CROATICA CHEMICA ACTA CCACAA **70** (1) 229–245 (1997)

ISSN–0011-1643
CCA–2418

Original Scientific Paper

A New Method for Data Analysis in Studies of Trace Metal Complexation*

Ivica Ružić and Jadranka Pečar

Ruđer Bošković Institute, Center for Marine Research, P.O.B. 1016, 10001 Zagreb, Croatia

Received February 2, 1996; accepted July 10, 1996

A new method for correction of the asymptote at high additions of trace metal during titration was developed for the van den Berg-Ružić plot. Using this method, a more accurate value for complexing capacity can be obtained from titration data. The accuracy of this parameter is a limiting factor for detailed studies of trace metal complexation in natural waters. In addition, a sequential analysis of the shape of titration curves is proposed, by which more than two different groups of complexes can be identified in the case of a large ratio between the complexing capacity and trace metal concentration originally present in the water sample. These procedures are illustrated by the analysis of synthetic titration curves for two and three groups of different complexes. The complexation parameters were reproduced with satisfactory accuracy in both cases. Simultaneous use of both the van den Berg-Ružić and Scatchard plots is suggested in order to test the agreement between the experimental titration data and theoretical predictions based on the complexation parameters determined.

INTRODUCTION

Interaction of trace metals with organic ligands in natural waters is a subject of great interest in scientific literature. This interaction is responsible for the fate of trace metals in an aquatic environment. This phenomenon has been studied experimentally by many authors.[1–4] A significant amount of experimental data is available in literature, obtained by direct titration

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

of natural water with and without pretreatment of samples. However, the analysis of such experimental data and their interpretation involve great difficulties due to the fact that natural organic material represents a complex system of binding sites.[5–9] First of all, natural organic material is a collection of a variety of different organic compounds and, in addition, individual organic macromolecules also include different types of binding sites. In Appendix A, it is shown that polynuclear complexes with several active sites per molecule will behave like a group of individual complexes whose partial stability constants will act as stability constants of individual sites. Therefore, instead of dealing with solutions of organic materials, we are investigating the so called »solution of binding sites« of different binding strengths measured through the corresponding stability constants. Then, the results of direct titration of natural waters with trace metals are the consequence of a whole series of possible interactions of trace metals with individual binding sites. An equilibrium model of such interactions could be based on the assumption that, in an aquatic system, binding sites could be treated in groups of similar binding strengths (the so called discrete model), or as a system of continuously distributed binding strengths. Both models have been examined and discussed by a number of authors.[5–9] Two methods for interpretation of the results of direct titration, based on the discrete model, have been reported so far in literature. Both of them make use of special plots in which the original experimental data (bound or free metal concentration vs. total metal concentration) are given in a linearized form for a single group of 1:1 binding sites.

Scatchard[10,11] proposed a plot of the ratio between bound (M_T – M) and free metal concentrations (M) *vs.* bound metal concentration:

$$(M_T - (M)) / (M) = K \cdot [L_T - (M_T - (M))] \tag{1}$$

where K is an overall conditional stability constant (obtained from the slope of the corresponding plot), and L_T and M_T are total concentrations of the available binding sites and metal, respectively. This plot is convenient for investigating the shape of the titration curve at lower additions of metal.

Later, van den Berg,[12–13] Ružić,[14–16] and Lee[17] independently proposed a plot of the ratio between free and bound metal concentration vs. free metal concentration.

$$(M) / (M_T - (M)) = ((M) + 1/K) / L_T \tag{2}$$

with a slope of $1/L_T$. This plot is convenient for investigating the shape of the titration curve at larger additions of metal. However, in the case of a single group of 1:1 binding sites of similar binding strengths both methods could be equally well used for the interpretation of direct titration data.

A problem arises in the interpretation of direct titration data when more than one group of binding sites can be detected. In such a case, in both plots, the overall conditional stability constant K becomes a function of free metal concentration. In Appendix B, a general expression for such a stability function is developed and should read:

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

$$[(\mathrm{M})^{n-1} / K_1^* + (\mathrm{M})^{n-2} / \overline{K_i K_j} + \ldots +1 / \Pi K_i \ ] \qquad (3)$$

where

$$K_1^* = L_\mathrm{T} / \Sigma(L_{\mathrm{T}i}/K_i), \text{ and } L_\mathrm{T} = \Sigma L_{\mathrm{T}i} \ .$$

Here, n is the number of different groups of binding sites, K_i, $L_{\mathrm{T}i}$ are individual complexation parameters and K_i^* and $\overline{K_i K_j}$ are different overall stability constants. The total concentration of all binding sites is the so called complexing capacity. Stabilities of all binding sites in a given concentration range could not be recognized from titration data.

