University of Medicine and Pharmacy[1], Tg.Mureş (Marosvásárhely) Romania, and INGENIEURBÜRO[2], München, Germany

Soft ligands – biodegradable complexants in plant cultivation and environmental protection

B. Tőkés[1], J. Száva[1], S. Duşa[1], A. Vintilă[1], G. Donáth-Nagy[1], G. Gál[1]

Received August 8, 2005, accepted September 6, 2005

Prof. Dr. Béla Tőkés, University of Medicine and Pharmacy, Department of Physical Chemistry, Str. Gh. Marinescu 38, 540139 Tg. Mureş (Marosvásárhely), Romania
btokes@umftgm.ro

Pharmazie 61: 166–170 (2006)

In this paper, the place and role of biodegradable complexants in light of modern plant cultivation criteria is discussed. From this point of view, micro- and macroelement (metal) chelators play a remarkable role. They are widely used in a variety of consumer products and processes, especially in agriculture. The great majority of the traditional chelators (e.g., EDTA) are, however, essentially nonbiodegradable. We have systematically investigated some new biodegradable ligands with polyaminopolycarboxylic structure. We conclude that these compounds may be considered as a special group of the soft (safer) chemicals proposed first by Bodor based on retrometabolic concepts. We studied the structural conditions of biodegradability, especially regarding the new ligands, by the physicochemical characterization of their complexes formed with micro- and macroelements. The study of the complicated equilibrium conditions required the prior determination of the stability constants of the metal aqua/hydroxo complexes and the protonation constants of the ligands as well. Knowledge of the pH-dependence of the proper competitive equilibriums allowed the optimization of the investigated complexation reactions. The protonation and stability constants obtained were compared with the adequate values of the traditional, but weakly biodegradable EDTA. Excellent or very good linear correlations have been found between the logarithms of the different ligands' protonation constants, but the slopes of the straight lines were different and generally smaller than EDTA's corresponding values. Presumably, the log stability constants ($\log K_{st}$) of complexes formed by different ligands (X_m, X_n) having analogue structures with a given metal-ion series (M_i, $i = 1, 2, \ldots$) are linearly correlated [i.e., $\log K(X_mM_i) = a \log K(X_nM_i) + b$], just as the stability constants of complexes formed by different ions (M_k, M_l) with a given ligand series (X_j, $j = 1, 2, \ldots$). Based on the rich data basis of EDTA, these regression equations allow the calculation of some still unmeasured stability constants of the new biodegradable complexes. In the analysis of structural effects we would like to especially highlight the role of stereochemical relations in addition to the usual structural elements. From this point of view, the eventual presence of chiral nitrogen atoms is of particular importance. The stability of these compounds increases in their complexed form. As a special research field, we have examined the possibility of obtaining metal ion buffers. These systems may get an important role both from theoretical and practical points of view.

1. Introduction

In our age, the environment and its inhabitants, including humans, are constantly affected by ever increasing amounts of manmade chemicals, which are used for a variety of purposes. Their design and use are most frequently goal-guided, but the necessity to decrease their toxicity and secondary effects to the possible minimum is stressed rarely, if ever. These effects appear frequently in complex relations and indirectly. From this point of view, it is important to discuss the place and role of numerous complexants in the light of modern plant cultivation as micro- and macroelement (metal) chelators. They are widely used in a variety of consumer products and processes, especially in agriculture. The great majority of traditional chelators (e.g., EDTA) are, however, essentially nonbiodegradable. The systematic study of some new biodegradable ligands with polyaminopolycarboxylic structures, which were projected, synthesized, and patented by J. Szava (INGENIEURBÜRO, München, Germany) (Száva 1999; Száva 2001), convinced us that these compounds may be considered as a special group of the soft (safer) chemicals proposed first by Bodor based on retrometabolic concepts (Bodor 1996).

