electropherogram (see Fig. 2C). The first peak is caused by the deficiency of the complex and the area is a measure of the bound ligand. The second negative peak originates from the deficiency of the total free ligand.

4. Experimental

4.1. Apparatus

The measurements were performed on a commercial CZE injection system (Prince, Lauer Labs, Emmen, Netherlands) in combination with on-column UV detection (linear 200, Linear Inst., Fremont, CA). The wavelength was set at 273 or 302 nm depending on the ligand. Platinum wire electrodes were inserted into the buffer vials for connection to the electrical circuit. Sample injection was carried by electromigration (2.4–60 s at

10 kV) at the anodic side. The analysis voltage ranged between 12–20 kV. The used fused silica capillary was 75 cm $\times$ 50 μ m i.d. and was obtained from Polymicro Technologies (Phoenix, AZ). The detection window was prepared by burning off locally the outer polyimide coating. The distance between the point of injection and the detection window was 41 cm. Before the experiments the capillary was always flushed for 20 min with 1 M KOH, water and buffer, respectively. The measurements were performed at room temperature.

4.2. Chemicals

Chloroacetic acid and 1,10-phenanthroline monohydrate were obtained from Aldrich (Bornem, Belgium). Sodium hydroxide and $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$ were purchased from Merck (Darmstadt). 2,2'-Bipyridyl was AnalaR grade (BDH, Poole) and recrystallized several times from methanol before use. All ligand solutions were prepared from doubly distilled water and kept in the dark.

4.3. Procedures

Solutions

Chloroacetic acid was chosen as buffer because of its negligible complexation strength with copper(II) and its acid strength. The ionic strength of all buffers was kept at 0.025 M. The pH of the buffer was adjusted with a solution of 0.1 M sodium hydroxide. On the basis of the complexation strength and $\text{p}K_a$ value of the ligand, the pH values of the copper–1,10-phenanthroline and copper–2,2'-bipyridyl system were set to 1.96 and 2.85, respectively. At these pH values no copper(II) hydroxide complex is formed.

In all experiments 5×10^{-5} M copper(II) was used except with the frontal analysis of the copper–1,10-phenanthroline system. In this experiment three different copper(II) concentrations, i.e. 4×10^{-5} , 5×10^{-5} , 6×10^{-5} M, were used to study the effect of metal concentration on the determination of the complex formation constants.

The ligand concentrations used range generally between 3×10^{-5} and 1.5×10^{-3} M.

Calculations

For the calculation of the stability constants the relation between of $\bar{n}$, the ratio of the concentration of the bound ligand and total metal, and the free ligand concentration has to be known. The free ligand concentration can be calculated from the total free ligand concentration, pH of the buffer and the pK_a value of the ligand. For 1,10-phenanthroline and 2,2'-bipyridyl the pK_a values are 4.98 and 4.47, respectively [6]. The bound ligand concentration is either directly known, as is the case in the Hummel-Dreyer method, or can be derived from the total free ligand concentration.

In the frontal analysis method the total free ligand concentration was determined from the height of the sample plateau and the height of the plateau obtained when injecting the same total ligand concentration in the buffer as used in the sample. The total free ligand is then calculated according to:

$$C'_L = \left(\frac{\text{sample height}}{\text{ligand height}} \right) C_L$$

where C_L is the total ligand concentration and C'_L the total free ligand concentration in the complex solution. The difference between C_L and C'_L is the bound ligand concentration.

In the Hummel-Dreyer method, the bound ligand concentration is determined from two separate injections: the sample, and the buffer only. From the areas of both negative peaks the bound metal concentration, C_b , can be calculated according to:

$$C_b = \left(\frac{A_1 - A_2}{A_2} \right) C_L$$

where A_1 is the area of the sample peak and A_2 the area of the buffer peak. C_L is the total ligand concentration in the solution put in the capillary. Since the ligand is present in a large excess, the free ligand concentration can be directly calculated from C_L .

In the vacancy peak method, the total free ligand concentration was determined by internal calibration. For that purpose increasing concentrations of ligand in the buffer were injected.

