Complex Formation of Selected Trivalent Metal Ions with (4*R*)-Thiazolidine-4-carboxylic Acid and Some of its Derivatives

P. BUTVIN, J. SVĚTLÍK, and D. TALIGOVÁ

Department of Pharmaceutical Analysis and Nuclear Pharmacy, Faculty of Pharmacy, Comenius University, SK-832 32 Bratislava

Received 12 December 1997

The complex formation of Al(III), Ga(III), In(III), Fe(III), La(III), and Ce(III) with (4*R*)-thiazolidine-4-carboxylic acid (TCA) and some of its 2-alkyl/aryl-substituted derivatives in aqueous solutions at ionic strength $I = 0.1$ mol dm^{-3} (NaCl) and temperature $\theta = 25$ °C was studied using the potentiometric titration method. The satisfying results were attained only for Al(III), Ga(III), and In(III) in reaction with TCA and its 2-alkyl-substituted derivatives. Other M(III) tested did not allow to evaluate their complex stability constants because of the precipitate creation or poor interaction. Further it was found that 2-aryl- or 2,2-dimethyl-substituted TCA derivatives are rapidly changed in aqueous solutions resulting in impossibility to determine their correct dissociation constants and hence to quantify the complex formation constants.

Complex formation abilities of the (4*R*)-thiazolidine-4-carboxylic acid (TCA) and its two simple analogues towards a group of divalent metal ions were studied by several authors [1—5]. Their results point at remarkably differentiated coordination properties and at not less interesting biological activities. Therapeutical action of TCA and related compounds according to certain scientists is influenced right by the ability to bind metal ions, the others [1] call for more detailed specification (*e.g.* formation of possible ternary complexes of the compounds in question with metal ions and proteins). In general, the ions of Ni(II), Zn(II), Cu(II), Cd(II), Mg(II), Ca(II), and Mn(II) form with these ligands the complexes mainly of ML, ML_2, and ML(OH) type. The order of complex stability constants has shown to be the same as that of other usual ligands bound to the central metal ion by oxygen and nitrogen atoms. It is believed that a sulfur atom in thiazolidine heterocycle significantly influences the complex formation while itself it does not take a part in the coordination. Authors of Ref. [6] have investigated the interaction of selected M(II) ions with several 2-aryl-substituted derivatives of TCA in mixed water—ethanol solutions. Their full and detailed results are, however, still not available to us. The TCA complexes with Co(III) and Fe(III) were studied by spectroscopic methods [7, 8]. The ions of Al, Ga, In, and other possible trivalent metals were not probably studied so far. With respect to the increasing meaning of mainly the ^{67,68}Ga and ^{111}In radionuclides [9], as well as to the wide-spectrum biological efficiency of compounds which are either thiazolidines modified in another way [10, 11] or they are a part of more complicated structures [12, 13], we have decided to study the TCA and some of its 2-substituted derivatives as the fundamental bidentate (tridentate) binding units for the above-mentioned trivalent metal ions in aqueous solutions by the potentiometric neutralization titration method.

EXPERIMENTAL

The L-thiazolidine-4-carboxylic acid (TCA) (98 %, Avocado, Research Chemicals Ltd., U.S.A.) was without additional purification taken into the experiments. The (2*RS*)-2-methyl (META), (2*RS*)-2-propyl (PRTA), (2*RS*)-2-butyl (BUTA), (2*RS*)-2-isobutyl (IBTA), 2,2-dimethyl (DMTA), and (2*RS*)-2-(2-hydroxyphenyl) (HPTA: expected tridentate ligand) derivatives of (4*R*)-thiazolidine-4-carboxylic acid were synthesized as in [14].

The other chemicals used (all anal. grade): NaOH (Salvus, Slovak Republic), NH_2SO_3H, NaCl (Chemapol, Czech Republic), $InCl_3$ (Aldrich, U.S.A.), $CeCl_3$ (Apolda, Austria); $Ga(NO_3)_3 \cdot 9H_2O$, $Al(NO_3)_3 \cdot 9H_2O$, $La(NO_3)_3 \cdot 6H_2O$, $Fe(NO_3)_3 \cdot 9H_2O$ (Lachema, Czech Republic). Concentrations of M(III) ions in stock solutions were determined by the complexometric titrations (for details see the relevant chapters in [15]).

