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# Analysis of Titration Curves of Lake Tjeukemeer Water With Cu(II). Identification of the Formation of More than Two Groups of Different Complexes*

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The results for direct titration of Lake Tjeukemeer water with Cu(II) are interpreted by using both the van den Berg-Ružić and Scatchard plots. The use of a new method for correction of asymptote at higher additions of metal proposed by Ružić and Pečar has been illustrated with these titration data. The analysis of reduced titration data obtained by extraction of the equation for weaker complexes from the original titration data has been proved to be a very powerful method by which the accuracy of determining complexation parameters for higher energy binding sites can be checked.

The following stability constants ($K_i$) and complexing capacities ($L_i$) are reported:

$K_1 = 1.661 \cdot 10^4$ dm$^3$ mol$^{-1}$, $L_{T1} = 7.978 \cdot 10^{-5}$ mol dm$^{-3}$
$K_2 = 3.014 \cdot 10^5$ dm$^3$ mol$^{-1}$, $L_{T2} = 1.278 \cdot 10^{-5}$ mol dm$^{-3}$
$K_3 = 1.588 \cdot 10^7$ dm$^3$ mol$^{-1}$, $L_{T3} = 5.432 \cdot 10^{-6}$ mol dm$^{-3}$
$K_4 = 6.432 \cdot 10^8$ dm$^3$ mol$^{-1}$, $L_{T4} = 5.996 \cdot 10^{-7}$ mol dm$^{-3}$

High concentration of organic matter in the Lake enabled direct titration with Cu(II) in a wide range of concentrations, so that four different binding strengths could be detected from the titration data.

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* Dedicated to Marko Branica on the occasion of his 65th birthday.

## INTRODUCTION

Investigation of the copper complexation parameters in Lake Tjeukemeer (Figure 1), a shallow lake in the Netherlands, indicated that more than one group of ligands was present in the water.[1] Since this lake is very rich in dissolved organic carbon (1.4 mM of C per water sample in this study), as compared to the natural copper concentration (44 nM of Cu; five orders of magnitude lower than C), it is possible to identify several groups of organic ligands forming complexes with this metal.

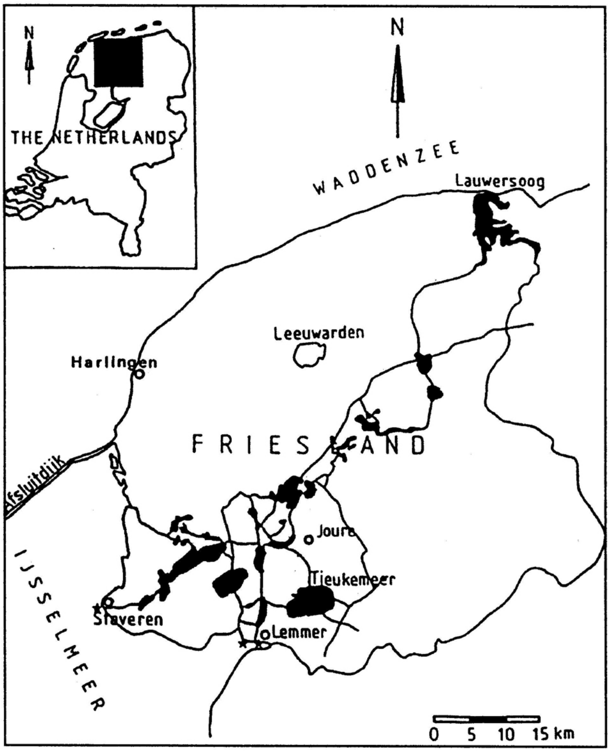


Figure 1. Map showing the location of Lake Tjeukemeer and Lake Ijsselmeer.

To obtain accurate complexation parameters, two methods should be used simultaneously. The van den Berg-Ružić plot can be used[2–4] to determine the complexing capacity of water samples (a plot of the ratio between concentrations of free and bound Cu(II) *vs.* the concentration of free Cu(II) ion, see Figure 2A):

$$(\mathrm{M}) / (M_\mathrm{T} - (\mathrm{M})) = ((\mathrm{M}) + 1/K) / L_\mathrm{T} \qquad (1)$$