Depending on the concentration, the peaks can be negative or positive. By plotting the area of the peaks versus the added ligand concentration, the total free ligand concentration can be determined from the intersection on the ligand axis. At this point the added ligand concentration cancels the area of the sample peak. From the total free ligand concentration the free ligand and bound ligand concentration can be calculated.

Fitting of the experimental data

The experimental data were fitted according to Eq. 1, using a non-linear regression curve fitting program based on the Levenberg-Marquardt algorithm [7]. The data were fitted supposing two complexation steps. Since any iterative parameter estimation method has to start with initial values, we used the mean values of β_1 and β_2 from literature as initial values. In order to test whether the selection of the initial values may influence the final values of the stability constants, the fitting was also performed with different initial values (differing a factor 5–50). Varying the initial values in that range did not influence the final values of the stability constants. Furthermore a weighting of the data points with the measurement error was considered. The weighting involves a division of the individual data points by its standard deviation of the replicates. For the frontal analysis and the Hummel-Dreyer method no significant difference in the measurement errors was found and therefore a weighting of the data points is not necessary. However, for the vacancy peak method the measurement errors were different over the pL range and weighting of the data points was essential.

The standard deviations of the calculated stability constants were estimated as described in Ref. [8].

5. Results and discussion

In order to judge the three different CZE methods on their merits, the complexation of copper(II) with 1,10-phenanthroline was extensively investigated. From explorative experiments it appeared necessary to do the measurements

according a stringent time and washing protocol to obtain reproducible data. To get some insight in the reliability of the calculated stability constants, all measurements were replicated at least in duplicate. From the experimental data the complex formation curves were computed by using the non-linear curve fitting program as described in procedures.

5.1. Experimental $\bar{n}$ values

For the calculation of the three stability constants, the experimental data points should cover the whole $\bar{n}$ range. Unfortunately with all three methods it seemed impossible to attain reliable values for high and small $\bar{n}$ values. The reasons for this can be attributed to the limited sensitivity of the detection system and the back ground noise.

Since the coordination of the third ligand to the metal is considerably sterically hindered, a relative high ligand concentration compared to the metal concentration has to be used in order to realise $\bar{n}$ values > 2 . This means that under these conditions only a small change in the total free ligand concentration will occur. This change is reflected in the difference of the height or the area of the peak/plateau and the reference peak/plateau. Consequently for the calculation of $\bar{n}$, two large values are subtracted and this appears to give an unacceptable large spreading in $\bar{n}$. Moreover, with the Hummel-Dreyer and the vacancy methods there is a high background detector signal due to the presence of the ligand in the capillary and this background signal causes noise which increases with increasing ligand concentration. With the Hummel-Dreyer method $\bar{n}$ values up to 2.2 were measured but the spreading in replicates was unacceptably large.

For the measurement of $\bar{n}$ values < 1 , ligand concentrations smaller than that of the copper have to be used. It can be pointed out that the experimental difficulties in the determination of small $\bar{n}$ values is due to the stability of the first complex and the relative weak formation of the competitive reaction, i.e., the small pK_a value of the acid. The degree of complexation is appreciable even in rather acidic solutions and this makes

it difficult to detect the very small total free ligand concentrations with the used detection system. It can be noticed that in principle lowering the pH can circumvent this problem but leads to excessive heat production in the capillary. The very small total free ligand concentration results for the vacancy method in very small peaks and in the frontal analysis in low plateaus. With the Hummel-Dreyer method measurable peak heights were found but the peaks become very broad and at very small ligand/metal concentration ratios even double peaks were monitored. The deformation of the peaks when the copper concentration is larger than that of the ligand, must be attributed to the exhaustion of the amount of ligand. In order to attain an equilibrium, the copper ions have to migrate a longer distance through the ligand zone and this broadens the negative deficiency peak considerably. In principle the area of the deformed negative peak reflects still the amount of bound ligand but it appeared impossible to integrate this area properly with the available integrator.

Due to the absence of ligand in the buffer the background noise with the frontal analysis is small and $\bar{n}$ values down to about 0.6 could be measured with sufficiently small spreading. On basis of the aforementioned limitations to measure $\bar{n}$ values > 2 , the experimental data points were fitted assuming only the first two complex formation steps.

5.2. Experimental and computed formation curves

The experimental data points and the computed formation curves of the three CZE methods are reflected in Figs. 3–5. The calculated stability constants, including the standard deviations and literature values, are tabulated in Table 1.