The potentiometric titrations were carried out on an automated equipment consisting of pH-meter OP-550-S (Radelkis, Hungary), electrodes Theta 90 (combined glass one, Elektrochemická čidla – Electrochemical sensors, Czech Republic), G202C (glass elec-

Table 1. Dissociation Constants of TCA and Some of its Derivatives

Acid	Constant	Value ± s.d.	Equilibrium	pK_{a1}	Ref.
TCA	pK_{a1}	6.191 ± 0.026		6.20(3)	[1]
META	pK_{a1}	6.176 ± 0.088		6.10(4)	[2]
PRTA	pK_{a1}	6.126 ± 0.104	$>NH_2^+ \leftrightarrow >NH + H^+$	6.10(9)	[3]
BUTA	pK_{a1}	6.087 ± 0.060			
IBTA	pK_{a1}	6.102 ± 0.081			
DMTA	pK_{a1}	?			
	pK_{a1}	9.185 ± 0.037	Ar—OH ↔ Ar—O^- + H^+?		
HPTA	pK_{a2}	?	$>NH_2^+ \leftrightarrow >NH + H^+$?		
	pK_{a3}	2.052 ± 0.110	—COOH ↔ —COO^- + H^+		

Table 2. Overall Complex Stability Constants $\log \beta_n$ of TCA and Some of its Derivatives with Selected M(III) Ions

M(III)	Complexes	4TCA	PRTA	BUTA	IBTA
Al	MLH	–	* –	* –	* –
	ML		–		
	ML_2	5.55	4.54	4.14	4.40
	ML_3	12.18	11.37	10.42	10.82
	$M_xL_y(OH)_z$			-1.65_{112}	-1.77_{112}
Ga	MLH				
	ML	–	–		
	ML_2	11.33	10.83	11.24	11.12
	ML_3	13.54	14.44	15.95	15.75
	$M_xL_y(OH)_z$			-2.75_{111}	
In	MLH	10.61	* 11.48	* 11.25	* 11.80
	ML	6.14	6.31	6.47	6.64
	ML_2	9.58	10.24	10.91	10.55
	ML_3	11.40	13.59	14.96	14.64
	$M_xL_y(OH)_z$	-3.60_{122}	-2.89_{122}	1.39_{122}	-3.05_{122}

*Evaluated from the reduced pH range data (up to pH = 8.5); $0.07 > \sigma_{pH\ fit} > 0.030$...

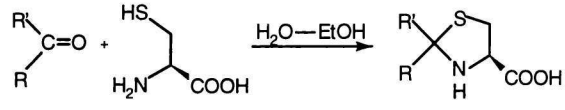
trode, Radiometer, Denmark) and OP718-P (SKE, Radelkis, Hungary), autoburette OP-930/1 (Radelkis, Hungary), thermostat UH-MLW, (Germany), magnetic stirrer MM6 (Poland), and computer PC 386 SX (LeNS, Slovak Republic).

Electrodes were calibrated with standard buffers of KH phthalate (c = 0.05 mol dm^{-3}, pH = 4.04 at 20 °C) and $Na_2B_4O_7$ (c = 0.1 mol dm^{-3}, pH = 9.18 at 20 °C). Buffer solutions with pH = 2.15 and 7.06 (Radelkis, Hungary) were used as working ones. The titrations were carried out in inert atmosphere of purified nitrogen (Tatragas, Slovak Republic) and at a constant ionic strength I = 0.1 mol dm^{-3} (NaCl) and θ = 25 °C.

Procedure: To a jacket titration vessel maintained at desired temperature there were pipetted calculated volumes of the ligand (metal) and NaCl solutions and filled up to 50 cm^3 with redistilled water. After everything had been prepared the titration has started *via* a command from the computer, and later automatically stopped according to the estimated NaOH titrant consumption. Experimental data (pH = f(V_{4NaOH})) were saved for the subsequent treatment by the PKAS, BEST, and SPE programs [16]. The respective results are given in Tables 1 and 2, and Figs. 1—4 as well.