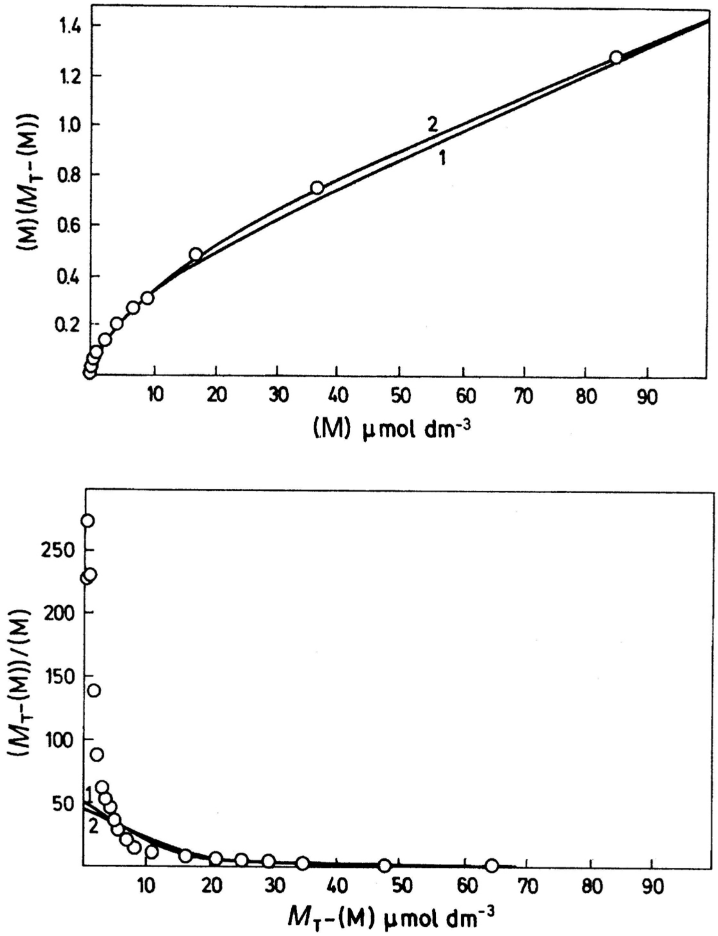


Figure 2. Original titration data: A. Van den Berg-Ružić-Lee Plot and B. Scatchard plot: Circles original data, lines theoretical predictions with complexation parameters for two weaker groups of complexes: 1. first estimate and 2. final values of complexation parameters after the correction procedure.

where (M) is the concentration of the free Cu(II) ion. $M_T$ and $L_T$ are the total concentrations of Cu(II) and organic ligands, respectively, and $K$ is the stability function which depends on the free Cu(II) ion concentrations:[5]

$$K = [(\mathrm{M})^{n-1} + (\mathrm{M})^{n-2} / K_2^* + \ldots K_1^* / \Pi K_i^*] /$$

$$[(\mathrm{M})^{n-1} / K_1^* + (\mathrm{M})^{n-2} / \overline{K_i K_j} + \ldots +1 / \Pi K_i\ ] \qquad (2)$$

where

$$K_1^* = \sum L_{Ti} \, / \, \sum (L_{Ti} \, / \, K_i).$$

Here $K_i$ and $L_{Ti}$ represent individual stability constants and total ligand concentrations, respectively, $\overline{K_i K_j}$ is the mean product of individual stability constants, $K_i^*$ are different apparent stability constants and n is the total number of different groups of complexes identified. This plot is more sensitive for presenting the part of titration curve near the equivalence point. The same plot can be used for the estimation of complexation parameters for two weaker groups of complexes. In order to avoid errors in the estimation of the asymptote of this plot, in the range of large additions of Cu(II), used in the analysis (discussed by Morel *et al.*[6–8]):

$$(\mathrm{M}) \, / \, (M_T - (\mathrm{M})) = ((\mathrm{M}) + 1/K_1^*) \, / \, L_T \tag{3}$$

a special method can be used for a more precise determination of the complexing capacity $L_T$ and apparent stability constant $K_1^*$ in this concentration range.[9]

The Scatchard plot[10,11] is more sensitive to the part of the titration curve at small additions of Cu(II) (a plot of the ratio between the concentrations of the bound and free Cu(II) vs. bound Cu(II) concentration, see Figure 2B):

$$((M_T - (\mathrm{M})) \, / \, (\mathrm{M}) = K \, [L_T - (M_T - (\mathrm{M}))]. \tag{4}$$

This plot is, therefore more convenient for identifying stronger groups of complexes. In an earlier paper, Ružić designed a method for a more accurate analysis of the Scatchard plot[9] which is based on the use of the results already obtained by analysis of the van den Berg-Ružić plot. The results of the titration of water samples from Lake Tjeukemeer by Cu(II) are analyzed in this paper by both of these methods.

## EXPERIMENTAL

At ten locations in Lake Tjeukemeer, samples were taken, using a perspex tube, from the upper meter of the water column and stored in polyethylene. In the laboratory, the samples were mixed and filtrated (0.2 μm). Then, 200 mL was used for the copper titration at 20 °C, pH = 6 (by addition of either hydrochloric acid or a sodium hydroxide solution using pH-stat) and at virtually constant ionic strength by adding up to 0.1 M $NaNO_3$. A calibrated ORION Ion Selective Electrode (ISE) was used for measuring the »free« copper ion concentration. After each addition, the pH was adjusted by the pH-stat and, when the system was in apparent equilibrium, the ISE-signal was read. Since total copper additions were so high that insoluble compounds might occur, the form of titration curve was checked for precipitation.

### *Analysis of the Van Den Berg-Ružić Plot*

The original titration data are presented by the Van den Berg-Ružić-Lee plot in Figure 2A. It is obvious that more than one group of complexes between Cu(II) and organic ligands are formed because this plot is not linear and the deviation from linearity appears in the right direction. The first estimate of the asymptote from the region of larger additions of Cu(II) ion in this plot can be described by the following Eq.:

$$(\mathrm{M}) / (M_\mathrm{T} - (\mathrm{M})) = 0.01121\,(\mathrm{M}) \cdot 10^6 + 0.3546 \qquad (5)$$

wherefrom $K_1^* = 3.164 \cdot 10^4$ dm$^3$ mol$^{-1}$ and $L_\mathrm{T} = 8.921 \cdot 10^{-5}$ mol dm$^{-3}$. By plotting the inverse value of the difference between the asymptote and the original titration curve *vs.* free Cu(II) concentration (according to the method proposed by Ružić[3]) the following straight line is obtained:

$$1 / \{[(\mathrm{M}) / (M_\mathrm{T} - (\mathrm{M}))]_\infty - (\mathrm{M}) / (M_\mathrm{T} - (\mathrm{M}))\} = 0.4526\,(\mathrm{M}) \cdot 10^6 + 2.984 \qquad (6)$$

wherefrom $K_2^* = 1.517 \cdot 10^5$ dm$^3$ mol$^{-1}$. Individual complexation can now be estimated using the procedure described earlier by Ružić[3,4] (see App. A):

$$\begin{array}{ll} K_1 = 2.637 \cdot 10^4\ \mathrm{dm^3\ mol^{-1}} & L_\mathrm{T1} = 7.430 \cdot 10^{-5}\ \mathrm{mol\ dm^{-3}} \\ K_2 = 3.308 \cdot 10^6\ \mathrm{dm^3\ mol^{-1}} & \underline{L_\mathrm{T2} = 1.492 \cdot 10^{-5}\ \mathrm{mol\ dm^{-3}}} \\ & L_\mathrm{T}\ = 8.922 \cdot 10^{-5}\ \mathrm{mol\ dm^{-3}}. \end{array}$$

Assuming that only two different groups of complexes are formed Eq. (2) is reduced to a simpler relation:

$$K = ((\mathrm{M}) + 1/K_2^*) / ((\mathrm{M}) / K_1^* + 1/K_1K_2)\ . \qquad (7)$$

Ružić and Pečar[5] designed a method for correcting $K_1^*$ and $L_\mathrm{T}$ values on the basis of Eq. (7):

$$\mathrm{d}[(\mathrm{M}) / (M_\mathrm{T} - (\mathrm{M})) / \mathrm{d}(\mathrm{M})] = [1 + \mathrm{d}(1/K) / \mathrm{d}(\mathrm{M})]L_\mathrm{T} \qquad (8)$$

where

$$\mathrm{d}(1/\mathrm{K}) / \mathrm{d}(\mathrm{M}) = (1/K_1^*K_2^* - 1 / K_1K_2) / ((\mathrm{M}) + 1/K_2^*)\ .$$

Using Eq. (8), one can correct the value for $L_\mathrm{T}$ (the accurate slope of the asymptote is $1/L_\mathrm{T}$) because the right side of this Eq. represents the first estimate of the slope of the asymptote. The intersection of the asymptote with the ordinate can be obtained from the last point in the titration curve in the following way:

$$(\mathrm{M}) / (M_\mathrm{T} - (\mathrm{M})) - (\mathrm{M}) / L_\mathrm{T} = [((\mathrm{M}) + 1/K_2^*) / ((\mathrm{M}) + K_1^* / K_1 K_2)] / L_\mathrm{T} \cdot K_1^* \qquad (9)$$

wherefrom the accurate intersection of the asymptote ($1 / L_\mathrm{T} \cdot K_1^*$) can be extracted. In this way, the Eq. (5) can be corrected and the analysis can be repeated producing a new set of individual complexation parameters. This procedure can be repeated until the results obtained are accurate enough. After more than thirty such iterations, the following final results have been obtained for two different groups of complexes:

$K_1 = 1.661 \cdot 10^4\ \mathrm{dm^3\ mol^{-1}}$  $L_{\mathrm{T}1} = 7.977 \cdot 10^{-5}\ \mathrm{mol\ dm^{-3}}$
$K_2 = 2.362 \cdot 10^6\ \mathrm{dm^3\ mol^{-1}}$  $L_{\mathrm{T}2} = 1.873 \cdot 10^{-5}\ \mathrm{mol\ dm^{-3}}$
$L_\mathrm{T} = 9.851 \cdot 10^{-5}\ \mathrm{mol\ dm^{-3}}$.

In Figure 3, both asymptote straight lines are plotted, the first estimate from the last two points (described by Eq. (5)) and the final one determined by the corrections described above. On the basis of the calculated individual complexation parameters, one can construct a theoretical titration curve according to the following equation:

$$(\mathrm{M}) / (M_\mathrm{T} - (\mathrm{M})) = 1 / [L_{\mathrm{T}1} / ((\mathrm{M}) + 1/K_1) + L_{\mathrm{T}2} / ((\mathrm{M}) + K_2)]. \qquad (10)$$

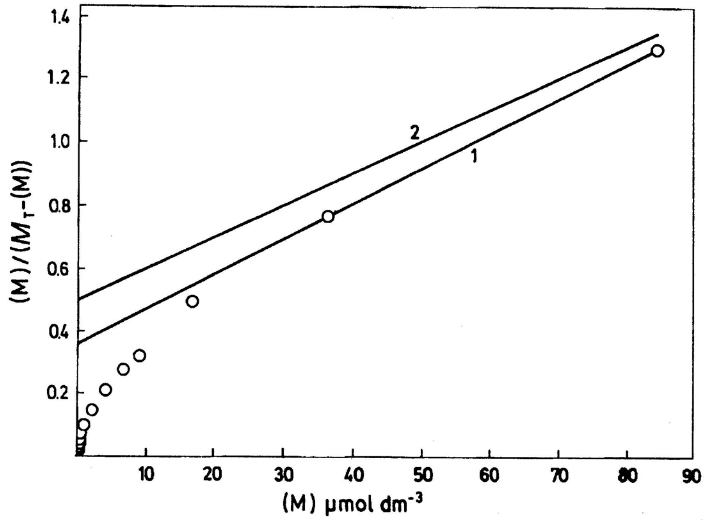


Figure 3. Comparison between the first estimate and the final asymptote of the Van den Berg-Ružić-Lee plot after the correction procedure.

