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ELECTROPHORETIC INVESTIGATIONS OF THE COMPLEXING OF CADMIUM AND ZINC WITH EDTA

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SUMMARY

The formation of EDTA complexes of ^{109}Cd and ^{65}Zn in KNO_3 solutions has been investigated by high-voltage paper electrophoresis. The concentration of EDTA was varied from $1\ \mu\text{M}$ to $10\ \text{mM}$, the concentrations of inactive cadmium in the systems were 0 , $0.1\ \text{mM}$ and $1\ \text{mM}$, the concentrations of inactive zinc were 0 , $1\ \mu\text{M}$, $10\ \mu\text{M}$ and $0.1\ \text{mM}$, and the pH of the system was 8.1 . The behaviour of cadmium and zinc was examined by measuring the dependence of their electrophoretic mobilities on the EDTA concentration and on the age of the system up to 5 days. From the results obtained, it is evident that in $0.1\ \text{M}\ \text{KNO}_3$ both ions have similar cationic mobilities. At very low and at very high concentrations of EDTA, cadmium and zinc give well-defined cationic and anionic zones, respectively. In the intermediate range of EDTA concentration, *i.e.*, in the transition region, ^{109}Cd gives well-defined zones with continuous change in electrophoretic mobilities, but ^{65}Zn gives very pronounced multi-zonal tailing. This indicates that EDTA reacts rapidly with cadmium, but rather slowly with zinc. From the results, the variation in effective stability constants of the cadmium-EDTA and zinc-EDTA complexes with the age of the system was calculated, and the values are compared with the effective stability constants obtained for both ions in sea water and $0.55\ \text{M}\ \text{NaCl}$ systems at pH 8.1 . The stability constants have also been calculated from the electrophoretic data using methods devised by other authors; these methods are discussed on the basis of the results obtained.

INTRODUCTION

Electrophoresis has been used in the study of metal-ion complexes in aqueous solutions by Shvedov and Stapanov¹ and by Jokl and his co-workers²⁻⁵, Jercan and Popa⁶, Pučar⁷, Musani-Marazović and Pučar⁸⁻¹⁰. This technique yields information on the physico-chemical behaviour of metal complexes, the number of stable species and the sign of the electrical charge on a complexed ion. From electrophoretic data, it is also possible to calculate the effective stability constants (conditional equilibrium constants) of a metal ion-ligand complex, even in such multicomponent systems as sea water.

The aim of this work was to compare the behaviour of Cd- and Zn-EDTA

complexes in a conventional supporting electrolyte KNO_3 at pH 8.1 and ionic strength 0.1 with previously published results⁸⁻¹⁰ on their behaviour in sea water.

From the variation in electrophoretic mobility with concentration of EDTA, effective stability constants could be calculated, and the values for both ions in sea water and in 0.55 M NaCl could be compared.

MATERIALS AND METHODS

The electrophoretic experiments were performed as described previously¹¹, except that the temperature of the gasoline was maintained constant by means of an ultra-thermostat^{12,13}.

Cadmium complexes

The supporting electrolytes used (each of ionic strength 0.1) were

- (a) $\text{KNO}_3 + 1 \text{ mM}$ barbitone,
- (b) $\text{KNO}_3 + 0.1 \text{ mM}$ $\text{Cd}(\text{NO}_3)_2 + 1 \text{ mM}$ barbitone, and
- (c) $\text{KNO}_3 + 1 \text{ mM}$ $\text{Cd}(\text{NO}_3)_2 + 1 \text{ mM}$ barbitone,

with or without EDTA; the ionic strength was kept constant by varying the KNO_3 concentration. The concentration of EDTA was varied from 1 μM to 10 mM, and the pH was adjusted to 8.1. For the filter paper used (Munktel 302), the correction factor due to the effect of the paper structure was 2.01; the free length and width of the paper strips was $66 \times 1.5 \text{ cm}$. The electrophoretic parameters were 2200 V and 11 mA per strip (current density per 1 cm width of strip, 7.3 mA), and the specific electric effect on the strips was 0.24 VA cm^{-2} . The duration of an experimental run was 40 min, the temperature of the strip was $25 \pm 0.1^\circ$, and the temperature of the gasoline was $19 \pm 0.1^\circ$.

The solutions of ^{109}Cd were prepared from an original solution of carrier-free ^{109}Cd in 0.5 M HCl (NEN Chemicals GmbH) by evaporation to dryness and adding the appropriate supporting electrolyte to the residue. The specific activities of these solutions were 1 $\mu\text{C}/20 \mu\text{l}$ (20 μl was the volume of solution applied to the starting point of each electrophoretic strip). The concentration of carrier-free ^{109}Cd added to the filter-paper strip at the point of application was *ca.* 35 nM.

Zinc complexes

The supporting electrolytes used (each of ionic strength 0.1) were

- (a) $\text{KNO}_3 + 1 \text{ mM}$ barbitone,
- (b) $\text{KNO}_3 + 1 \mu\text{M}$ $\text{Zn}(\text{NO}_3)_2 + 1 \text{ mM}$ barbitone,
- (c) $\text{KNO}_3 + 10 \mu\text{M}$ $\text{Zn}(\text{NO}_3)_2 + 1 \text{ mM}$ barbitone, and
- (d) $\text{KNO}_3 + 0.1 \text{ mM}$ $\text{Zn}(\text{NO}_3)_2 + 1 \text{ mM}$ barbitone,

with or without EDTA. The concentration of EDTA was varied from 1 μM to 1 mM. The electrophoretic parameters were 1100 V and 5.7 mA per strip (current density per 1 cm width of strip 3.6 mA) and the specific electric effect on the strips was 0.06 VA cm^{-2} . The duration of an experiment was 36 min, the temperature of the gasoline was $23.3 \pm 0.1^\circ$, and the other conditions were as for the cadmium complexes.