RESULTS AND DISCUSSION

The synthesis of 2-substituted derivatives of TCA is simply feasible by reaction of L-cysteine and a carbonyl compound according to the scheme [14]



where R′ = R = H (TCA), R′ = R = CH_3 (DMTA), R′ = H, R = CH_3 (META), C_3H_7 (PRTA), C_4H_9 (BUTA), iso-C_4H_9 (IBTA), 2-(C_6H_4)OH (HPTA).

Identity of the synthesized TCA derivatives was confirmed by comparison of their melting points: HPTA: m.p. = 163—165 °C (164—166 °C [14]), META: m.p. = 162—164 °C (161—163 °C [17]), PRTA: m.p. = 170—171 °C (168—169 °C [17]), BUTA: m.p. = 162—163 °C (163—164 °C [17]), DMTA: m.p. = 131—133 °C

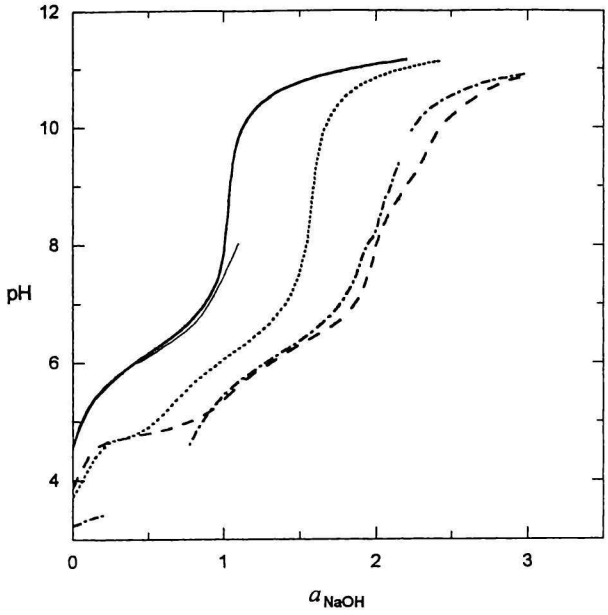


Fig. 1. Titration curves of TCA alone and in the presence of selected M(III) ions. $c_{NaOH} = 0.1012$ mol dm^{-3}, $I = 0.1$ mol dm^{-3} (NaCl), $\theta = 25$ °C. Remark: Fe(III) curve not drawn because of precipitate formation at pH > 5. —— TCA, -- TCA Al (3 1), - - - TCA Ga (3 1), TCA In (6 1), —— TCA La(Ce) (3 1).

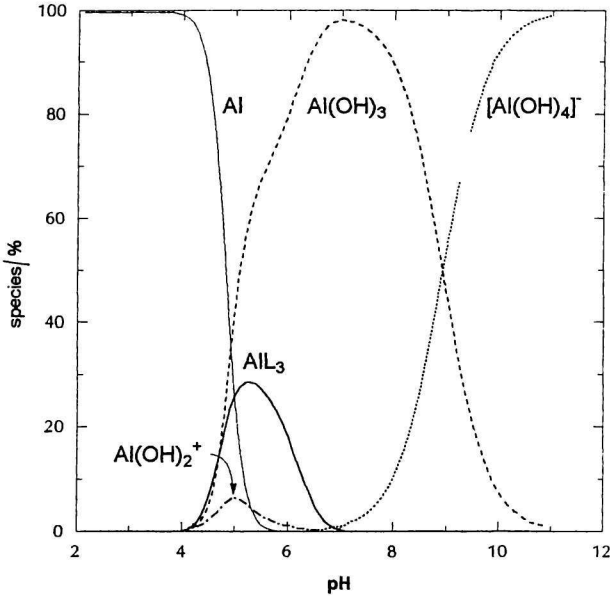


Fig. 2. Species distribution plot of the TCA—Al(III) system.