This approach emphasizes that the basic ideas on drug toxicity and development can be extended to commercial chemical substances as well; the main difference being that the intrinsic toxicity or selectivity term of the substance

itself (Tc) is usually less important as many of the commercial chemical substances have no strong receptor binding capacity. Thus, the main issue becomes the intrinsic toxicity of the metabolic products of commercial substances. As the most important characteristic of a drug is its therapeutic index (TI), the safety index (SI) should be most important property of any commercial chemical substance:

1. Drug (D) toxicity:
   $T(D) = T^D(i) + T(A_1, A_2, \ldots, A_n) + T(M_1, M_2, \ldots, M_n) + T(I^*_1, I^*_2, , I^*_n)$
   $T^D(i)$: intrinsic toxicity – selectivity
   $A_1, A_2, \ldots, A_n$: analog metabolites with activities of the type of D
   $M_1, M_2, \ldots, M_n$: other metabolites
   $I^*_1, I^*_2, \ldots, I^*_n$: reactive intermediates
   TI = TD/ED: therapeutic index
   T = Toxicity
   TD = Toxic Dose
   ED = Effective dose (dose needed to elicit desired therapeutic response)
2. Chemicals (C) toxicity:
   $T(C) = TC(i) + T(C_1, C_2, \ldots, C_n) + T(M_1, M_2, \ldots, M_n) + T(I^*_1, I^*_2, \ldots, I^*_n)$
   $TC(i) \ll T^D(i)$
   reactive intermediates
   $I^*_1, I^*_2, \ldots, I^*_n$: most important
   SI = TD/EmD: safety index
   EmD: maximum environmental dose

The mentioned biodegradable ligands group (EDTA analogues) is important for introduction in plant cultivation of complexant agents which are unable to return in soil nutrition elements. Although complexants used for similar purposes at present are, in general, not toxic, because of their weak degradability, they will get together with microelements in the soil or water, where they will accumulate, pollute the environment, and cause effects that are yet incalculable (Labádi and Száva 2001; Nowack 2003; Pettit and Powell 1997a, 1997b; Pitter et al. 1999; Sykora et al. 2001; Száva 1999, 2001). Another aspect that underlines the necessity of employing adequate complexants is the need to avoid the precipitation of the interaction products of the components, since the insoluble substances are not useful for plants. The resulting complexes are soluble in water.

The biodegradability, that is the reactions that a compound may undergo, is determined by its molecular structure (Boethling 1996). Hundreds of transformations have been described, but almost all can be classified broadly as oxidative, reductive, hydrolytic, or conjugative. In the last decades, it has been shown that relatively small changes in molecular structure can appreciable alter the susceptibility of chemicals to biodegradation. These studies have resulted in several "rules of thumb" concerning the effects of chemical structure on biodegradability. The following molecular features generally increase resistance to aerobic biodegradation:

- halogens, especially chlorine and fluorine;
- chain branching, especially quaternary carbon and tertiary nitrogen;
- nitro, nitroso, azo, arylamino groups;
- polycyclic and heterocyclic residues;
- aliphatic ether bonds.

In most cases the mechanism by which increased resistance to biodegradation is conferred is not known in detail. In contrast, biodegradability is usually enhanced by the presence of potential sites of enzymatic hydrolysis (e.g., esters, amides), by the introduction of oxygen in the form of hydroxyl, aldehydic, or carboxylic acid groups, and by the presence of unsubstituted linear alkyl chains and phenyl rings, which represent possible sites for attack by oxygenase. It is easy to observe the analogy between the soft chemicals and the biodegradable ones.

The effect of solubility involves one or more of the following:

(1) microbial bioavailability
(2) rate of solubilization, which may control the rate of biodegradation
(3) low aqueous concentration (for chemicals soluble to the extent of only a few micrograms per liter or less, this concentration may be too low for optimal function of cellular enzymes or transport systems).

At present, it can be stated that:

(a) highly substituted structures are likely to be less rapidly biodegraded than much simpler compounds
(b) for very insoluble chemicals, replacement of a given functional group with one that increases solubility may also result in enhanced biodegradability.