The solutions of ^{65}Zn were prepared from an original solution of carrier-free ^{65}Zn in 1 M HCl (NEN Chemicals GmbH) by evaporation to dryness and adding the

supporting electrolyte to the residue. The specific activities of these solutions were $1 \mu\text{C}/20 \mu\text{l}$, and the concentration of carrier-free ^{65}Zn was *ca.* 20 nM.

The behaviour of ^{109}Cd and ^{65}Zn was studied by measuring the change in electrophoretic mobility of carrier-free ^{109}Cd and ^{65}Zn with the concentration of EDTA and the age of the system (from 0 to 5 days).

The electrophoretic mobilities (in $\text{mm V}^{-1}\text{h}^{-1} \text{cm}$) were corrected as described earlier¹¹; these mobility values can easily be transformed into the usual dimensions ($\text{cm}^2 \text{V}^{-1} \text{sec}^{-1}$) by multiplying by 0.278×10^{-4} (see ref.¹¹).

The variation in effective stability constants of the cadmium-EDTA and zinc-EDTA complexes and the number of ligands (*n*) per atom of cadmium or zinc with the age of the system were calculated from the electrophoretic data according to the method developed by Pučar⁷, using the C.I.I. Model 90.40 computer at the "Rudjer Bošković" Institute.

RESULTS

The electrophoretic behaviour of cadmium and zinc was dependent on the concentration of EDTA in all the systems investigated. Both ions behaved as cations at lower concentrations of EDTA, and as anions at higher concentrations of EDTA; in the "transition region", cadmium and zinc changed their electrophoretic mobilities from cationic to anionic in nature. The age of the system was significant only with ^{65}Zn .

Cadmium complexes

Fig. 1a shows the electrophoretic mobilities of ^{109}Cd in KNO_3 -EDTA systems as a function of the log. of the concentration of EDTA; the age of the system had no significant effect. In Figs. 1b and 1c, the electrophoretic mobilities of ^{109}Cd are presented as a function of the log. of the concentration of EDTA in the systems with 0.1 mM and 1 mM inactive $\text{Cd}(\text{NO}_3)_2$ added, respectively.

Comparison of Figs. 1b and 1c shows that the transition region of complexing was shifted to higher concentrations of EDTA when $\text{Cd}(\text{NO}_3)_2$ solution was added to the system. In the system with 0.1 mM $\text{Cd}(\text{NO}_3)_2$ added, the transition region lay between 20 μM and 0.2 mM EDTA (Fig. 1b). For the system with 1 mM $\text{Cd}(\text{NO}_3)_2$ added (Fig. 1c), the transition region was shifted between 0.17 and 1.8 mM EDTA.

Zinc complexes

Fig. 2a shows the behaviour of ^{65}Zn in KNO_3 -EDTA systems. In the transition region, cationic and anionic "tailing" occurs, giving ill-defined electrophoretic zones. At EDTA concentrations greater than 146 μM , there was no change in the anionic electrophoretic mobility of ^{65}Zn .

Figs. 2b, 2c and 2d show the electrophoretic mobilities of ^{65}Zn as a function of the log. of the concentration of EDTA when 1 μM , 10 μM and 0.1 mM $\text{Zn}(\text{NO}_3)_2$ were added to the system.

When the concentration of zinc in the system was 1 μM (Fig. 2b), ^{65}Zn behaved as a cation up to an EDTA concentration of 10 μM , and in the transition region between 46.5 μM and 0.333 mM EDTA, ^{65}Zn showed pronounced "tailing" in both cationic and anionic directions.