Ružić[15,18] proposed a method for determination of individual complexation parameters (two pairs of stability constants and total ligand/site concentrations) in the case when only two different binding sites could be discerned from the titration data. In that case, the stability function K could be written in the following way:

$$K = ((\mathrm{M}) + 1/K_2^*) / ((\mathrm{M})/K_1^* + 1/K_1K_2) \qquad (4)$$

where K_1 and K_2 are individual stability constants, and K_1^*, K_2^* are two different overall values:

$$K_1^* = L_\mathrm{T} / (L_{\mathrm{T}_1}/K_1 + L_{\mathrm{T}_2}/K_2)$$

and

$$K_2^* = L_\mathrm{T} / (L_{\mathrm{T}_1}/K_2 + L_{\mathrm{T}_2}/K_1) \ .$$

Here, L_{T_1}, L_{T_2} are total concentrations of individual sites and $L_\mathrm{T} = L_{\mathrm{T}_1} + L_{\mathrm{T}_2}$ is the so called complexing capacity. At very low metal concentrations, the stability function becomes a true constant:

$$\mathrm{K} \approx K_1K_2/K_2^* = (L_{\mathrm{T}_1}K_1 + L_{\mathrm{T}_2}K_2) / L_\mathrm{T} = K_0^* \qquad (5)$$

while at very high metal concentrations, the stability function also becomes a true constant:

$$K \approx K_1^* \ .$$

As mentioned by Morel *et al.*[19] the difficulty of analyzing the titration curve is that very often it is not possible to perform direct titration at extremely high metal concentrations. Because of this, no accurate asymptote of the titration curve can be obtained in the linearized plot. The purpose of this paper is to overcome this difficulty and to develop a new, more accurate, method for the determination of individual complexation parameters from titration data presented in the van den Berg-Ružić plot.

DEVELOPMENT OF THE NEW METHOD

The asymptote for large additions of metal for relation (2) can be written in the following way:

$$(\mathrm{M}) / (M_\mathrm{T} - (\mathrm{M})) = ((\mathrm{M}) + 1/K_1^*) / L_\mathrm{T} \,. \tag{6}$$

This asymptote is estimated from data in the region of moderately large addition of metal where the slope of the titration curve does not coincide exactly with the $1/L_\mathrm{T}$ value (see curve 1 from Figure 1). The lower asymptote represents the first estimate. It is a straight line taken from the experimental points (in Figure 1. the last two points on the right side of the plot).

A better estimate of the real asymptotic slope of the titration curve can be written in the following way:

$$\mathrm{d}[(\mathrm{M}) / (M_\mathrm{T} - (\mathrm{M}))] / \mathrm{d}(\mathrm{M}) = \mathrm{d}[((\mathrm{M}) + 1/K) / L_\mathrm{T}] / \mathrm{d}(\mathrm{M}) = (1/L_\mathrm{T}) \cdot [1 + \mathrm{d}(1/K) / \mathrm{d}(\mathrm{M})] \tag{7}$$

and from Eq. (4) it follows that:

$$\mathrm{d}(1/K) / \mathrm{d}(\mathrm{M}) = (1/K_1^* K_2^* - 1/K_1 K_2) / ((\mathrm{M}) + 1/K_2^*)^2 \,.$$

Therefore, the slope of the titration curve can be corrected to obtain a more accurate estimate of the real slope of the asymptote in the following way:

$$1/L_\mathrm{T} \approx \{[(\mathrm{M}) / (M_\mathrm{T} - (\mathrm{M}))] / \mathrm{d}(\mathrm{M})\} / [1 + (1/K_1^* K_2^* - 1/K_1 K_2) / ((\mathrm{M}) + 1/K_2^*)^2] \,. \tag{8}$$

From the first estimate of the asymptote, the complete set of complexing parameters (not accurate enough) is determined. Then, instead of $1/L_\mathrm{T} \approx \approx \{ \ldots \}$, where the bracket from the Eq. (8) represents the initial slope, *i.e.* the first derivative of $(\mathrm{M}) / (M_\mathrm{T} - (\mathrm{M}))$ at large additions of metal, is corrected and the new slope value is obtained using Eq. (8) from the old slope divided by [...] bracket.

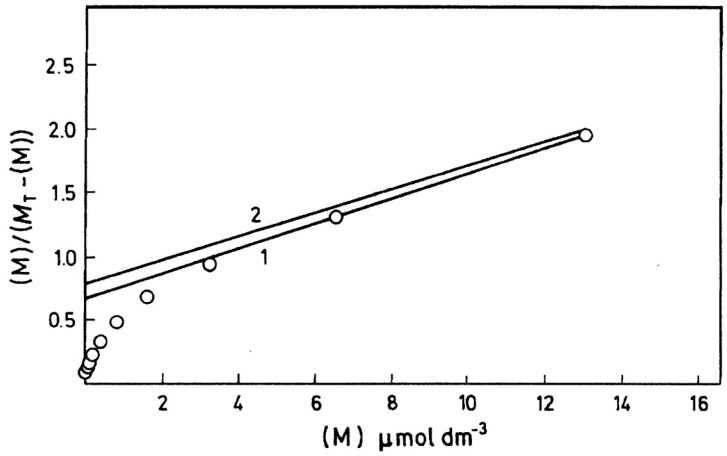

Figure 1. Van den Berg-Ružić plot for synthetic titration data (circles) for two different groups of binding ligands (K_1 = 0.1 dm^3 mol^{-1}, L_{T_1} = 10 mol dm^{-3}, K_2 = 10 dm^3 mol^{-1}, L_{T_2} = 1 mol dm^{-3}). Line 1 first estimate and line 2 final estimate of complexation parameters after the correction procedure.