The ability to predict relative rates of biodegradation from a chemical structure alone would greatly facilitate the design of safer chemicals. One of the approaches used develops some mathematical models capable of such predictions. Fragment contribution methods have been used for many years in chemical engineering, but only more recently in environmental chemistry. The basic premise is that the activity of interest is a function of the contribution of one or more molecular substructures or fragments of which the molecule is composed, and there is no interaction between fragments. In the ideal situation, each fragment of the model would have a clear mechanistic relationship to the activity of interest, which is understood at the molecular level. This situation, however, rarely, if ever is realized. Fortunately, it is of no major consequence as long as:

(1) a set of measured values ("training set") of adequate size exists for model development, and
(2) a reasonably comprehensive set of structural fragments associated in some way with activity can be identified.

There are a few models developed for predicting biodegradability. Two of the models (based on linear or nonlinear algorithms) classify chemicals as easily or not easily biodegradable, and another two (for primary and ultimate biodegradation) make semi-quantitative estimates of aquatic biodegradation rates. Data are retrieved from BIODEG, a component of the Environmental Fate Data Base that contains extracted biodegradation data on more than 800 organic chemicals. Each chemical is assigned a qualitative descriptor, such as BR (biodegrades rapidly) or BSA (biodegrades slowly even with acclimation), and a reliability code (screening studies, grab sample studies, and so on). Biodegradability and reliability codes are also assigned for an overall assessment, and this biodegradability endpoint was used in modeling.

What the two classification-type models predict is the probability that the chemical is in the BR category. The models predict biodegradation category correctly for approximately 90%. This level of accuracy is similar to that of other published models for predicting biodegradability that use different training sets and statistical methods. Both the sign and relative magnitudes of the coefficients are generally consistent with expectation.

There is no need to use the particular models, or any models for that matter, to make an educated guess about the biodegradability of an untested compound. But the fragment contribution models do provide a rapid, convenient, and systematic way to accomplish this in a way that is

Table 1: Structural fragments and coefficients

Structural fragment	BIODEG models		Survey models	
	Linear coeff.	Nonlinear coeff.	Primary coeff.	Ultimate coeff.
Aliphatic OH	0.159	1.118	0.129	0.160
Aliphatic NH_2, NH	0.154	1.110	0.043	0.024
Aliphatic O=COH	0.073	0.643	0.386	0.365
Tertiary amine	−0.205	−2.223	−0.288	−0.225
carbon with 4 single bonds and no H	−0.184	−1.723	−0.153	−0.212

consistent with knowledge in the field. Molecular structure is the only input needed and is entered from the PC keyboard via the chemical's SMILES (Simplified Molecular Information and Line Entry System) notation. Some, frequently used data are shown in Table 1.

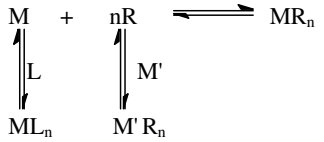
Lacking specific knowledge about the environmental behavior of a chemical, the way to design more biodegradable chemicals is to incorporate molecular features such as ester linkage, carboxylic and hydroxyl groups, and exclude halogens, quaternary carbons, nitro groups and other similar groups. The positive and negative features presented in Table 1 are good starting points.

Studies of EDTA-homologues highlighted the fact that the biological stability of these compounds depends on the substituents linked to the nitrogen atoms and a decreasing order of biodegradability could be established:

$-H, -COCH_3, -CH_3, -C_2H_5, -CH_2CH_2OH, -CH_2COOH$, or: $-NH\ -CO\ -CH_3$, $-N(COCH_3)_2$, and similarly: $-NH-CH_2-COOH$, $-N(CH_2-COOH)_2$. It was also shown that the biodegradability of heavy metal complexes is better than that of the complexants themselves. For example, the biodegradability of the EDTA–Fe^{3+} complex is about 80% while of EDTA itself is 20–25%. Some complexes from polyaminopolycarboxylic acids are optically active, and their isomers biodegrade in different extent (Schowanek et al. 1997).