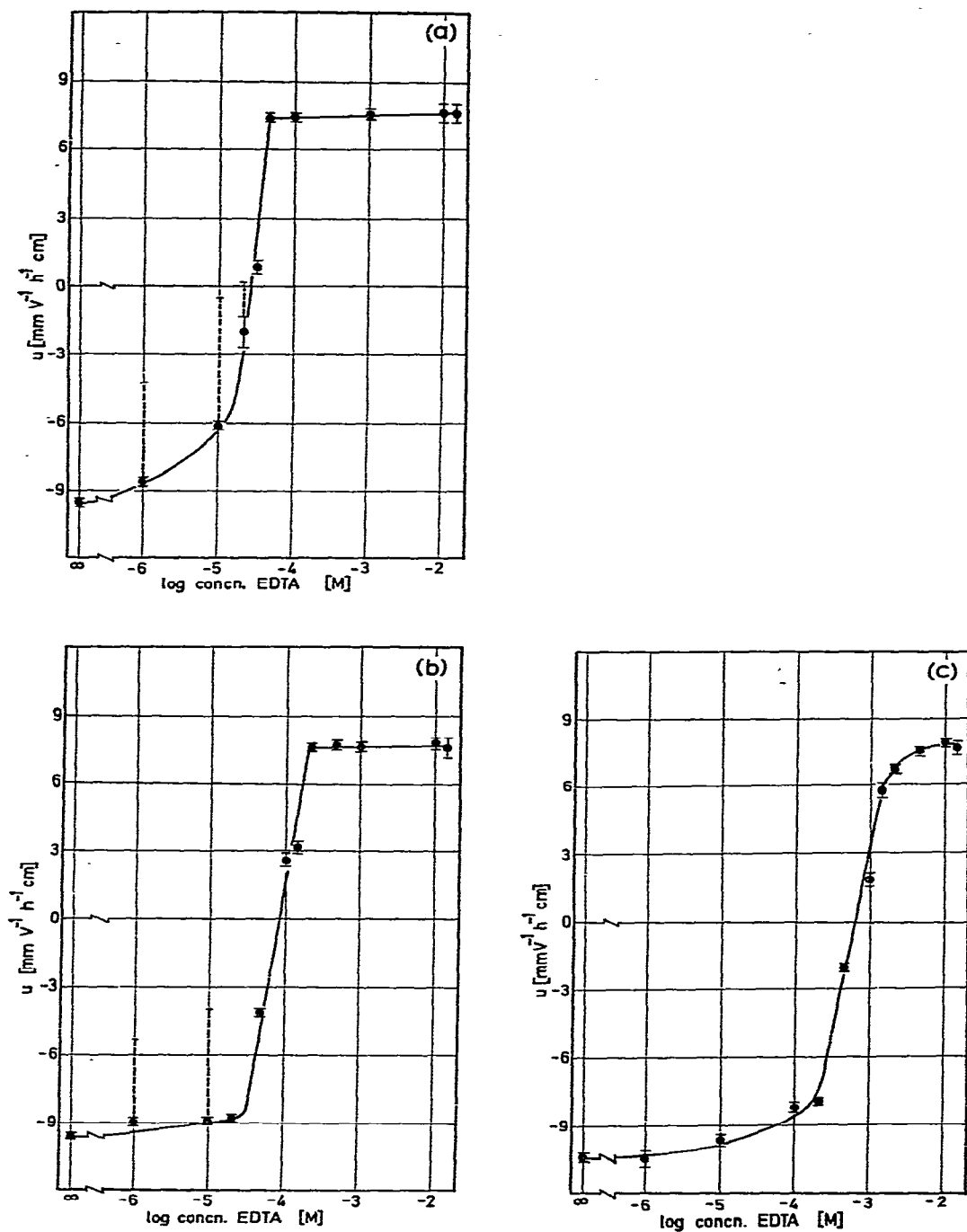


Fig. 1. Dependence of electrophoretic mobility of ^{109}Cd on age and EDTA concentration in KNO_3 medium (pH 8.1; ionic strength, 0.1). (a) $\text{KNO}_3 + 1 \text{ mM barbitone}$; (b) $\text{KNO}_3 + 0.1 \text{ mM Cd(NO}_3)_2 + 1 \text{ mM barbitone}$; (c) $\text{KNO}_3 + 1 \text{ mM Cd(NO}_3)_2 + 1 \text{ mM barbitone}$. The curves do not change over the period 0–5 days.