When the slope of the asymptote is corrected one can also estimate the intersection of the asymptote with the ordinate (M) / (M_T – (M)). The intersection of the tangent of the corrected slope with the ordinate can be written in the following way:

$$(M) / (M_T - (M)) - (M) / L_T = 1/KL_T \ .$$

Corrected intersection of the asymptote with the ordinate should be $1/K_1^* L_T$. Hence, the estimate of this intersection is:

$$1/K_1^* L_T \approx [(M) / (M_T - (M)) - (M)/L_T] \cdot K/K_1^* =$$
$$[(M) / (M_T - (M)) - (M)/L_T] \cdot ((M) + 1/K_2^*) / ((M) + K_1^*/K_1K_2) \ . \qquad (9)$$

The first estimate of the intercept is made using the first asymptote $1/K_1^* L_T \approx$ [...] where the bracket from the Eq. (9) represents the initial estimate of the intercept (using the old slope $1/L_T$ inside this bracket). Then the new intercept value is corrected by multiplying the old intercept by

$$((M) + 1/K_2^*) / ((M) + K_1^*/K_1K_2)$$

according to Eq. (9). The new L_T value is obtained form the new slope (as the inverse value of the slope), and new K_1^* value is obtained from the new

intercept and new L_T value. In the next step, new values for L_T and K_1^* should replace old values and the procedure should be repeated till stable new values are obtained (about 30 iterations should be enough for most cases).

This procedure should work well if only two different groups of binding sites could be detected from the direct titration data. In the cases where more than two groups of different binding sites can be detected, one can expect the influence of stronger binding sites on the precision of the complexation parameters determined. In order to test the proposed procedure, we will analyze the relevant synthetic titration curves.

It is also possible to develop similar corrections for the Scatchard plot whose asymptote in the range of higher metal concentrations can be described with the following Eq.:

$$(M_T - (M)) / (M) = K_1^* \cdot [L_T - (M_T - (M))] \ . \tag{10}$$

The slope of the Scatchard plot can be written in the following way:

$$\mathrm{d}[(M_T - (M)) / (M)] / \mathrm{d}(M_T - (M)) = [L_T - (M_T - (M))] \cdot \mathrm{d}K / \mathrm{d}(M_T - (M)) - K \tag{11}$$

and from Eq. (4) it follows that:

$$\mathrm{d}K / \mathrm{d}(M_T - (M)) = - \mathrm{dK/d(M)} = (1/K_1^*K_2^* - 1/K_1K_2) / ((M)/K_1^* + 1/K_1K_2)^2 \ . \tag{12}$$

Therefore, one can correct the slope of the Scatchard plot by combining Eqs. (4), (11) and (12) in the following way:

$$K_1^* \approx \mathrm{d}[(M_T - (M)) / (M)] / \mathrm{d}(M_T - (M)) / [1 + A \cdot (1 - B)] \tag{13}$$

where

$$A = (1/K_1^*K_2^* - 1/K_1K_2) / ((M) + K_1^*/K_1K_2)$$

and

$$B = [L_T - (M_T - (M))] / ((M)/K_1^* + 1/K_1K_2) \ .$$

The interception with the ordinate can be obtained using the interception with the abscissa in the form of a product ($L_T \cdot K_1^*$). However, the Scatchard plot is less sensitive than the van den Berg-Ružić plot in the range of higher additions of metal and, therefore, the former is more suitable for the determination of L_T and K^* values.

TESTING OF THE NEW METHOD USING WITH SYNTHETIC DATA

In order to test the new method, we used the synthetic titration curve obtained with by following Eq.:

$$(\mathrm{M}) / (M_T - (\mathrm{M})) = 1 / [L_{T_1} / ((\mathrm{M}) + 1/K_1) + L_{T_2} / ((\mathrm{M}) + 1/K_2)] \; . \quad (14)$$

It is known that stronger binding sites are less abundant in natural waters and, therefore, we used the following complexation parameters for generation of synthetic data:

$$K_1 = 0.1 \text{ dm}^{-3} \text{ µmol}^{-1} \qquad L_{T_1} = 10 \text{ µmol}^{-1} \text{ dm}^{-3}$$
$$K_2 = 10 \text{ dm}^{-3} \text{ µmol}^{-1} \qquad L_{T_2} = 1 \text{ µmol}^{-1} \text{ dm}^{-3}$$
$$L_T = 11 \text{ µmol}^{-1} \text{ dm}^{-3}$$

In order to test the influence of higher binding strengths, additional synthetic titration data generated by the following Eq. have been used:

$$(\mathrm{M}) / (M_T - (\mathrm{M})) = 1 / [L_{T_1} / ((\mathrm{M}) + 1/K_1) + L_{T_2} / ((\mathrm{M}) + 1/K_2) + L_{T_3} / ((\mathrm{M}) + 1/K_3)] \; . \quad (15)$$

Complexation parameters for the higher binding sites used for the generation of synthetic data using Eq. (15) were:

$$K_3 = 1000 \text{ dm}^{-3} \text{ µmol}^{-1} \qquad L_{T_3} = 0.1 \text{ µmol}^{-1} \text{ dm}^{-3}$$
$$L_T = 11.1 \text{ µmol}^{-1} \text{ dm}^{-3}$$

In Figure 1, titration data generated by Eq. (14) are presented in the van den Berg-Ružić plot. Such synthetic data were analyzed using the new method described here. Also in Figure 1, the first estimate and the final asymptote (obtained by the above mentioned corrections) are compared. As one can clearly see there is a significant difference between these two asymptotes indicating that the new method will be a useful tool for the estimation of complexation parameters. In Table I, a summary of the results obtained by the analyses of synthetic data for the two groups of complexes is presented. Complexation parameters are obtained using the procedure described earlier by Ružić.[15,16] The inverse value of the difference between the asymptote and the original titration curve is plotted *vs.* free metal concentration and the resulting straight line, together with the asymptote, is used to obtain individual complexation parameters. After the correction described in this paper, a fairly good estimate of the complexation parameters for the first weaker group of complexes can be obtained. Obviously, the first esti-