2. Investigations, results and discussion

The complexity of the investigated competitive equilibriums:



was described by usual relationships and parameters (absolute and effective stability constants, partial molar fractions, consecutive and overall stability constants, the same parameters for the principal and auxiliary complexing processes, etc.) (Beck 1965; Dudutz 1976; Kékedy 1979).

Some of results will be examined. Since the most important secondary processes in the complexing reactions between metal ions and weak acids as ligands are the formation of aqua- and then hydroxocomplexes, and the proton change at the ligand, respectively, it is obvious that the principal equilibrium is substantially affected by pH-modifications even as the complex-stability must have a maximum at a certain pH value. That is, with the increase of pH, the hydroxo-complex stability increases, which hinders the formation of the main complex, but at the same time, the ratio of the active (deprotonated) ligand form also increases, wich facilitates the principal complexation. The function describing the two opposing effects must have a pH-optimum:

$$\beta'_{MR_n} = \beta_{MR_n} \cdot \alpha_M \cdot \alpha_R^n = \frac{\beta_{MR_n}}{\left(\sum_{i=0}^{p} \beta_i^{OH} \cdot K_w^i \cdot [H^+]^{-1}\right) \cdot \left(\sum_{i=0}^{q} \beta_i^H \cdot [H^+]^i\right)^n}$$

that can be obtained from the first derivative of this function at its zero value:

$$\left(\frac{\beta_1^{OH}K_w}{[H^+]^2} + \frac{2\beta_2^{OH}K_w^2}{[H^+]^3} + \frac{3\beta_3^{OH}K_w^3}{[H^+]^4} + \frac{4\beta_4^{OH}K_w^4}{[H^+]^5}\right) \times \left(1 + \beta_1^H[H^+] + \beta_2^H[H^+]^2 + \beta_3^H[H^+]^3 + \beta_4^H[H^+]^4\right) - n\left(1 + \frac{\beta_1^{OH}K_w}{[H^+]} + \frac{\beta_2^{OH}K_w^2}{[H^+]^2} + \frac{\beta_3^{OH}K_w^3}{[H^+]^3} + \frac{\beta_4^{OH}K_w^4}{[H^+]^4}\right) \times \left(\beta_1^H + 2\beta_2^H[H^+] + 3\beta_3^H[H^+]^2 + 4\beta_н[H^+]^3\right) = 0$$

To test this statement, we compared the calculated values with the data known from the literature and measured in our laboratory, respectively, for EDTA and some metal ions; e.g.:

$[Cu\ (OH)_4]^{2-}$: $lgK^{OH}{}_1 = 7.9$; $lgK^{OH}{}_2 = 7.3$; $lgK^{OH}{}_3 = 3.8$; $lgK^{OH}{}_4 = 3.0$

$[Zn\ (OH)_4]^{2-}$: $lgK^{OH}{}_1 = 6.5$; $lgK^{OH}{}_2 = 6.0$; $lgK^{OH}{}_3 = 5.7$; $lgK^{OH}{}_4 = 4.0$

EDTA: $lgK^{H}{}_1 = 10.27$; $lgK^{H}{}_2 = 6.17$; $lgK^{H}{}_3 = 2.68$; $lgK^{H}{}_4 = 2.00$

In this case: $n = q = 1$.

The agreement between the calculated and the more laboriously obtained experimental results is very good (pH_{opt} between 8–9). It is interesting, although not surprising, that if only the first step of the auxiliary complex-formation ($\beta_1^{OH} = K_1^{OH}$ and $\beta_1^H = K_1^H$) is considered, approximately correct values are obtained:

$$\frac{\beta_1^{OH}K_w}{[H^+]}\left(1 + \beta_1^H[H^+]\right) - \left(1 + \frac{\beta_1^{OH}K_w}{[H^+]}\right)\beta_1^H = 0$$

$$[H^+]_{opt} = \left(K_w \frac{\beta_1^{OH}}{\beta_1^H}\right)^{1/2}$$

The dissociation and the protonation constants of the new, biodegradable polyaminopolycarboxylic acids investigated agree satisfactorily with the corresponding EDTA's values (Table 2).