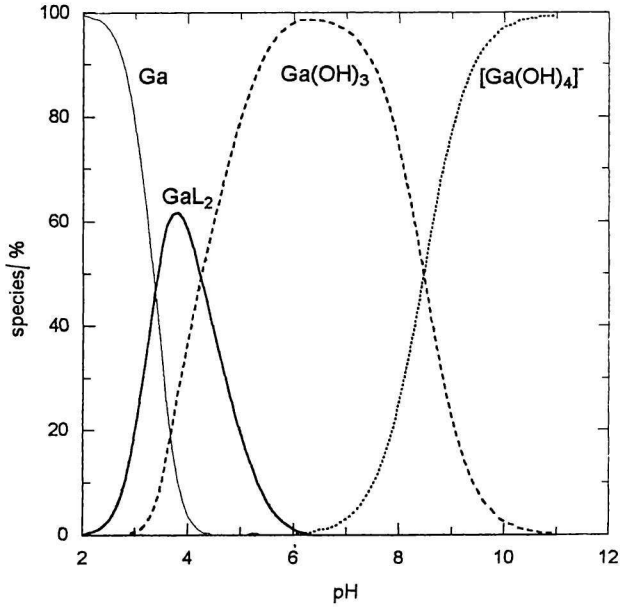


Fig. 3. Species distribution plot of the TCA—Ga(III) system.

(134—136 °C [17]), IBTA: m.p. = 163—165 °C (154 °C, decomp. [18]).

The NaOH titrant quality and that of the electrode systems were tested by a Gran method [16]. Its results have shown that titrant did not contain more than 0.2 % of carbonates (during all experiments), and the electrode systems measured the pH with sufficient sensitivity and accuracy (system G202C + OP718-P was better at the pH level > 9 and was used for the verification of doubtful outputs).

Acid-Base Properties

Having twenty 2-substituted TCA derivatives synthesized, the TCA itself and six related compounds, *i.e.* META, PRTA, BUTA, IBTA, DMTA, and HPTA were subjected to the study. The others exert very low solubility in water (addition of ethanol suppressed it even more). Freshly prepared solutions of the ligands (in approximate concentration of 5×10^{-3} mol dm^{-3} dissolved at room temperature except of HPTA with $c = 2.5 \times 10^{-3}$ mol dm^{-3} dissolved at 37—40 °C) were always taken into the experiments. Their exact concentrations were evaluated from the titrant end-point consumption by the controlling program.

The dissociation constants were computed by the PKAS program (Table 1). The lowest pK_a values for all acids were obtained by the titration of equimolar mixtures of their solutions with HCl. These were practically the same (2.00 ± 0.08), which means that the carboxylic group ionization is not influenced by the 2-alkyl/aryl substitution. The mean pK_a values corresponding to the equilibrium $> NH_2^+ \leftrightarrow > NH + H^+$ were after the 2-alkyl substitution of TCA slightly decreased, but within the determined deviations. On the other hand, compounds obtained by the alkylation of EDTA ethylene skeleton exert expected, *i.e.* an increased mean value of the constant belonging to that type of equilibrium [19] due to the influence of the $+I$ effect of an alkyl substituent. The missing values for DMTA and HPTA in Table 1 (?) were determined to

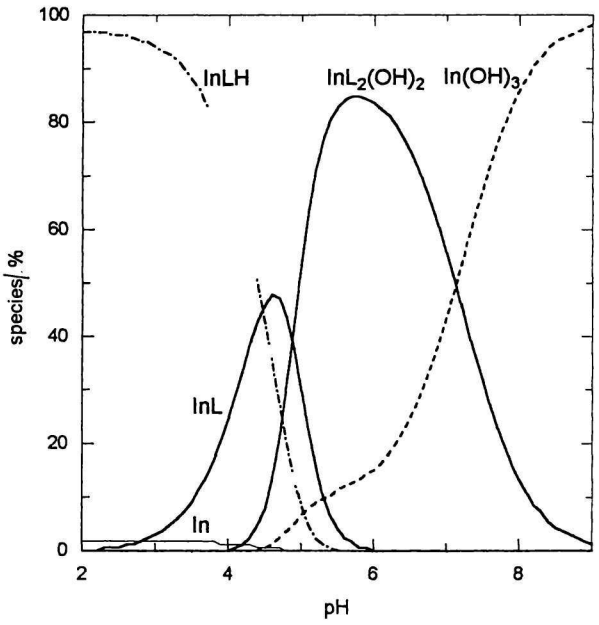


Fig. 4. Species distribution plot of the TCA—In(III) system.