TABLE I

Summary of the results obtained by the analysis of synthetic data generated by Eq. (14) for two different groups of complexes

Complexation parameters	Original values	First estimate	Final values after corrections (30 iterations)
K_1	0.1	0.1335	0.1050
	10	9.547	9.900
K_2	10	19.22	10.73
	1	0.6763	0.9511
L_T	11	10.22	10.85

mate of complexation parameters is not satisfactory and, in addition, the error in estimation of the complexation parameters for the stronger group of ligands is significantly higher than that for the group of weaker ligands. In order to test the possibility of increasing the accuracy of the analyses of titration data, the complexation parameters determined by the final theoretical prediction for the first weaker group of ligands are extracted from the original titration data in the following way:

$$(M_T - (M)) / (M) - L_{T_1} / ((M) + 1/K_1) = F[(M)] \; . \qquad (16)$$

The new function $F[(M)]$, contains information on the complexation parameters for the stronger group of ligands. In Figure 2, this function is used as the ordinate in the Scatchard plot. One can clearly notice the very good agreement between the reduced titration data (after extraction of the theoretical prediction for the weaker group of ligands) and the theoretical prediction for the stronger group of ligands, pointing to the absence of higher energy binding ligands. This means that, if only two groups of ligands are present, the new method produces a fairly good estimate of complexation parameters for both groups of ligands. In Figures 3A and 3B, original titration data are compared with the final theoretical prediction for both groups of ligands. The agreement between them is satisfactory.

Using Eq. (15), a new set of synthetic titration data including the influence of a third group of high energy binding ligands, was generated. The Van den Berg-Ružić-Lee plot almost coincides with the corresponding plot generated by Eq. (14) for only two groups of ligands (see Figure 1). As shown in Figure 4., the Scatchard plot is significantly different. While in the case of only two weaker groups of ligands the interception with the ordinate gives $(M_T - (M)) / (M) = L_{T_1} \cdot K_1 + L_{T_2} \cdot K_2 = 1$, in the case of three different groups of ligands $(M_T - (M)) / (M) = L_{T_1} \cdot K_1 + L_{T_2} \cdot K_2 + L_{T_3} \cdot K_3 = 11$ (about one order of magnitude higher value). The results of the analysis of titration data

for three groups of ligands are summarized in Table II. Complexation parameters are obtained by the same procedure as in the case of two groups of ligands. After the corrections described in this paper, a fairly good estimate of complexation parameters for the first weaker group of complexes is obtained. Then one can check the influence of higher energy binding ligands on the complexation parameters for the second group of complexes using ex-

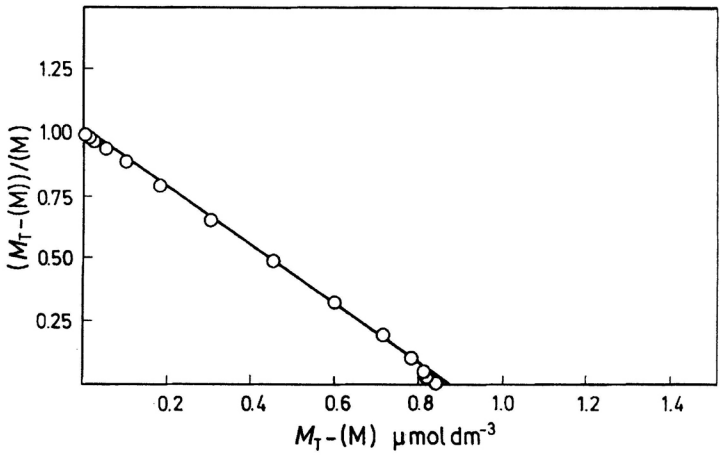

Figure 2. Scatchard plot for reduced synthetic data obtained by extraction of theoretical prediction for the first weaker group of binding ligands from original synthetic data from Figure 1 (circles). The line represents the theoretical prediction for the remaining stronger group of binding ligands.

TABLE II

Summary of the results obtained by the analysis of synthetic data generated by Eq. (15) for three different groups of complexes

Complexation parameters	Original values	First estimate	Final values after corrections (30 iterations)	After extraction of the first group of complexes
K_1	0.1	0.1335	0.1050	–
	10	9.547	9.900	–
K_2	10	19.22	10.73	10.88
	1	0.6763	0.9511	0.9627
L_{12}	11	10.22	10.85	10.88
K_3	1000	–	–	1017
	0.1	–	–	0.09832
L_T	11.1	–	–	10.98