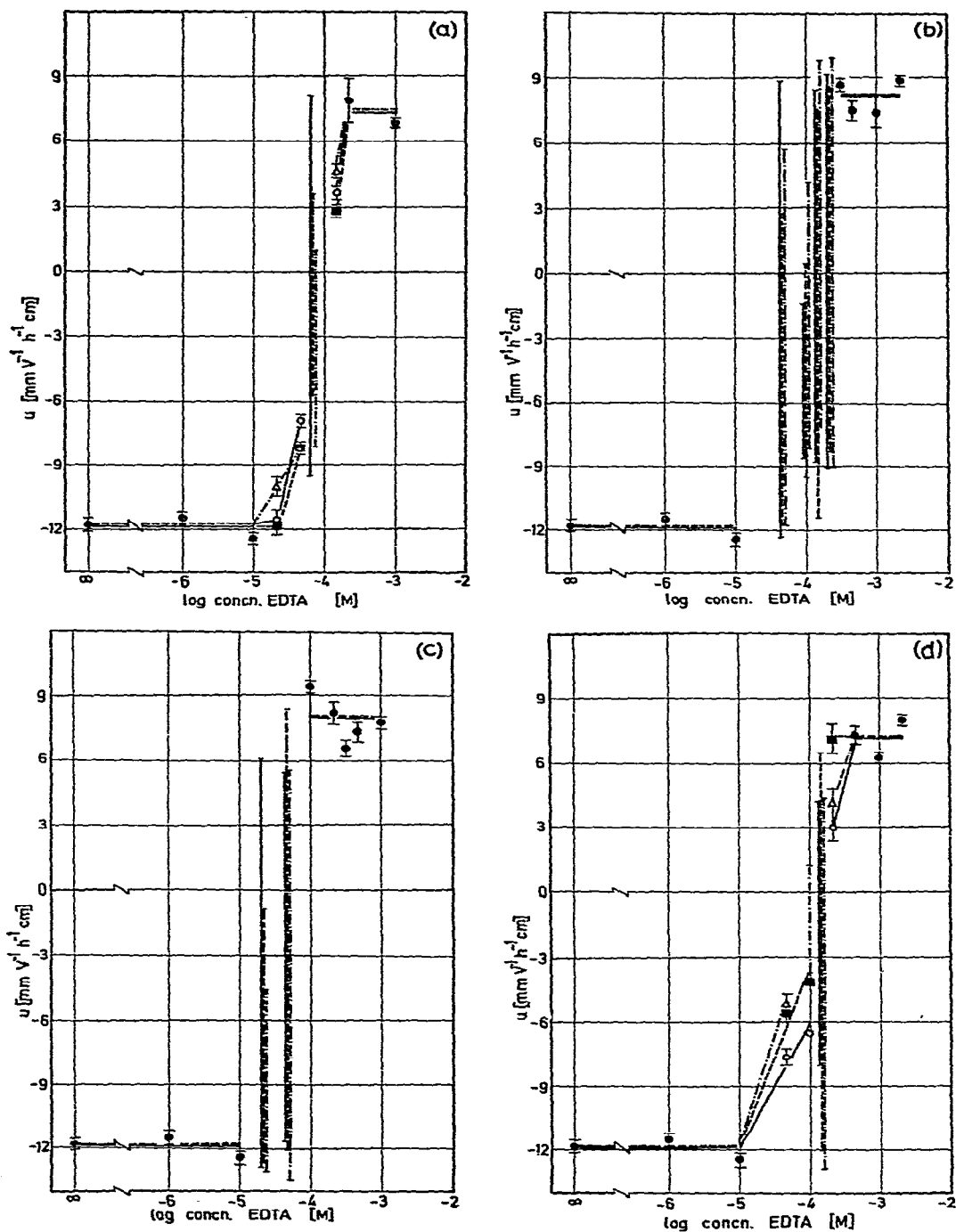


Fig. 2. Dependence of electrophoretic mobility of ^{65}Zn on age and EDTA concentration in KNO_3 medium (pH 8.1; ionic strength, 0.1). (a) KNO_3 + 1 mM barbitone; (b) KNO_3 + 1 μM $\text{Zn}(\text{NO}_3)_2$ + 1 mM barbitone; (c) KNO_3 + 10 μM $\text{Zn}(\text{NO}_3)_2$ + 1 mM barbitone; (d) KNO_3 + 0.1 mM $\text{Zn}(\text{NO}_3)_2$ + 1 mM barbitone. \circ - \circ , 0th day; \blacksquare - \blacksquare , 1st day; \triangle - \triangle , 5th day; \bullet - \bullet , 0-5th day.

Fig. 2c shows the behaviour of ^{65}Zn in systems with $10\ \mu\text{M}$ $\text{Zn}(\text{NO}_3)_2$; ^{65}Zn migrates cationically at EDTA concentrations up to $10\ \mu\text{M}$, and at EDTA concentrations higher than $0.1\ \text{mM}$, it migrates anionically.

With $0.1\ \text{mM}$ $\text{Zn}(\text{NO}_3)_2$ added (Fig. 2d), the transition region lies between $10\ \mu\text{M}$ and $0.215\ \text{mM}$ EDTA. Above $0.465\ \text{mM}$ EDTA, ^{65}Zn shows a well-defined anionic zone independent of the age of the system.

Evaluation of the experimental data for fast complexing⁷

When the concentration of a ligand A in a system is gradually increased, and the concentration of the metal species and the pH are kept constant, complexing of metal with ligand occurs and the charge on the metal species changes from $z+$ to $(z+ + nz-)$. Processes with a fast rate of complexing are characterised by the fact that, in the transition region of ligand concentration, the mobility of the metal species (\bar{u}) changes continuously from the maximum cationic mobility of the metal ion in the given media ($u-$) to the maximum anionic mobility of the metal-ligand complex ($u+$), and it is impossible to separate the complexed and uncomplexed forms by electrophoresis.

For calculating the effective stability constant, K , the equation

$$\frac{[\text{MA}_n^{z+ + nz-}]}{[\text{M}^{z+}][\text{A}^{z-}]^n} = K \quad (1)$$

could be written as follows

$$\frac{C_X}{(C_M - C_X)(C_A - nC_X)^n} = K \quad (2)$$

where

$$C_X = C_M \cdot \frac{\bar{u} - u-}{u+ - u-} \quad (3)$$

In eqns. 2 and 3, C_M , C_A and C_X represent, respectively, the total concentrations of metal, ligand and complexed metal in the system, and \bar{u} , $u-$ and $u+$ represent the electrophoretic mobilities of the zone of the metal in the transition region of ligand concentration, the uncomplexed metal (M^{z+}) and the electrophoretic mobility of the complexed metal $\text{MA}_n^{z+ + nz-}$.

The electrophoretic mobility of the metal species can be presented in diagrams as a function of the total ligand concentration. In most cases, graphs of \bar{u} against $\log C_A$ will give a straight line in the transition region, which is suitable for the statistical evaluation of experimental results. Other functions, such as $\bar{u} = f(C_A)$ can also be used if necessary. Eqns. 2 and 3 have been combined, and a computer program was devised for evaluating the results. For a given system, C_M , $u-$ and $u+$ are constants and C_A , \bar{u} and n are variables, where $\bar{u} = f(C_A)$. The value of $\log K$ should satisfy the relationship $\log K_{n=\text{constant}} = f(\bar{u}) = \text{constant}$.