be $pK_{a1} \approx 8.12$ and $pK_{a2} \approx 7.98$, respectively. It is evident that these constants for that equilibrium are almost of 2 units higher than those of TCA and its 2-alkyl derivatives, being practically equal to corresponding pK_a of free cysteine (8.18 [20]). Therefore it is likely that almost immediately after the dissolution of DMTA or HPTA in water the change of their original structures takes place. Neither the experiments led in mixed solvent (water ethanol (v/v) = 9 1 and 3 1 – at latter the HPTA starts to precipitate off the solution) did not suppress it. The hydrolysis of 2-aryl-substituted thiazolidines at pH $\geqq$ 11 is known (at simultaneous heterocycle opening [21, 22]).

Since it had been impossible to obtain the dissociation constants for DMTA and HPTA as well defined ligands, they were excluded from the effort to quantify their complex formation abilities. META is just investigated.

Complex Formation Properties

TCA, PRTA, BUTA, and IBTA were studied in the reaction with Al(III), Ga(III), In(III), La(III), Ce(III), and Fe(III) ions. The three mutual mole ratios of metal to ligand M L = 1 2, 1 3, and 1 6, respectively, were used. The typical titration curves of TCA itself and in the presence of the ions studied are shown in Fig. 1. The types of complexes and their overall stability constants ($\log \beta_n$) were determined by the BEST program, which iteratively computes a mathematical model of the titration curve after addition of the known dissociation constant(s) and first estimates of other input parameters to the experimental data files. If a computed curve fits tightly with the experimental one (expressed as a $\sigma_{pH\ fit}$), the computed $\log \beta_n$ may be regarded as true. The suitability of BEST (establishing the initial chemical equilibrium models) was tested on the Ni(II)—TCA at experimental conditions described in [1]. Obtained $\log \beta_n$ were very close (devs. $\leqq$ 2.2 %) to those published there. The $\log \beta_n$ for TCA and its three 2-alkyl derivatives complexes with Al(III), Ga(III), and In(III) are listed in Table 2.

Only the experiments for the Ga(III)—TCA systems could be evaluated without difficulties. In the other cases, where the precipitate formation or anomalous courses (partly reproducible number and depth of the pH "down jumps" – observed only for the TCA derivatives), both starting at the pH of 8.5—9.5, occurred, it was necessary to use either the experimental titration curves (data) for reduced pH range, or to treat those acquired for the metal-to-ligand ratio of 1 6, where these phenomenons were suppressed. Only two types of successive "normal" complexes, *i.e.* ML_2 and ML_3 were detectable for TCA and its three tested derivatives with Al(III) and Ga(III), while In(III) gave all three types ML, ML_2, and ML_3 preceded by hydrido complex MLH. The prolongation of 2-alkyl substituent of the TCA derivatives brought about the slight increase of the Ga and In $\log \beta_{ML_3}$ values while those of Al became a little decreased. Indium gave, in general, lower $\log \beta_n$ values than those for Ga—TCA (derivatives) systems.

Treatment of the same final input data files (used in BEST) by the SPE program gave the species distributions data. Fig. 2 is showing that only the species of AlL_3 are visibly present. At pH $\geqq$ 7 there exist only inorganic aluminium species. Fig. 3 for Ga(III) species distribution is showing practically the same picture, its system contains mainly the products of hydrolysis. Fig. 4 depicts that from all successive classical complexes only the InL distribution could be visibly plotted. In acidic region predominate the InLH, in neutral one $InL_2(OH)_2$, and then $In(OH)_3$. PRTA and IBTA have shown very similar species distributions, BUTA gave slightly higher percent of $InL_2(OH)_2$ (with a maximum of 94 % at pH = 5.5) while that of $In(OH)_3$ was there decreased. Although the relative abundance of all Al(III) and Ga(III) species in the systems containing TCA and its derivatives differs mutually to some extent (BUTA and IBTA hydroxo complexes distribution is not drawn in Figs. 1 and 2, respectively), it was found that alkan-1-yl 2-substitution of TCA does not exert a significant influence upon the formation and percent of normal successive complexes being of dominant interest.