From these figures it can be seen that the experimental points with the Hummel-Dreyer method fall well on the fitted line but with the frontal and vacancy method the experimental points scatter significantly around the fitted line. The reason for the scattering of the experimental points must be partly attributed to the manual operation of the measurements and temperature

Fig. 3. Experimental datapoints and computed formation curve of copper(II)–1,10-phenanthroline with the frontal analysis method. Copper(II) concentration: $\square = 4 \times 10^{-5}$; $\bullet = 5 \times 10^{-5}$; $\triangle = 6 \times 10^{-5}$ M.

Fig. 4. Experimental datapoints and computed formation curve of copper(II)–1,10-phenanthroline with the Hummel-Dreyer method.

fluctuations in the room. Less scattering can be expected with a completely automated well thermostated CZE system.

From Table 1 it can be seen that the values of the stability constants, calculated with the three methods, fall well within the range of previously reported literature values. The standard deviation is the smallest with the frontal analysis method and is significantly larger with the Hummel-Dreyer and vacancy method. On basis of the good fit of the experimental points with the Hummel-Dreyer method as shown in Fig. 4, the worse standard deviation of the Hummel-Dreyer

method might be a surprise for the reader but can be explained by the lack of $\bar{n}$ values below 1 (as is also the case with the vacancy method). This introduces a larger uncertainty in the calculation of the stability constants. It must be noticed that due to the laborious internal calibration, less data points were measured with the vacancy peak method. This certainly leads to larger standard deviations for the stability constants.

In a separate experiment the effect of the copper concentration on the complex formation was investigated with the frontal analysis method

Table 1

Calculated stability constants of the copper(II)–1,10-phenanthroline and copper(II)–2,2′-bipyridyl complexes and reported literature values

Ligand		Frontal Analysis	Hummel-Dreyer Method	Vacancy peak Method	Reported values [6]
1,10-Phenanthroline	$\log \beta_1$	8.3 ± 0.09	8.9 ± 0.17	8.4 ± 0.20	7.4–9.1
	$\log \beta_2$	15.0 ± 0.10	15.6 ± 0.16	15.0 ± 0.19	15.7–16.0
2,2′-Bipyridyl	$\log \beta_1$	7.5 ± 0.10			6.3–8.2
	$\log \beta_2$	13.1 ± 0.10			13.6–13.7

Fig. 5. Experimental datapoints and computed formation curve of copper(II)–1,10-phenanthroline with the vacancy peak method.

and the experimental points are included in Fig. 3. As can be seen all data points fall close to the same line, which largely ensures that under the selected experimental conditions the complex formation is independent of the copper concentration.

In order to demonstrate that the CZE method also works for other complexes the stepwise complex formation of copper(II) and 2,2'-bipyridyl was determined with the frontal analysis. The computed formation curve is shown in Fig. 5 and the values of the calculated stability constants are included in Table 1. The determined values fall well in the range reported in literature.

6. Conclusions

From the results so far some preliminary conclusions can be drawn. CZE is in principle an attractive and simple technique to determine stability constants of metal complexes. The method

is so far only suited for complexation with neutral ligands. The main drawback is the lack of concentration sensitivity of the applied on-column UV detection. Improvements in concentration sensitivity can be expected when using UV detection cells with a longer lightpath such as a Z- or U-shaped [9] cell or a bubble cell from Hewlett-Packard (Waldbronn, Germany). Also the application of fluorescence and electrochemical detection for certain application looks attractive.

Of the three investigated CZE methods, viz. frontal analysis, Hummel-Dreyer and the vacancy peak methods, at this moment the frontal analysis method appears to be the most reliable method because $\bar{n}$ values < 1 can be determined.

Since all CZE measurements were performed manually it can be expected that more reliable results can be obtained with an automated CZE system. Research in that direction, including an investigation to select the optimal experimental points for the fitting via an experimental design approach, is now undertaken.

Fig. 6. Experimental data points and computed formation curve of copper(II)–2,2'-bipyridyl with the frontal analysis.

Acknowledgments

The authors thank Prof. Dr. H. Poppe for his valuable remarks during the preparation of the manuscript.

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