With the ^{109}Cd systems, the values for u were taken directly from the transition

region of the experimental curves; for ^{65}Zn systems, the values for \bar{u} were taken from the straight line connecting the cationic and anionic parts of the complexing curves, e.g., in Fig. 2c, the points $C_A = 10 \mu\text{M}$, $u^- = 11.8$, and $C_A = 0.1 \text{ mM}$, $u^+ = 8.1$ were joined.

Figs. 3 and 4 show some examples of results obtained by computing $\log K$ against electrophoretic mobilities for values of n between 0.33 and 3; these results refer to the experiments presented in Figs. 1a, 1b, 1c (Fig. 3) and 2d (Fig. 4). Since the value K depends on the number (n) of ligands chelated to one metal ion, the correct values of K and n are represented by the curve that gives constant values of K in diagrams of $\log K$ vs. electrophoretic mobility. The "straight" lines obtained show deviations at their ends because experimental curves expressed in the form $\bar{u} = f(\log \text{EDTA concentration})$ do not fit completely to straight lines, and only the middle parts of the curves can be taken into account.

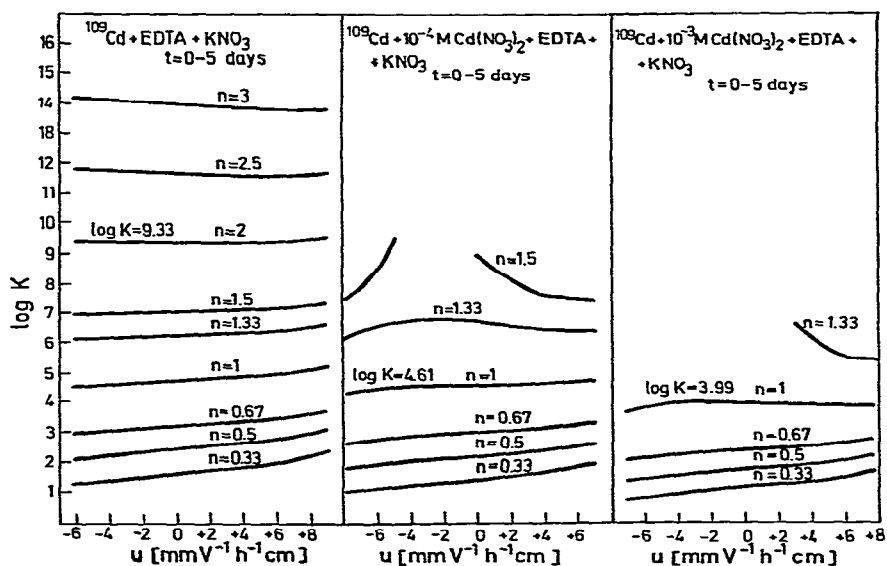


Fig. 3. Dependence of effective stability constants of the Cd-EDTA complexes in KNO_3 medium on the electrophoretic mobility of ^{109}Cd and the number of ligands (n).

In order to compare the effective stability constants of metal-EDTA complexes in different systems, and determined by other methods, the values $\log K_{n=1}$ are introduced, although this value should not be assumed to be the same as the actual $\log K$ value. The values $\log K_{n=1}$ are convenient for comparison because the absolute stability constants are usually calculated for $n = 1$.

DISCUSSION

The radionuclides ^{109}Cd and ^{65}Zn behave in 0.1 M potassium nitrate as cations with almost identical electrophoretic mobilities. From our previous results obtained

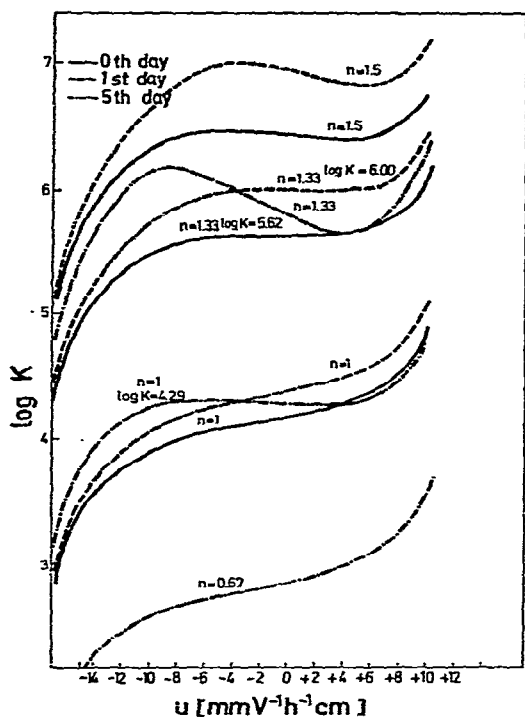


Fig. 4. Dependence of effective stability constants of the Zn-EDTA complexes in KNO_3 medium ($\text{Zn} + 0.1 \text{ mM Zn}(\text{NO}_3)_2 + \text{EDTA} + \text{KNO}_3$) on the electrophoretic mobility of ^{65}Zn , the number of ligands (n) and the age of the system.