An excellent linearity between the logarithms of protonation constants was observed; nevertheless the pK_a values of polyaminopolycarboxylic acids studied vary less abruptly than the similar EDTA's parameters.

Table 2: Acidity constants of polyaminopolycarboxylic acids

Acid	pK_1	pK_2	pK_3	pK_4	pK_5
EDTA	2.00	2.68	6.17	10.27	
DMTETA	2.6	3.0	3.5	7.6	
DMTPA	3.4	4.0	9.8	10.4	10.5
METTA	3.2	3.6	6.6	9.6	
	(2.7	3.5	6.0	9.6)*	
MDTA	3.0	3.2	5.2	9.3	
HEMDTA	3.2	3.6	6.6		

* Independent experimental values obtained by another research team.

$$pK_{a(DMTETA)} = (0.45 \pm 0.05)\, pK_{a(EDTA)} + (3.02 \pm 0.26)$$
$$n = 4; \quad r = 0.988; \quad s_0 = 0.44$$
$$pK_{a(DMTPA)} = (0.90 \pm 0.26)\, pK_{a(EDTA)} + (2.12 \pm 1.64)$$
$$n = 4; \quad r = 0.925; \quad s_0 = 1.73$$
$$pK_{a(META)} = (0.785 \pm 0.021)\, pK_{a(EDTA)} + (1.60 \pm 1.34)$$
$$n = 4; \quad r = 0.992; \quad s_0 = 0.14$$
$$pK_{a(MDTA)} = (0.760 \pm 0.86)\, pK_{a(EDTA)} + (1.15 \pm 0.53)$$
$$n = 4; \quad r = 0.988; \quad s_0 = 0.12$$

According to a generally known rule, the stability constants (lgK_{st}) of complexes formed between different ligands and the same metal ions are correlated linearly with one another (Table 3 and the corresponding correlative equations).

Table 3: Stability of some new complexes (lgK_{st})

Me^{n+} Acid	Ca^{2+}	Mn^{2+}	Cu^{2+}	Fe^{3+}	Fe^{2+}	Mg^{2+}	Zn^{2+}
EDTA	10.6	13.6	18.6	25.1	14.3	8.7	16.5
METTA	7.5	9.0	16.0	20.0	(10.6)	(5.5)	(12.6)
MDTA	6.94	10.22	7.31	(16.57)	(9.76)	4.19	10.80

$$lgK_{st(METTA)} = (0.920 \pm 0.108)\, lgK_{st(EDTA)} - (2.52 \pm 1.93)$$
$$n = 4; \quad r = 0.987; \quad s_0 = 1.18$$
$$lgK_{st(MDTA)} = (0.63 \pm 0.097)\, lgK_{st(EDTA)} - (-0.76 \pm 1.37)$$
$$n = 5; \quad r = 0.966; \quad s_0 = 0.80$$

Since EDTA complexes were thoroughly investigated, and there is a rich corresponding database in the literature (Anderegg 1997; Arena et al. 1983; Danielle et al. 1994; Delgado et al. 1997; De Robertis et al. 1987; De Stefano et al. 1987, 1993; Dietz 1987; Frimmel et al. 1989; Kari and Giger 1996), the above equations allow the prediction of the stability of analog complexes for the present experimentally unknown cases (e.g., the constants shown in the brackets).