In Figure 2A, both theoretical curves are plotted on the basis of the first and final estimations of individual complexation parameters. One can clearly recognize a much better fit of experimental data with the corrected theoretical titration curve.

### *Analysis of the Scatchard Plot*

The original titration data are also presented by the Scatchard plot in Figure 2B, together with the theoretical plots based on final individual complexation parameters obtained from the van den Berg-Ružić plot and relation (10). At lower additions of Cu(II), there is obviously a significant discrepancy between the experimental data and the theoretical prediction based on the assumption that only two different groups of complexes are formed. The overall complexation parameters at lower additions of Cu(II) for two weaker groups of complexes are the following:

$$K_{12} = K_1 K_1 / K_2^* = (K_1 L_{T1} + K_1 L_{T2}) / L_T = 0.4626 \cdot 10^6 \text{ dm}^3 \text{ mol}^{-1}$$

and

$$L_T = 9.851 \cdot 10^{-5} \text{ mol dm}^{-3}. \tag{11}$$

Using the analysis proposed earlier by Ružić,[9] one can determine complexation parameters for the third group of stronger complexes. By plotting the ratio between the theoretical ordinate in the Scatchard plot for two weaker complexes and the difference between the original data and such theoretical prediction, the following straight line is obtained, as shown in Figure 4:

$$[(M_T - (\mathrm{M}))]_\propto / \{(M_T - (\mathrm{M})) / (\mathrm{M}) - [(M_T - (\mathrm{M})) / (\mathrm{M})]_\propto\} = 3.895(\mathrm{M}) \cdot 10^7 + 0.1046 \tag{12}$$

wherefrom $K_3^* = 3.526 \cdot 10^7$ dm$^3$ mol$^{-1}$. The individual complexation parameters can be calculated now:

$$\begin{array}{ll} K_{12} = 4.571 \cdot 10^5 \text{ dm}^3 \text{ mol}^{-1} & L_{12} = 9.735 \cdot 10^{-5} \text{ mol dm}^{-3} \\ K_3 = 3.769 \cdot 10^8 \text{ dm}^3 \text{ mol}^{-1} & \underline{L_{T3} = 1.159 \cdot 10^{-6} \text{ mol dm}^{-3}} \\ & L_T = 9.851 \cdot 10^{-5} \text{ mol dm}^{-3}. \end{array}$$

Of course, $K_{12}$ and $L_{12}$ are overall parameters ($L_{12} = L_{T1} + L_{T2}$). On the basis of individual complexation parameters one can calculate the theoretical prediction for the Scatchard plot in the following way:

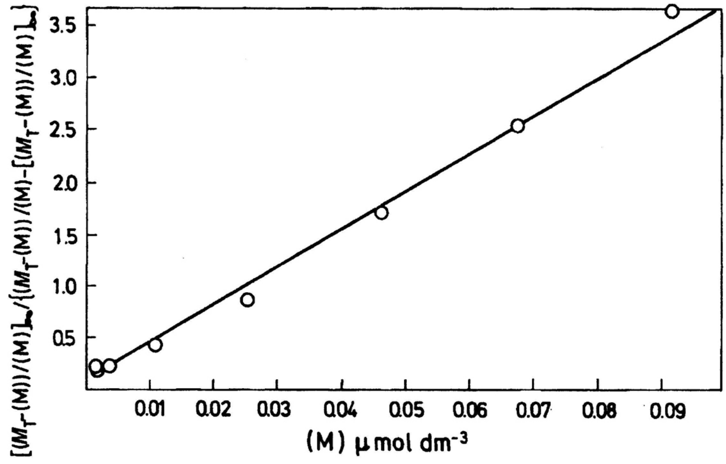


Figure 4. Analyses of the Scatchard plot according to Eq. (12).

$$(M_T - (M)) / (M) = L_{T1} / ((M) + 1/K_1) + L_{T2} / ((M) + 1/K_2) + L_{T3} / ((M) + 1/K_3). \quad (13)$$

In Figure 5, this theoretical prediction is plotted and one can see that the agreement with the experimental data is not satisfactory.

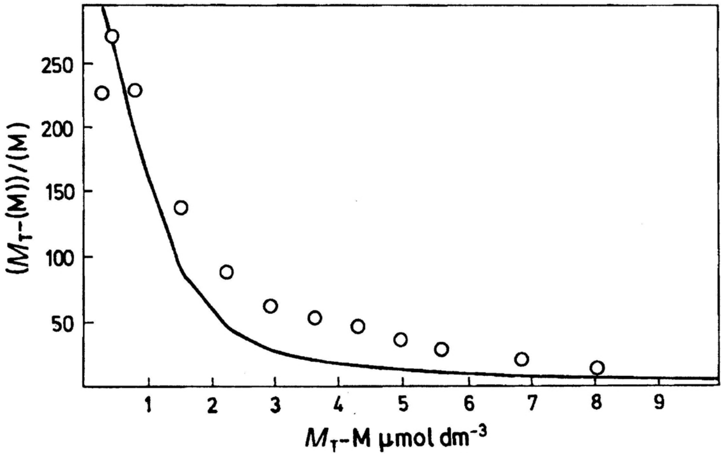


Figure 5. Comparison of experimental data with theoretical prediction for three groups of different ligands generated by Eq. (13).