in chloride medium (sea water and 0.55 M sodium chloride), it is evident that ^{109}Cd has a low cationic mobility, showing formation of chloro- and/or dichloro-complexes¹⁰. In contrast, ^{65}Zn has a cationic mobility corresponding to that of uncomplexed divalent cations⁹. From a study of the hydrolysis of zinc in aqueous solutions, in which the tyndallometric method was used as well as high-voltage paper electrophoresis¹⁴, we conclude that, in the systems investigated, ^{65}Zn behaves as a divalent cation with no hydrolytic species present. According to earlier results^{9,14}, in sea water and in sodium chloride solutions, ^{65}Zn behaves as divalent cation. In these experiments, ^{65}Zn was introduced in solutions in ionic form, and only the behaviour of ionic zinc was studied. For this reason, no conclusions could be formed as to the state of the zinc present in sea water. This was confirmed by the results of Piro *et al.*¹⁵, who found that, in natural sea water at pH 8, 10–20% of the zinc was in ionic form (including labile complexes), 30–50% was in particulate form and 40–50% was in complexed form; these workers also established that, even after 1 year, radioactive ionic zinc does not enter into equilibrium with naturally complexed stable zinc, although it is partly transformed into particulate form. According to Florence and Batley¹⁶, measurement of the recovery of ionic zinc added to the solution bears no relationship to the recovery of the metal original present in the water sample.

Our present results, as well as those published earlier^{9,10}, show that, in the systems without EDTA or at very low or very high concentrations of EDTA, both

radionuclides behave in the same way, giving well-defined cationic and anionic zones, respectively. In the intermediate range of EDTA concentrations, ^{109}Cd and ^{65}Zn differ slightly in behaviour. ^{109}Cd gives well-defined zones with continuous change in electrophoretic mobilities, whereas ^{65}Zn gives very pronounced "tailing", consisting of poorly-defined multi-zones instead of defined zones. This indicates that the interaction of cadmium with EDTA is fast, whereas the reaction-rate for zinc is fairly slow. The effect of age of the system shows that the rate of formation of cadmium-EDTA complexes is higher than that of the zinc complexes, because there is no change in the electrophoretic mobility of cadmium with the age of the system in the intermediate range of EDTA concentration.

When the rate of interaction is slow, complexed and uncomplexed species present in solution exhibit defined zones with constant electrophoretic mobilities; in the transition region of ligand concentration, only the intensity of the zones changes⁷.

From the data in Figs. 2b and 2c, the effect of the age of the system corresponding to 0 and 1 day could not be evaluated in the form of stability constants and number of ligands, although the effects of aging were evidenced in the transition region by the different forms of tailing.

By increasing the metal-ion concentration in the system, the concentration of EDTA needed for total complexing of the metal increases; at lower concentrations of metal, relatively more EDTA is needed than at higher metal contents, where complexing with EDTA is almost stoichiometric. This is in agreement with literature data^{17,18}. For comparison, the results already published and those obtained as described here are shown in Tables I and II.

The fact that values for the effective stability constants assuming $n = 1$ ($\log K_{n=1}$) are almost the same shows that cadmium and zinc behave similarly in the EDTA-containing medium. At low metal-ion concentration in the system, the number of ligands, n , exceeds 1, thus increasing the value of the effective stability constant. This could be attributed to increasing competition between ligand ions for a metal

TABLE I

EFFECTIVE STABILITY CONSTANTS (K) AND NUMBERS OF LIGANDS (n) OF ^{109}Cd -EDTA COMPLEXES IN VARIOUS MEDIA

<i>Electrolyte</i>	<i>Ionic strength</i>	<i>Age of system (days)</i>	<i>n</i>	<i>Log K</i>	<i>Log K[*]_{n=1}</i>	<i>Temp. (°C)</i>	<i>Ref.</i>
Sea water + EDTA	0.74	0	2.5	10.92	(4.34)	21	10
		1	2.5	10.93	(4.35)	21	
		7	1.33	5.54	(4.16)	21	
Sea water + 0.1 mM Cd(NO ₃) ₂ + EDTA	0.74	0-7	1.33	6.30	(4.44)	21	10
KNO ₃ + EDTA	0.1	0-5	2	9.33	(4.50)	25	**
KNO ₃ + 0.1 mM Cd(NO ₃) ₂ + EDTA	0.1	0-5	1	4.61	(4.61)	25	**
KNO ₃ + 1 mM Cd(NO ₃) ₂ + EDTA	0.1	0-5	1	3.99	(3.99)	25	**
0.55 M NaCl + EDTA	0.55	0-10	2.5	8.52	(3.35)	21	10

* The effective stability constant when the number of ligands per atom of cadmium is 1.

** This paper.

TABLE II

EFFECTIVE STABILITY CONSTANTS (*K*) AND NUMBERS OF LIGANDS (*n*) OF ⁶⁵Zn-EDTA COMPLEXES IN VARIOUS MEDIA

<i>Electrolyte</i>	<i>Ionic strength</i>	<i>Age of system (days)</i>	<i>n</i>	<i>Log K</i>	<i>Log K[*]_{n=1}</i>	<i>Temp. (°C)</i>	<i>Ref.</i>
Sea water + EDTA	0.74	0-10	1.5	4.25	(4.25)	21	8**
KNO ₃ + EDTA	0.1	0	1.5	6.32	(4.32)	25	***
		1	1.5	6.18	(4.14)	25	
		5	2	8.34	(4.20)	25	
		0-5	1.33	5.65	(4.26)	25	***
KNO ₃ + 10 μM Zn(NO ₃) ₂ + EDTA	0.1	0-5	1.5	6.94	(4.60)	25	***
KNO ₃ + 0.1 mM Zn(NO ₃) ₂ + EDTA	0.1	0	1.33	5.62	(4.10)	25	***
		1	1.33	6.00	(4.32)	25	
		5	1	4.29	(4.29)	25	
		0-10	1.5	5.52	(3.68)	21	8**

* The effective stability constant when the number of ligands per atom of zinc is 1.