It is interesting that the same correlations between the parameters of the new ligands are better compared to EDTA:

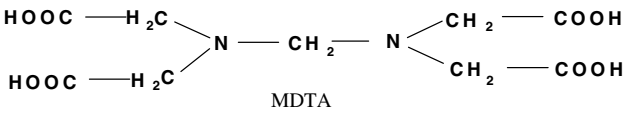
$$lgK_{St(MDTA)} = (0.929 \pm 0.092)\, lgK_{St(HEMDTA)} + (0.061 \pm 1.43)$$
$$n = 5; \quad r = 0.981; \quad s_0 = 0.88$$
$$lgK_{St(MDTA)} = (2.00 \pm 0.16)\, lgK_{St(DMTETA)} - (8.06 \pm 1.84)$$
$$n = 5; \quad r = 0.994; \quad s_0 = 0.58$$
$$lgK_{St(MDTA)} = (1.19 \pm 0.13)\, lgK_{St(METPA)} - (3.45 \pm 2.00)$$
$$n = 5; \quad r = 0.976; \quad s_0 = 0.99$$
$$lgK_{St(DMTETA)} = (0.468 \pm 0.045)\, lgK_{St(HEMDTA)} + (4.28 \pm 0.73)$$
$$n = 5; \quad r = 0.986; \quad s_0 = 0.36$$
$$lgK_{St(HEMDTA)} = (1.27 \pm 0.13)\, lgK_{St(METPA)} - (3.40 \pm 1.96)$$
$$n = 5; \quad r = 0.974; \quad s_0 = 0.97$$
$$lgK_{St(DMTETA)} = (0.550 \pm 0.105)\, lgK_{St(METPA)} + (3.31 \pm 1.63)$$
$$n = 5; \quad r = 0.950; \quad s_0 = 0.68$$

One hypothesis is that the differences may be explained with the different experimental conditions. According to general expectations, approximately linear correlations exist between stability constants measured for some metal ions with different ligands; e.g.:

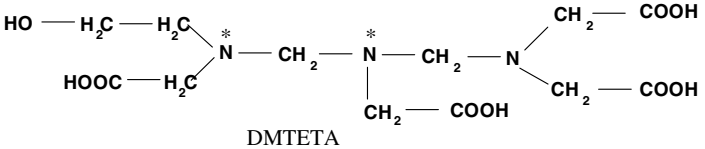
$$lgK_{St(Cu)} = (1.50 \pm 0.26)\, lgK_{St(Zn)} - (1.65 \pm 4.06)$$
$$n = 5; \quad r = 0.981; \quad s_0 = 0.88$$
$$lgK_{St(Cu)} = (0.552 \pm 0.158)\, lgK_{St(FeIII)} + (6.80 \pm 2.99)$$
$$n = 5; \quad r = 0.86; \quad s_0 = 1.35$$

In the analysis of structural effects, we would like to especially highlight the role of stereochemical relations in addition to the usual structural elements discussed in the introductive part. From this point of view, the eventual presence

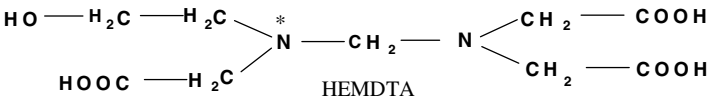
The abbreviations denote the following new compounds:



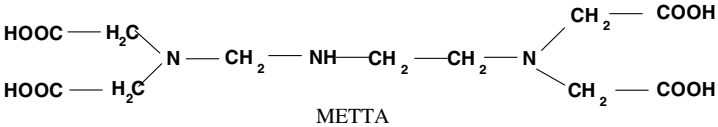
MDTA



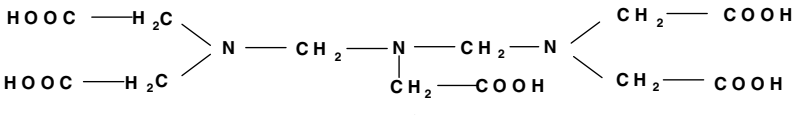
DMTETA



HEMDTA



METTA



METPA

of chiral nitrogen atoms is of particular importance, such as in DMTETA and HEMDTETA. The separation of the chiral isomers increases in their complexed form. Nevertheless, these questions require further research.