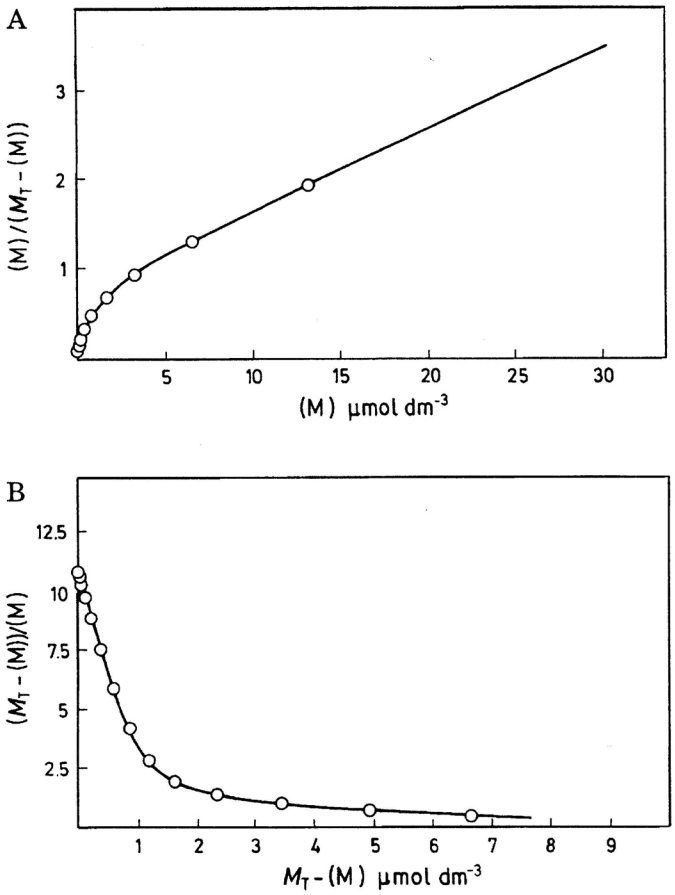

Figure 3. Comparison of original synthetic titration data (circles) with the theoretical prediction for two different groups of binding ligands (line, parameters from Table I). A. Van den Berg-Ružić-Lee plot, and B. Scatchard plot.

traction of the first weaker group of complexes from the original titration data. Reduced titration data can be analyzed again by the procedure described earlier by Ružić[11,12] for two groups of different complexes. In Figure 5, the corresponding Scatchard plot is presented. The correction described in this paper produces fairly good complexation parameters for the second group of ligands from the reduced titration data. Again, the accuracy of the complexation parameters for the third stronger group of complexes can be checked by extraction of two weaker groups of ligands from the original titration data in the following way:

$$(M_T - (M)) / (M) - L_{T_1} / ((M) + 1/K_1) - L_{T_2} / ((M) + 1/K_2) = G[(M)] \quad . \quad (17)$$

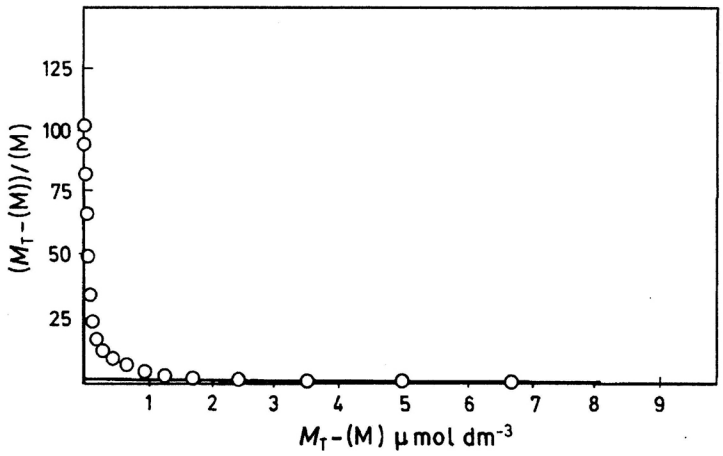

Figure 4. Scatchard plot for synthetic titration data (circles) for three different groups of binding ligands (K_1 = 0.1 dm^3 mol^{-1}, L_{T_1} = 10 mol dm^{-3}, K_2 = 10 dm^3 mol^{-1}, L_{T_2} = 1 mol dm^{-3}, K_3 = 1000 dm^3 mol^{-1}, L_{T_3} = 0.1 mol dm^{-3}). The line represents the theoretical prediction for the weaker group of binding ligands.

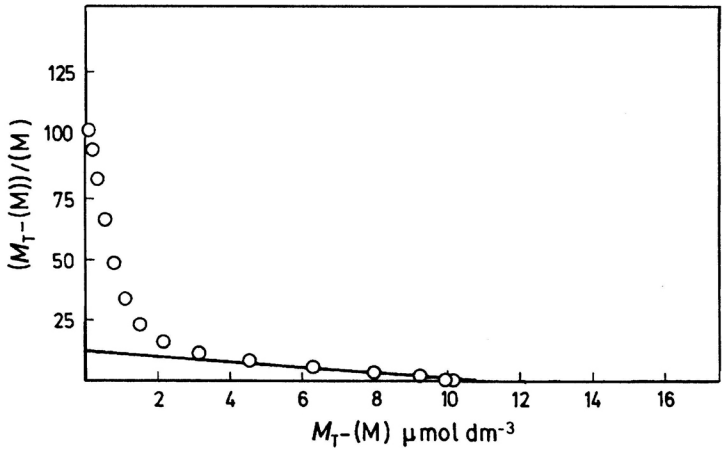

Figure 5. Scatchard plot for reduced synthetic titration data obtained by extraction of the theoretical prediction for the weaker group of binding ligands from the original synthetic titration data from Figure 4 (circles). The line represents the theoretical prediction for the second group of stronger binding ligands.