** For experimental results, see ref. 9.

*** This paper.

ion. Generally, the values of $\log K_{n=1}$ show that, at low concentrations of metal ions (micro-constituents), the stability of the EDTA complexes decreases; thus, special attention should be paid to the treatment of complex-formation at low metal-ion concentration.

With increasing cadmium nitrate concentration beyond 1 mM or zinc nitrate concentration beyond 0.1 mM, an apparent decrease in stability constant (due to competition of EDTA with impurities introduced by the reagents) is observed; this was markedly confirmed for 0.55 M sodium chloride⁸. In this respect, sea water, a relatively concentrated solution (ionic strength 0.74), had a relatively low content of impurities that interacted with EDTA; this is not so with high-purity chemicals.

Our calculated values for the stability constants of the cadmium- and zinc-EDTA complexes (see Tables I and II) differ from the data of Schwarzenbach and his co-workers^{19,20}. For this reason, we have re-calculated, from our experimental data, the thermodynamic stability constants of these complexes according to the method developed by Shvedov and Stepanov¹, *viz.*

$$\log K = \log a + \log b + \log f \quad (4)$$

in which *a* (the activity of the free ligand EDTA⁴⁻) is defined by

$$a = \frac{C_{\text{EDTA}} K_1 K_2 K_3 K_4}{(H^+)^4 + (H^+)^3 K_1 + (H^+)^2 K_1 K_2 + (H^+) K_1 K_2 K_3} \cdot f_{\text{EDTA}^{4-}} \quad (5)$$

where *K*₁, *K*₂, *K*₃ and *K*₄ are the acid dissociation constants of EDTA, *C*_{EDTA} is the total concentration of EDTA in the system and (*H*⁺) is the hydrogen-ion activity.

The value of *b* is given by

$$b = \frac{\mu_{\text{MEDTA}^{2-}} - \mu_c}{\mu_c - \mu_{\text{M}^{2+}}} \quad (6)$$

where $u_{\text{MEDTA}^{2-}}$, $u_{\text{M}^{2+}}$ and u_c are the electrophoretic mobilities of the metal-EDTA complex, the free metal ion and the metal species in the transition region of ligand concentration, respectively, and the value of f by

$$f = \frac{f_{\text{M}^{2+}}}{f_{\text{MEDTA}^{2-}}} \quad (7)$$

$f_{\text{M}^{2+}}$ and $f_{\text{MEDTA}^{2-}}$ are the activity coefficients of the free and complexed metal ion, respectively.

From Table III, it is evident that the values of the stability constants calculated according to Pučar⁷ (effective stability constants) and according to Shvedov and Stepanov¹ (thermodynamic stability constants) differ only for the pH factor, α , (the pH being 8.1), because $\log K_{\text{eff}} = \log K_{\text{aps}} - \log \alpha$ (see ref. 21). For a divalent-cation complex (MEDTA²⁻), K_{aps} is approximately equal to K_{therm} because eqn. 7 reduces to 1. The factor α depends on the pH and the acid dissociation constant.

At the EDTA concentration where 50% of the metal is complexed, the effective stability constant of the metal-EDTA complex is approximately equal to the negative value of this EDTA concentration [$\log K_{n=1} \approx -\log C_A$ (compare columns 3 and 6 in Table III)].

Jokl and Majer⁵ also calculated the absolute stability constants of cadmium- and zinc-EDTA complexes by using paper electrophoresis. From experimental data, Jokl *et al.*⁴ calculated the curve of u (electrophoretic mobility) against $\log A$ (free-ligand concentration), for which the value of A depended greatly on pH. According to their conclusions, $\log K \approx -\log A$ at the point u (see ref. 19) (the mean value of the electrophoretic mobilities of two metal species, *e.g.*, free metal and metal complex).

TABLE III

STABILITY CONSTANTS OF Cd- AND Zn-EDTA COMPLEXES IN POTASSIUM NITRATE SOLUTIONS OF pH 8.1 AND IONIC STRENGTH 0.1

Species	Electrolyte	$\log K_{n=1}^*$	$\log K_{n=1} \div \log \alpha^{**}$	$\log K$ (= $-\log a$) ^{***}	$\log C_{\text{EDTA}}^{\S}$
¹⁰⁹ Cd	KNO ₃ + EDTA	4.50	6.67	6.77	-4.60
¹⁰⁹ Cd	KNO ₃ + 0.1 mM Cd(NO ₃) ₂ + EDTA	4.61	6.78	6.28	-4.12
¹⁰⁹ Cd	KNO ₃ + 1 mM Cd(NO ₃) ₂ + EDTA	3.99	6.16	5.45	-3.28
⁶⁵ Zn	KNO ₃ + EDTA	4.20	6.37	6.27	-4.10
⁶⁵ Zn	KNO ₃ + 1 μM Zn(NO ₃) ₂ + EDTA	4.26	6.43	6.40	-4.23
⁶⁵ Zn	KNO ₃ + 10 μM Zn(NO ₃) ₂ + EDTA	4.60	6.77	6.67	-4.50
⁶⁵ Zn	KNO ₃ + 0.1 mM Zn(NO ₃) ₂ + EDTA	4.29	6.46	6.30	-4.13

* Effective stability constants calculated according to Pučar⁷ (see Tables I and II).