### *Separation of Individual Groups of Ligands from the Titration Curve*

Analysis of the van den Berg-Ružić plot, after correction of the asymptote in the range of higher metal concentrations, gave fairly accurate results for the complexation parameters of the weaker group of bounding ligands. To increase the accuracy of the analysis in the range of lower metal concentrations, we decided to subtract the theoretical prediction for the weaker group of ligands from the total titration curve in the following way:

$$(M_{\mathrm{T}} - (\mathrm{M})) / (\mathrm{M}) - L_{\mathrm{T1}} / ((\mathrm{M}) + 1/K_1) = F[(\mathrm{M})]. \qquad (14)$$

The new function $F[(\mathrm{M})]$ should contain the information on all the other stronger binding ligands. Now, we can again perform the analysis in the Van den Berg-Ružić-Lee plot using $1/F[(\mathrm{M})]$ as the ordinate. This procedure has been proposed by Ružić and Pečar.[5] First, we should determine the asymptote of such a new plot and then perform the analysis of the plot according to relation (6). The results of the analysis of such reduced titration data can be summarized by the following complexation parameters:

$$\begin{array}{ll} K_2 = 3.014 \cdot 10^5\ \mathrm{dm^3\ mol^{-1}} & L_{\mathrm{T2}} = 1.278 \cdot 10^{-5}\ \mathrm{mol\ dm^{-3}} \\ K_3 = 1.759 \cdot 10^7\ \mathrm{dm^3\ mol^{-1}} & \underline{L_{\mathrm{T2}} = 6.032 \cdot 10^{-6}\ \mathrm{mol\ dm^{-3}}} \\ & L_{23} = 1.181 \cdot 10^{-5}\ \mathrm{mol\ dm^{-3}}. \end{array}$$

One can clearly see that the overall stability constant $K_{12}$, obtained by the analysis of the Scatchard plot, is controlled mainly by the second group of bounding ligands (with the stability constant $K_2$). However, since $K_{12} > > 3.014 \times 10^5\ \mathrm{dm^3\ mol^{-1}}$, the first estimate of $K_2$ was obviously inaccurate (more than one order of magnitude higher). This can be easily explained by the influence of high energy bounding ligands which are present in lake water and which have been disregarded in the first attempt to analyze titration data. In Figures 6A and 6B, both the Van den Berg-Ružić-Lee and Scatchard plots are presented for titration data from which the contribution of the first weaker group of ligands has been extracted using the corresponding theoretical lines constructed from the complexation parameters determined above. Once again, the van den Berg-Ružić plot (Figure 6A) shows excellent agreement with the experimental points, proving that the parameters of the weaker group of bounding ligands are sufficiently accurate. From the corresponding Scatchard plot (Figure 6B), one can see that more than three groups of bounding ligands can be identified from the original titration data. This is the reason for the poor agreement between titration data in the previous Scatchard plot shown in Figure 5. This procedure was repeated so that the theoretical prediction for the first two groups of bounding ligands was subtracted from the original titration data:

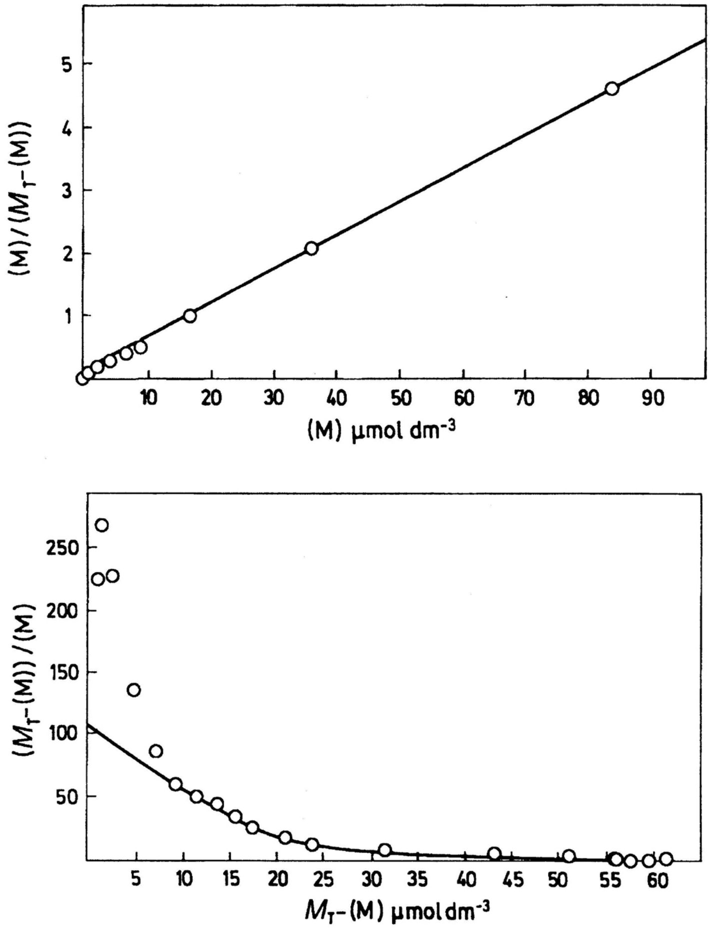


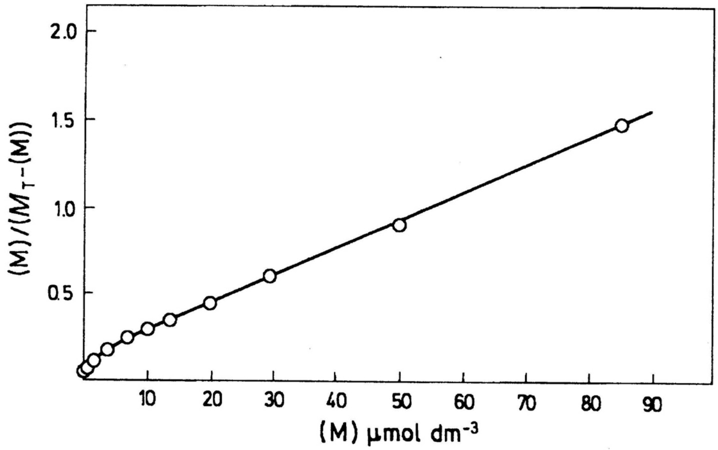
Figure 6. Reduced original titration data obtained by extraction of the theoretical prediction for the first weaker group of bounding ligands: A. Van den Berg-Ružić-Lee plot and B. Scatchard plot; Circles reduced titration data, lines theoretical predictions for the second and third group of bounding ligands.

$$
\begin{aligned}
&(M_T - (\mathrm{M})) / (\mathrm{M}) - L_{T1} / ((\mathrm{M}) + 1/K_1) - \\
&\qquad L_{T2} / ((\mathrm{M}) + 1/K_2) = G[(\mathrm{M})]. \qquad (15)
\end{aligned}
$$

The new function $G[(\mathrm{M})]$ should contain information about the third and, eventually, other high energy bounding ligands. We can now perform the analysis of the Van den Berg-Ružić-Lee plot using $1/G[(\mathrm{M})]$ as the ordinate. The results of this repeated procedure can be summarized in the following set of complexation parameters:

$$K_3 = 1.588 \cdot 10^7 \text{ dm}^3 \text{ mol}^{-1} \qquad L_{T3} = 5.432 \cdot 10^{-6} \text{ mol dm}^{-3}$$
$$K_4 = 6.341 \cdot 10^8 \text{ dm}^3 \text{ mol}^{-1} \qquad L_{T4} = 5.996 \cdot 10^{-7} \text{ mol dm}^{-3}$$
$$L_T = 6.032 \cdot 10^{-6} \text{ mol dm}^{-3}.$$

The previously determined $K_3$ value was a mean between $K_3$ and $K_4$ values obtained here. In Figures 7A and 7B both the van den Berg-Ružić and Scatchard plots are presented for such reduced titration data (generated by



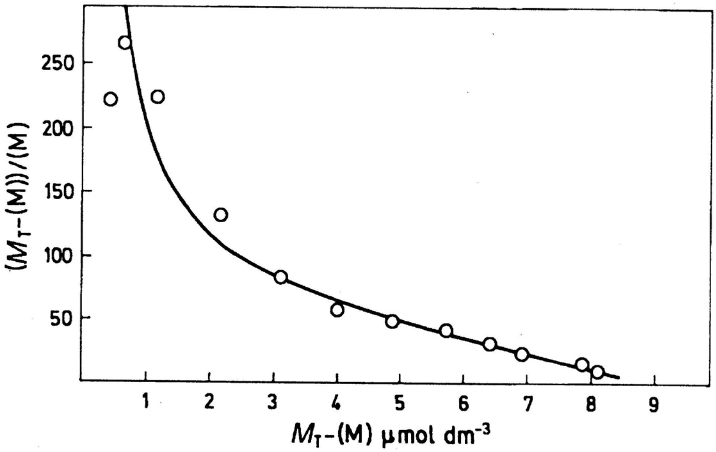


Figure 7. Reduced original titration data obtained by extraction of the theoretical prediction for the two weaker groups of bounding ligands: A. Van den Berg-Ružić-Lee plot, B. Scatchard plot; Circles reduced titration data, lines theoretical predictions for the two stronger groups of bounding ligands.

expression (15)) with the corresponding theoretical lines for high energy binding ligands. This time, the agreement between the titration data and theoretical predictions is very good. To make a final test of the validity of the complexation parameters determined, we subtracted the theoretical prediction for three weaker groups of bounding ligands from the original titration data:

$$\begin{aligned}(M_T - (\mathrm{M})) / (\mathrm{M}) - L_{T1} / ((\mathrm{M}) + 1/K_1) - L_{T2} / ((\mathrm{M}) + 1/K_2)\\ - L_{T3} / ((\mathrm{M}) + 1/K_3) = H[(\mathrm{M})].\end{aligned} \tag{16}$$

Agreement between the theoretical prediction for the last stronger group of bounding ligands and reduced titration data generated by expression (16) is good in both van den Berg-Ružić and Scatchard plots. Therefore, we can prepare the final set of complexation parameters:

$$\begin{array}{ll} K_1 = 1.661 \cdot 10^4 \ \mathrm{dm^3\ mol^{-1}} & L_{T1} = 7.978 \cdot 10^{-5} \ \mathrm{mol\ dm^{-3}} \\ K_2 = 3.014 \cdot 10^5 \ \mathrm{dm^3\ mol^{-1}} & L_{T2} = 1.278 \cdot 10^{-5} \ \mathrm{mol\ dm^{-3}} \\ K_3 = 1.588 \cdot 10^7 \ \mathrm{dm^3\ mol^{-1}} & L_{T3} = 5.432 \cdot 10^{-6} \ \mathrm{mol\ dm^{-3}} \\ K_4 = 6.432 \cdot 10^8 \ \mathrm{dm^3\ mol^{-1}} & \underline{L_{T4} = 5.996 \cdot 10^{-7} \ \mathrm{mol\ dm^{-3}}} \\ & L_T = 9.859 \cdot 10^{-5} \ \mathrm{mol\ dm^{-3}}. \end{array}$$

In Figures 8A and 8B original titration data are presented in both van den Berg-Ružić and Scatchard plots with the corresponding theoretical lines generated from the final complexation parameters determined here. Very good agreement between titration data and theoretical predictions is noticeable in both plots.