The new function G[(M)] contains information on the complexation parameters for the third stronger group of complexes. This function can be used as the ordinate in the Scatchard plot and, in that case, it coincides well with the theoretical prediction for the third group of ligands. In order to

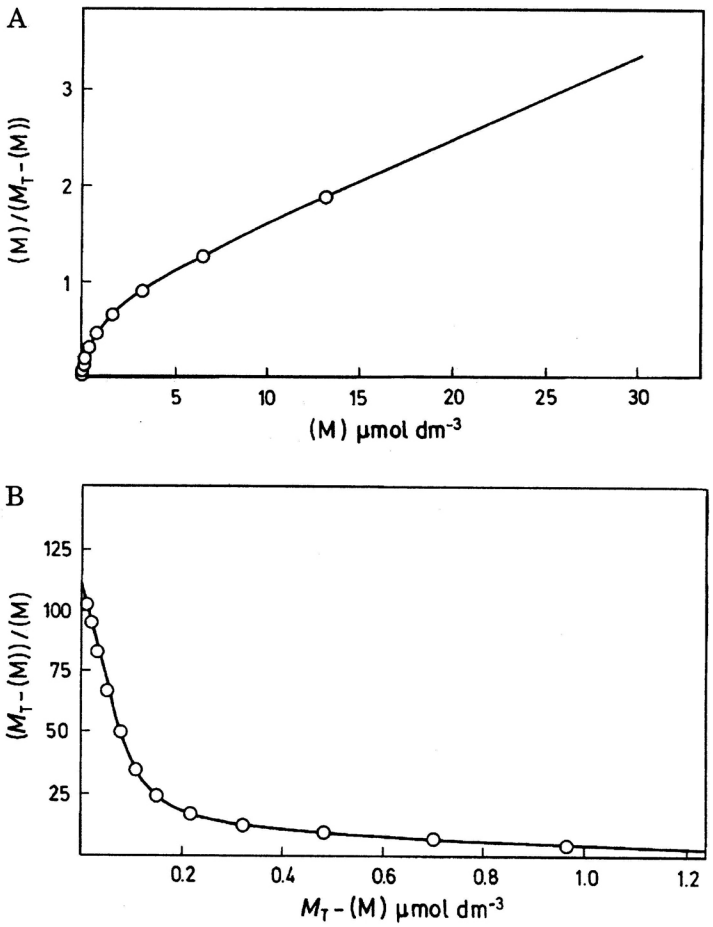

Figure 6. Comparison of the original synthetic titration data (circles) with theoretical prediction for three different binding ligands (line, parameters from Table II). A. Van den Berg-Ružić-Lee plot, and B. Scatchard plot.

check the validity of the complexation parameters determined, the theoretical prediction for all three groups of ligands is compared with the original titration data in Figures 6A and 6B, showing good agreement.

DISCUSSION

Traditional methods for the determination of complexation parameters (complexing capacity and stability constants) used so far are not accurate enough to produce good agreement between experimental points and theo-

retical predictions. In this paper, we propose a new method for the correction of the asymptote in the range of high additions of trace metals. Using this method, a fairly good estimate of complexation parameters for the weaker group of complexes can be obtained. Van den Berg-Ružić plot can be used for this purpose because it is more sensitive in the range of higher additions of metal where the influence of the weaker complexes can be well seen. In order to check the accuracy of the complexation parameters for higher energy binding sites, one should calculate the reduced titration data by extraction of the weaker complexes from the original titration data, as described with Eq. (16). Such reduced titration data should be compared with the theoretical prediction for high energy binding sites. If good agreement is reached this means that from the titration data only two different groups of ligands can be identified. If the agreement is not good enough, this means that a third group of even stronger complexes could be identified. In such a case, the reduced titration data should be analyzed by the method proposed in this paper under the assumption that again only two different groups of complexes could be identified from such reduced titration data. This procedure should be repeated until the good agreement between reduced titration data and the theoretical prediction for the high energy binding sites is reached. A good example is the analysis of the titration curves of Lake Tjeukemeer water with Cu(II) developed by Verweij and Ružić.[20] In their work, four different groups of ligands have been successfully identified using the analysis proposed in this paper. The number of different groups of ligands which can be identified from titration data depends on the ratio between the complexing capacity of the system and the total metal concentration originally present, before addition of metal into the system investigated. Trace metals are usually present in natural waters in concentrations between 10^{-8} and 10^{-7} mol dm^{-3}. Therefore, for complexing capacities between 10^{-7} and 10^{-6} mol dm^{-3}, in most cases a single group of complexes can be identified by direct titration of such natural waters. Two different groups of binding ligands can be identified if the ratio between the complexing capacity and the total metal concentration originally present in the system is higher than 10. Higher energy binding sites can be identified only if the complexing capacity is significantly higher than 10^{-7} mol dm^{-3}. Several authors proposed the use of nonlinear regression in order to determine complexing parameters.[21,22] The main disadvantage of these methods is that a number of different stabilities should be assumed in order to define the analytical expression by which the regression analysis can be performed. However, the number of different stabilities is chosen arbitrarily and this can influence the results obtained. Once the number of different stabilities is chosen, all of them are treated equally as if they had the same degree of importance in the treatment of experimental data. The fact is that, at higher additions of trace metal, the weakest complexes play the most important role. First of all because they are the most abundant, and second because they contribute to the overall value K_1^* as a sum of inverse values of individuals stabilities.