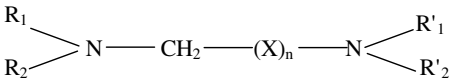
The following conclusions could be drawn:

1. From the pH-dependence of the stability of some new biodegradable complexes, the optimal experimental conditions can be predicted and the corresponding pH values can be calculated.
2. The established regression equations for the structural effects allow the calculation of some still unmeasured stability constants of the new biodegradable complexes.
3. Biodegradability in the case of chiral complexes raises special possibilities. From the compounds studied, two (HEMDTA and DMTETA) can have N-centered asymmetry; presumable their chiral structure is stabilized, but the biodegradability of the isomers will be different (Siemion et al. 1984).
4. Biodegradability may be considered as one of the characteristic properties of the soft compounds.

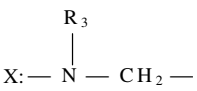
3. Experimental

3.1. Chemicals

The complex-genator metal ions (Ca^{2+}, Mg^{2+}, Cu^{2+}, Mn^{2+}, Zn^{2+}, Fe^{2+}, Fe^{3+}) were introduced in the system in water soluble salts. The biodegradable ligands (polyaminopolycarboxylic acids) were synthesized by Dr. E. J. Száva INGENIEUBÜRO (München). Their biodegradability (measured by OECD 301B method, in closed system) was 80% or above. Their general formula is:



Where R_1, R'_1, R_2, R'_2 are mutually independent hydroxyalkylic (C_{2-5}) or carboxyalkenylic (C_{2-4}) group;



is one of earlier groups or H; $n = 0$ or 1.

The investigated compounds were presented previously. For comparison, the measurements were carried out with EDTA also. The titrant solutions were prepared with KOH, of analytical purity. The constant ionic strength was assured with KCl a.p.

3.2. Apparatus, instruments

Multimeter, model Consort 835, glass-electrodes P10NB, Potentiometer (pH-meter) Jenway, Spectrophotometer Jenway 6405 UV-VIS.

3.3. Measurements

The experiments were made under optimized conditions as described previously (Boţone 2004; Dudutz 1976; Gherasim 2004; Kosztelnik 2004; Timaru 2004).

This research paper was presented during the 5th Conference on Retrometabolism Based Drug Design and Targeting, May 8–11, 2005, Hakone, Japan.

References

Anderegg G (1997) Critical survey of stability constants of EDTA complexes. Chemical data series. No. 14, Oxford, Pergamon Press, 250–258.

Arena G, Musumeci S, Purello R (1983) Calcium and magnesium – EDTA complexes stability constants and their dependence on temperature and ionic strength. Termochim Acta 61: 129–138.

Beck M (1965) Komplex egyensúlyok kémiája. Akadémiai Kiadó, Budapest.

Bodor N (1996) Design of biologically safer chemicals based on retrometabolic concepts. In De Vito SC, Garrett RL (eds.) Designing safer chemicals. Green chemistry for pollution prevention, Amer Chem Soc, Washington, p. 84–115.

Boethling RS (1996) Designing Biodegradable Chemicals. In De Vito SC, Garrett RL (eds.) Designing safer chemicals. Green chemistry for pollution prevention, Amer Chem Soc, Washington, p. 157–171.

Boţone DM (2004) Determinarea constantelor de protonare ale unor acizi poliaminopolicarboxilici. Diploma work, University of Medicine and Pharmacy, Tg-Mureş.

Danielle PG, De Stefano C, Penestri C, et al. (1994) Weak complex formation in aqueous solution. Curr Top Sol Chem 1: 95–106.

Delgado R, Do Carmo FM, Quintino S (1997) Redox method for the determination of stability constants of some trivalent metal complexes. Talanta 45: 451–462.

De Robertis A, De Stefano C, Rigano C, et al. (1987) The determination of formation constants of weak complexes by potentiometric measurements: Experimental procedures and calculation methods. Talanta 34: 933–938.