## DISCUSSION

The analysis of the titration data obtained for water samples taken from the Lake Tjeukemeer clearly illustrate that, using only one of the two different methods for the analysis of such data, at the most two groups of different complexes can be identified. Using the van den Berg-Ružić plot, only two weaker groups of complexes could be identified (complexation parameters for these complexes are $K_1$, $L_{T1}$, $K_2$ and $L_{T2}$). Using the Scatchard plot from the experimental titration data, one can recognize the formation of stronger complexes if they are not masked by the amount of Cu(II) originally present in water samples. In natural waters, characterized by the relatively low content of dissolved organic material in comparison with the amount of trace metal originally present in the water, the available range of trace metal concentration is not wide enough and then at the most two and very often

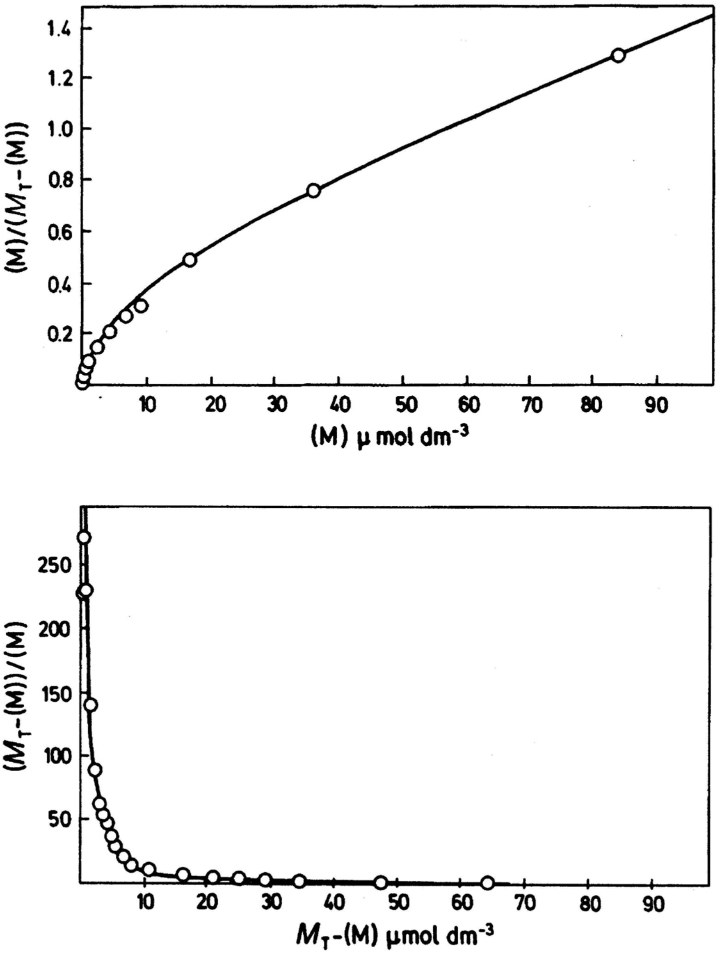


Figure 8. Comparison of original titration data (circles) with theoretical prediction for four different groups of bounding ligands (lines): A. Van den Berg-Ružić-Lee plot and B. Scatchard plot

only one single group of complexes can be identified from experimental data. For example, Moffett and Zika reported two groups of complexes for the titration of sea water with Cu(II) from the Biscayne Bay.[12] We analyzed their data using both of the above mentioned plots. Theoretical prediction based on data from the van den Berg-Ružić plot agreed well with the experimental data in the Scatchard plot ($K_1 = 2.75 \cdot 10^9$ dm$^3$ mol$^{-1}$, $L_{T1} = 1.11 \cdot 10^{-7}$ mol dm$^{-3}$ and $K_2 = 1.50 \cdot 10^{12}$ dm$^3$ mol$^{-1}$, $L_{T2} = 3.53 \cdot 10^{-9}$ mol dm$^{-3}$: original parameters reported by Moffett and Zika are close to these numbers with the exception of $K_1$ which they reported ten times higher probably due to a typing error[12]). Therefore, in this case only two groups of complexes have been identified. Plavšić *et al.* reported only one group of complexes in water samples from the Adriatic Sea[13] ($K = 6.4 \cdot 10^7$ dm$^3$ mol$^{-1}$ and $L_T = 1.5 \cdot 10^{-7}$ mol dm$^{-3}$). In this paper it is demonstrated that the new method proposed by Ružić and Pečar[5] can be used to produce more accurate complexation parameters. In this way, part of the criticism reported earlier by Morel[8] (see Figure 2B from Ref. 8) is resolved. However, the rest of his comments are not justified because the extrapolation should obviously be used in the concentration range near the equivalence point (and for that purpose one should use the part of the titration curve with $(M) / (M_T - (M)) > 1$, which is not the case at all with Figure 2A from Ref. 8). It has been demonstrated in this paper that both the van den Berg-Ružić and Scatchard plots should be used for complete interpretation of titration data in cases where the content of dissolved organic material is significantly higher that $10^{-7}$ mol dm$^{-3}$, as suggested earlier by Ružić and Pečar.[5] The complexation data reported in this paper are consistent with the results of other research conducted so far in Lake Tjeukemeer.[14] However, by a detailed analysis of the titration data, a fourth group of high energy bounding ligands (with a stability constant of more than four orders of magnitude higher than the stability constant of the most abundant weaker bounding ligands) is identified in a concentration of about 5% of the total complexing capacity. Titration data reported in this paper have a special value because the range of free Cu(II) concentrations is more than four orders of magnitude wide. In our next paper, we will try to reinterpret these data using the model of continuous distribution of stability constants.[15–17]