** Absolute stability constants calculated according to Schwarzenbach and Flaschka²¹ (at pH 8.1, $\log \alpha = 2.17$).

*** Thermodynamic stability constants calculated according to Shvedov and Stepanov¹: $\log K = -\log a$ because $\log b = 0$ (when 50% of the metal is complexed) and $\log f = 0$ (the activity coefficients of M²⁺ and MEDTA²⁻ are equal). See eqns. 4, 6 and 7.

† Concentration of EDTA when 50% of the metal is complexed (taken from the diagrams).

This means that, for experiments at pH 8.1, it is impossible to obtain values for the absolute stability constants of the cadmium- and zinc-EDTA complexes as cited in literature by using the equations of Shvedov and Stepanov¹ or those of Jokl *et al.*⁴. To obtain the value $\log K_{Cd-EDTA} = 16.5$ at pH 8.1, the concentration of EDTA must be approximately 0.01 pM; at this EDTA concentration, metal ions at concentrations between 10 nM and 1 mM can not be complexed with EDTA to the extent of 50%. This means that electrophoretic experiments should be done at pH values less than 4.

It should be noted that, according to the literature²³, zinc forms a simple $Zn-EDTA^{2-}$ complex in the pH range 4–11; if this is true, it should be irrelevant at which of these pH values the experiments are performed in order to calculate the stability constants of the zinc-EDTA complex.

Shvedov and Stepanov¹ calculated the stability constants of lanthanide-EDTA complexes; the origin of the main part of the numerical value of the thermodynamic stability constants obtained was that part of eqn. 4 corresponding to the activity of the free ligand $EDTA^{4-}$ (*i.e.*, $\log a$) at a pH between 2 and 3.

We would like to point out the difference between the experimental conditions used in our method and in the other methods already discussed. While in other methods optimal conditions for the appearance of a certain metal-ligand complex were used, we use the conditions present in natural aquatic systems, *e.g.*, sea water. In this sense, our calculation of the effective stability constants takes into account the actual conditions in the investigated systems, *viz.*, pH, ionic strength, actual ionic species present, possible formation of metal-ligand complexes in proportions other than 1:1, possible mixed-ligand complexes, intermediary and concurrent reactions, and instances in which rather low concentrations of metal ions to be investigated (10 nM) are present in concentrated mixed-electrolyte solutions.

REFERENCES

- 1 V. P. Shvedov and A. V. Stepanov, *Radiokhimiya*, 1 (1959) 162.
- 2 V. Jokl, *J. Chromatogr.*, 13 (1964) 451.
- 3 V. Jokl, *J. Chromatogr.*, 14 (1964) 71.
- 4 V. Jokl, J. Majer, H. Scharf and H. Kroll, *Mikrochim. Acta*, (1966) 63.
- 5 V. Jokl and J. Majer, *Chem. Zvesti*, 19 (1965) 249.
- 6 E. Jercañ and Gr. Popa, *J. Chromatogr.*, 42 (1969) 89.
- 7 Z. Pučar, *Thalassia Jugosl.*, 7 (1971) 639.
- 8 Lj. Musani-Marazović, *Ph.D. Thesis, University of Zagreb*, 1972.
- 9 Lj. Musani-Marazović and Z. Pučar, *Rapp. Comm. Int. Mer Médit.*, 20 (1972) 701.
- 10 Lj. Musani-Marazović and Z. Pučar, *Thalassia Jugosl.*, 9 (1973) 101.
- 11 Z. Pučar, *Anal. Chim. Acta*, 17 (1957) 476.
- 12 M. Petek, *M.Sc. Thesis, University of Zagreb*, 1971.
- 13 M. Ishikawa and Z. Pučar, *J. Radioanal. Chem.*, 11 (1972) 197.
- 14 B. Pokrić and Z. Pučar, *J. Inorg. Nucl. Chem.*, 33 (1971) 445.
- 15 A. Piro, M. Bernhard, M. Branica and M. Verži, *Radioactive Contamination of the Marine Environment*, IAEA, Vienna, STI/PUB/313, p. 29.
- 16 T. M. Florence and G. E. Batley, *Talanta*, 23 (1976) 179.
- 17 D. Maljković, *M.Sc. Thesis, University of Zagreb*, 1967.
- 18 D. Maljković and M. Branica, *Limnol. Oceanogr.*, 16 (1972) 779.
- 19 G. Schwarzenbach and E. Freitag, *Helv. Chim. Acta*, 34 (1951) 1503.
- 20 G. Schwarzenbach, R. Gut and A. Anderegg, *Helv. Chim. Acta*, 37 (1954) 937.
- 21 G. Schwarzenbach and H. Flaschka, *Complexometric Titrations*, Methuen, London, 1969.
- 22 K. Krishnan and R. A. Plane, *J. Amer. Chem. Soc.*, 90 (1968) 3195.