De Stefano C, Princi P, Rigano C, et al. (1987) Computer analysis of equilibrium data in solution ESAB2M: An improved version of the ESAB Programm. Ann Chim (Rome) 77: 643–675.

De Stefano C, Mineo P, Rigano C, et al. (1993) Ionic strength dependence of formazion constants. XVII. The calculation of equilibium concentrations and formation constants. Ann Chim (Rome) 83: 243–277

Dietz F (1987) New results concerning with EDTA content in drinking water. GWF-Wasser/Abwasser GWF 128: 286–288

Dudutz GyK (1976) Contribuţii la studiul unor compuşi ai acidului boric cu substanţe polihidroxilate sau tioderivaţi. PhD Thesis. Institute of Medicine and Pharmacy, Cluj-Napoca.

Gherasim DC (2004) Determinarea spectrofotometrică a constantelor de formare a unor complecşi poliaminopolicarboxilici, cu diferiţi ioni metalici. Diploma work, University of Medicine and Pharmacy, Tg-Mureş

Frimmel FH, Grenz R, Kordik E, Dietz F (1989) NTA and EDTA in surface waters in Germany. Vom Wasser 72: 175–184.

Kari FG, Giger W (1996) Speciation and fate of ethylenediamintetraacetate (EDTA) in municipal waste-water treatmant. Water Res 30: 122–134.

Kékedy L (1979) Fejezetek a korszerű analitikai kémiából. Dacia Könyvkiadó Kolozsvár, p. 65–66

Kezerian C, Ramsey WM (1964) Bis-adduction products and methods of preparing same. USP 3: 158–635.

Kosztelnik K (2004) Determinarea spectrofotometrică în UV a unor liganzi poliaminopolicarboxilici. Diploma work, University of Medicine and Pharmacy, Tg-Mureş.

Labádi I, Száva J (2001) Új biológiailag könnyen lebontható szerves komplexképző anyagok kifejlesztése és laboratóriumi vizsgálata. XXXVI. Komplexkémiai Kollokvium, Pécs

Nowack B (2003) Enviromental chemistry of phosphonic acids. Environ Sci Technol 37: 147–173

Pettit D, Powell KK (1997a) Stability constants database. Academic Software, IUPAC, Othey, UK, p. 25–30.

Pettit D, Powell K K (1997b) Stability constants database. Academic Software, IUPAC, Othey, UK, p. 132–139.

Pitter P, Sykora V, Bittnerova I (1999) Biological degradation of organic complexing agents and their legislation in Czech Republic. Berichte Nr. 153 aus Wassergüte- und Abfallwirtschaft, Technische Universität München

Schowanek D, Feijtel T, Perkins CM, Martmann FA, Federle TW, Larson RJ (1997) Biodegradation of [*S,S*], [*R,R*] and mixed stereoisomers of ethylene diamine disuccinic acid (EDDS), a transition metal chelator. Chemospere 34: 2375–2391.

Siemion IZ, Kubik A, Jezowska-Bojczuk M, Kolowski H (1984) The absolute configuration of the chiral nitrogen atom of proline residue in the metal complexes of oligopeptides. J Inorg Biochem 22: 137–141

Sykora V, Pitter P, Bittnerova I, Lederer T (2001) Biodegradability of ethylenediamine-based complexing agents. Wat Res 35: 2010–2016

Száva EJ (1999) Introduction of a new multipurpose chelating agent and its recommended field of application. 8th International Trace Element Symposium, Budapest.

Száva EJ (2001) Új környezetbarát ipari komplexképzők előállítása és vizsgálata. Erdélyi Magyar Műszaki Tudományos Társaság VII. Vegyész Konferencia, Kolozsvár.

Timaru A (2004) Determinarea constantelor de stabilitate ale unor complecşi biodegradabili, prin metoda potenţiometrică. Diploma work, University of Medicine and Pharmacy, Tg-Mureş.