The method proposed here takes into account this fact. In addition the use of continuous models of binding strengths has been proposed by several authors.[23] For this purpose, a series of different assumed distribution functions or inverse methods for obtaining such a distribution function from the original experimental data have been proposed.[24–26] It has been demonstrated that none of the assumed distribution functions (except for the Boltzmann distribution function) produce accurate results.[27] At the same time, determinations of the distribution function from the experimental results are often unreliable, unstable and yield very different results even when applied to the same experimental data.[28] Therefore, the method proposed here has definite advantages in cases where the titration window limiting the number of individual stabilities which could be generated by a discrete model of stabilities is relatively small. In cases where the titration window is wide enough (too many individual stabilities should be determined), the continuous model of stabilities based on the Boltzmann distribution function should be used.

APPENDIX A.

Let us assume that the trace metal M is bound to a macromolecular ligand with several active sites per molecule. Total and free concentrations for the trace metal and macromolecular ligand are M_T, (M), L_T and (L), respectively. The concentration of an individual plynuclear complex is (M_iL). In such a case, one can write the following balance Eq.:

$$M_T - (M) = \Sigma_i \cdot (M_iL) \tag{A1}$$

and

$$L_T - (L) = \Sigma\,(M_iL)\,. \tag{A2}$$

Individual partial stability constants can be defined in the following way:

$$k_i = (M_iL) / (M_{i-1}L)(M) \tag{A3}$$

Combining Eqs. (A1), (A2) and (A3) yields the following result:

$$(M_T - (M)) / (M) = L_T \cdot \Sigma i \cdot (M)^{i-1} \Pi k_j / [1 + \Sigma(M)^i \cdot \Pi k_j]\,. \tag{A4}$$

After some rearrangements, one can rewrite Eq. (A4) in the following way:

$$(M_T - (M)) / (M) = L_T \cdot \Sigma k_i \cdot \prod_{j=i} (1 + k \cdot (M)) / \Pi\,(1 + k_i \cdot (M)) =$$

$$L_T \cdot \Sigma\, 1 / ((M) + 1/k_i)\,. \tag{A5}$$

The complexing capacity for such a polynuclear ligand is $(n \cdot L_T)$, where n is the number of active sites per molecule. Hence, one can conclude that the macromolecule forming polynuclear complexes in binding trace metals behaves as an equivalent of n individual ligands whose stability constants are partial stability constants of individual binding sites.

APPENDIX B.

Let us assume that the trace metal M is bound into different type of complexes of different binding strengths at individual binding sites whose total and free concentrations are L_{Ti} and (L_i), respectively. Total and free metal concentrations are M_T and (M) and the corresponding balance equation should read:

$$M_T - (M) = \Sigma (L_{Ti} - (L_i)) . \tag{B1}$$

An individual stability constant can be defined in the following way:

$$K_i = (L_{Ti} - (L_i)) / (M)(L_i) \tag{B2}$$

$$\begin{aligned}(M_T - (M)) / (M) &= \Sigma L_{Ti} / ((M) + 1/K_i) = \\ &[(M)^{n-1} \cdot \Sigma L_{Ti} + (M)^{n-2} \cdot \Sigma\Sigma L_{Ti}/K_j + \ldots + \Sigma L_{Ti}K_i / \Pi K_j] / \\ &[(M)^n + (M)^{n-1} \cdot \Sigma 1/K_i + (M)^{n-2} \cdot \Sigma 1/K_iK_j + \ldots \\ &+ 1/\Pi K_i] = L_T / ((M) + 1/K)\end{aligned} \tag{B3}$$

where

$$L_T = \Sigma L_{Ti} \tag{B4}$$

$$\begin{aligned}K = &[(M)^{n-1} \cdot \Sigma 1/K_i - (\Sigma\Sigma(L_{Ti}/K_j) / \Sigma L_{Ti}) + (M)^{n-2} \cdot \\ &(\Sigma 1/K_iK_j - (\Sigma\Sigma\Sigma L_{Ti}/K_iK_j) / \Sigma L_{Ti} + \ldots + 1/\Pi K_i)] / \\ &[(M)^{n-1} + (M)^{n-2} \cdot (\Sigma\Sigma L_{Ti}/K_j) / (\Sigma L_{Ti} + \ldots + \\ &\Sigma L_{Ti}L_i / \Pi K_j)] .\end{aligned} \tag{B5}$$

After some rearrangements, one can rewrite expression (B5) in the following way:

$$\begin{aligned}K = &[(M)^{n-1} + (M)^{n-2}/K_2^* + \ldots {}^*K_1^* / \Pi K_1^*] / \\ &[(M)^{n-1}/K_1^* + (M)^{n-2} / \overline{K_i}\overline{K_j} + \ldots 1/\Pi K_i] /\end{aligned} \tag{B6}$$

where

$$K_1^* = \Sigma 1/K_i - (\Sigma\Sigma L_{Ti}/K_j) / \Sigma L_{Ti} = \Sigma(L_{Ti}/K_i) / \Sigma L_{Ti} .$$

At higher additions of metal, the overall stability function becomes a true constant K^*. The sum of individual site concentrations L_T is known as the complexing capacity of the system.

Acknowledgment. – The authors gratefully acknowledge the support of the National Science Fund of Croatia.