We should mention, however, that the question of the physical-chemical nature of the interaction of Cu with ligands is the most relevant one. Of course, this is also the most difficult question, and we believe that this question is yet to be answered properly in the future. Our recent paper[17] is partially dedicated to this problem. The aim of this paper is to improve the fitting of experiments if a discrete model of stabilities is chosen for the interpretation of these experiments. The method proposed in this paper puts more weigh on the region of the titration curve where weaker complexes have dominant influence ($L_T/K_1^* = \sum L_i / K_i$, weaker complexes are more abundant, therefore the effect of their abundance is increased when divided by lower strength, consequently, smaller corrections are needed and more accurate results are obtained), while the methods MINEQ, MICROQL and FITEQL use the same statistical criteria for the entire titration curve and for practical reasons they will always neglect the strongest complexes in the region where individual complexes are of comparable influence ($L_T \cdot K_1^* = \sum L_i \cdot K_i$, stronger complexes are less abundant, therefore, their higher strength is somewhat compensated by their smaller abundance).[5,18–21]

## APPENDIX A.

From Eq. (2), assuming the existence of two different groups of complexes between Cu(II) ion and organic ligands, one can write the following simpler equations:[3,4]

$$K_1^* = (L_{T1} + L_{T2}) / (L_{T1} / K_1 + L_{T2} / K_2) \tag{A1}$$

and

$$K_2^* = K_1 K_2 (L_{T1} + L_{T2}) / (L_{T1} K_1 + L_{T2} K_2). \tag{A2}$$

Combining Eqs. (A1) and (A2), one can derive the expression for the product and the sum of individual stability constants:

$$K_1 K_2 = 1 / (K_1^* + K_2^* - (L_{T1} + L_{T2}) / \text{slope}) \tag{A3}$$

where »slope« corresponds to the plot described by Eq. (6), and

$$K_1 + K_2 = K_1 K_2 (1/K_1^* + 1/K_2^*). \tag{A4}$$

From the values for $K_1 K_2$ and $(K_1 + K_2)$, one can calculate the individual values for stability constants $K_1$ and $K_2$. Then, $L_{T1}$ and $L_{T2}$ can be obtained from equations (A1) and (A2).

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## SAŽETAK

### Analiza krivulja titracije vode jezera Tjeukemeer sa Cu(II). identifikacija stvaranja više od dvije skupine različitih kompleksa

*Wilko Verweij and Ivica Ružić*

Interpretirani su rezultati direktne titracije uzoraka vode iz jezera Tjekemeer s Cu(II) koristeći metode zasnovanima na dijagramima koje su predložili van den Berg-Ružić i Scatchard. Na takovim eksperimentalnim podacima ilustrirana je upotreba nove metode za korekciju asimptote titracijske krivulje kod visokih dodataka metala koju su predložili Ružić i Pečar. Analizom tzv. reduciranih podataka dobivenih ekstrakcijom jednadžbe za slabije komplekse iz originalnih podataka titracije pokazalo se da je na taj način moguće znatno povećati preciznost određivanja parametara kompleksiranja za aktivna mjesta visokih energija vezanja. Određene su slijedeće konstante stabilnosti ($K_i$) i kapaciteti kompleksiranja ($L_i$):

$$K_1 = 1.661 \cdot 10^4\ \mathrm{dm^3\ mol^{-1}},\quad L_{T1} = 7.978 \cdot 10^{-5}\ \mathrm{mol\ dm^{-3}}$$
$$K_2 = 3.014 \cdot 10^5\ \mathrm{dm^3\ mol^{-1}},\quad L_{T2} = 1.278 \cdot 10^{-5}\ \mathrm{mol\ dm^{-3}}$$
$$K_3 = 1.588 \cdot 10^7\ \mathrm{dm^3\ mol^{-1}},\quad L_{T3} = 5.432 \cdot 10^{-6}\ \mathrm{mol\ dm^{-3}}$$
$$K_4 = 6.432 \cdot 10^8\ \mathrm{dm^3\ mol^{-1}},\quad L_{T4} = 5.996 \cdot 10^{-7}\ \mathrm{mol\ dm^{-3}}$$

Visoke koncentracije organske tvari u jezeru Tjeukemeer omogućile su direktnu titraciju s Cu(II) u širokom koncentracijskom području tako da su iz tih titracijskih podataka određene četiri različite jakosti vezanja.