REFERENCES

1. P. C. Singer (Ed.), *Trace Metal and Metal Organic Interactions in Natural Waters*, Ann Arbor Science, Ann Arbor, MI, 1973.
2. C. J. M. Kramer and J. C. Duinker (Eds.), *Complexation of Trace Metals in Natural Waters*, Proc. of the Intern. Symp., The Netherlands Institute for Sea Research of Texel, May 2–6, 1983, Martinus Nijhoff/Dr. W. Junk, The Hague, 1984.
3. J. Buffle, *Complexation in Aquatic Systems: An Analytical Approach*, Horwood, Chichester, 1988.
4. G. E. Batley (Ed.), *Trace Element Speciation: Analytical Methods and Problems*, CRC Press, 1989.
5. D. C. Melchior and R. L. Bassett (Eds.), *Chemical Modeling of Aqueous Systems II*, ACS Symp. Series 416, ACS, 1990.
6. D. A. Dzombak, W. Fish, and F. M. M. Morel, *Environ. Sci. Technol.* **20** (1986) 669–675.
7. W. Fish, D. A. Dzombak, and F. M. M. Morel, *Environ. Sci. Technol.* **20** (1986) 676–683.
8. I. Ružić, *Marine Chemistry* **53** (1996) 1–15.
9. I. Ružić, *Anal. Chim. Acta* **313** (1995) 139–143.
10. G. Scatchard, *Ann. N. J. Acad. Sci.* **51** (1947) 660–672.
11. G. Scatchard, J. S. Coleman, and A. L. Shen, *J. Am. Chem. Soc.* **79** (1957) 12–20.
12. C. M. G. van den Berg and J. R. Kramer, *Anal. Chim. Acta* **106** (1979) 113–120.
13. C. M. G. van den Berg, *Marine Chemistry* **11** (1882) 307–322.
14. I. Ružić, *Thalas. Jugosl.* **16** (1980) 325.
15. I. Ružić, *Anal. Chim. Acta* **140** (1982) 99–113.
16. I. Ružić, *Kinetics of complexation and determination of complexation parameters in natural waters,* Proc. of the Intern. Symp. on Complexation of Trace Metals in Natural Waters, C. J. M. Kramer and J. C. Duinker (Eds.), The Netherlands Institute for Sea Research of Texel, May 2–6, 1983, Martinus Nijhoff/Dr. W. Junk, The Hague, 1984, pp. 130–147.
17. J. Lee, *Water. Res.* **17** (1983) 501–510.
18. I. Ružić, 1987, *Environ. Sci. Technol.* **21** (1987) 1132–1135.
19. F. M. M. Morel, D. A. Dzombak, and W. Fish, *Environ. Sci. Technol.* **21** (1987) 1135–1136.
20. W. Verweij and I. Ružić, *Croat. Chem. Acta* **70** (1996) 419–434.
21. J. C. Westall, J. C. Zachary, and F. M. M. Morel, *MINEQL* – A computer program for the calculation of chemical equilibrium of aqueous systems, Techn. Note No. 18, Water Quality Lab., Dept. of Civil Eng., MIT, Cambridge, MA, 1976.
22. J. Westall, *MICROQL–II.*, Techn. Rep. EAWAG, Dubendorf, Switzerland, 1979.

23. D. S. Gamble, A. W. Underdown, and C. H. Langford, *Anal. Chem.* **52** (1980) 1901–1908.
24. F. Karush and M. Sonenberg, *J. Am. Chem. Soc.* **71** (1949) 1369–1376.
25. E. M. Perdue and C. R. Lytle, *Environ. Sci. Technol.* **17** (1983) 654–660.
26. W. H. van Riemsdijk, J. C. M. de Wit, L. K. Koopal, and G. H. Bolt, *J. Colloid Interface Sci.* **116** (1987) 511–522.
27. I. Ružić, *Fizika (Zagreb)* **A3** (1994) 177–192.
28. M. Černik, N. Borkovec, and J. Westall, *Environ. Sci. Technol.* **29** (1995) 413–425.

SAŽETAK

Nova metoda za analizu podataka u studiju kompleksiranja tragova metala

Ivica Ružić i Jadranka Pečar

Razvijena je nova metoda za korekciju asimptote u dijagramu van den Berg-Ružić za visoke dodatke tragova metala pri direktnoj titraciji prirodnih voda. Tom se metodom mogu dobiti precizniji podaci za vrijednosti kapaciteta kompleksiranja. Preciznost određivanja tog parametra predstavlja ograničavajući faktor u detaljnom ispitivanju kompleksiranja tragova metala u prirodnim vodama. Pored toga predložena je i sekvencijalna metoda analize oblika titracijske krivulje, kojom je moguće identificirati više od dvije različite skupine kompleksa u slučajevima velikih kvocijenata kapaciteta kompleksiranja i koncentracije tragova metala originalno prisutnih u uzorcima prirodnih voda. Te su procedure ilustrirane analizom sintetskih krivulja titracije za dvije i tri skupine različitih kompleksa. Parametri kompleksiranja reproducirani su sa zadovoljavajućom preciznošću u oba slučaja. Preporučena je istovremena primjena obiju metoda u kojima se koriste dijagrami koje su opisali van den Berg-Ružić i Scatchard kako bi se pouzdanije testiralo slaganje između eksperimentalnih titracijskih krivulja i teorijskih predviđanja dobivenih na osnovi ranije određenih parametara kompleksiranja.