According to the results summarized in Table 2 and inspection of the species distribution plots it may be stated that TCA and its 2-substituted derivatives tested are perhaps disadvantageous to insert the trivalent metal ions (*e.g.* radionuclides of Ga and In as potential radiopharmaceuticals) into the biological sys-

tems in the form of sufficiently stable "normal" complexes, because in the physiologic region of pH there exist the more component mixtures containing mainly hydroxo complexes and other products of hydrolysis. Maybe it is a consequence of low basicity of the imino-nitrogen atom in the thiazolidine ring and impossibility to form more chelate rings per a ligand molecule. One of the most stable complexes of these metal ions is formed by *N*,*N*′-dipyridyloxyethylenediamino-*N*,*N*′-diacetic acid (PLED [23]) with $\log K_{ML}$ for Ga(III) and In(III) equal to 36.55 and 36.89, respectively. La(III), Ce(III), and Fe(III) do not react or very little, not measurably by the method used. Maybe the experimental conditions (*e.g.* use of NaCl as ionic background salt) were not much suitably selected (likely for the Ga(III) and In(III) as well), but there was an effort to hold them so as to be similar with those published in [1, 2] to enable some comparison, which supports the idea about the observable differences in the metal complex formation abilities of the TCA type ligands.

TCA and related compounds are, however, attractive for the biological testing not only as complexes with metal ions. One of them is a compound named as pidotimod [10, 11] being an *N*-acylated derivative of TCA. Its pK_a as reported (3.03 at 24 °C) belongs to the deprotonation/protonation equilibrium on a secondary amido group in the pyroglutamic acyl part. From the point of view of the pidotimod structure and low value of its second dissociation constant, one cannot suppose the formation of any stable metal complexes, thus the published wide-spectrum biological activity is mediated by the other mechanism(s) than that assumed for TCA and its derivatives possessing unchanged donor groups.

CONCLUSION

The aim was to study the metal complex formation of the (4*R*)-thiazolidine-4-carboxylic acid and some of its derivatives with selected trivalent metal ions at the conditions used in earlier studies and approaching to those for biological environment. This has led to the determination of dissociation constants, the overall metal complex stability constants, the types of complexes, and the species distributions for the TCA and its three derivatives, *i.e.* PRTA, BUTA, IBTA with Al(III), Ga(III), and In(III) using experimental data from the automated potentiometric neutralization titration method, treated by the standard computing programs PKAS, BEST, and SPE.

Ions Fe(III), La(III), and Ce(III) did not reveal measurable affinity towards these ligands, which might support the earlier assumption about the complex formation selectivity of the TCA type ligands. In the physiological region of pH the given compounds, however, form predominantly the hydroxo complexes and other products of hydrolysis, therefore they do not seem to be the advantageous scavengers of meaningful M(III) ions in a biological system. But at the radiolabelling there are used just very small amounts of radionuclides with great excess of complexing compounds [9], so that it may positively alter the formation of useful complex species and reduce that of undesired ones (good labelling of some TCA derivatives with ^{299m}Tc was already carried out [24]).

Simultaneously it was found that the TCA derivatives which were substituted at the C-2 position with other (bulky) radicals instead of n-alkyl or strongly electron-attracting ones, are rapidly changed along with heterocycle opening when dissolved in water medium. In general, one cannot exclude that the presence of certain metal ions in freshly prepared solutions of some unstable ligands may invoke the complex formation with the original ligand structures or that they might be renewed owing to the chelation phenomenon. But the potentiometric neutralization titration and subsequent evaluation without a chance to obtain the correct dissociation constants do not allow to use the method there.

Acknowledgements. This work is a part of the solution of the grant task No. 1/1218/94.

REFERENCES

1. Kurzak, B., Kroczewska, D., Morawiec, J. P., and Tatarowski, T. *Bull. Pol. Acad. Sci., Chem. 39*, 177 (1991).
2. Huang, Z. X., May, P M., Williams, D. R., and Gosalvez, M., *Inorg. Chim. Acta 56*, 41 (1981).
3. Fazakerley, G. V., Jackson, G. E., and Linder, P W. *J. Inorg. Nucl. Chem. 38*, 1397 (1976).
4. Tatarowski, T., Kubiak, M., Glowiak, T., Morawiec, J. P., Kozlowski, H., and Gosalvez, M., *Inorg. Chim. Acta 93*, 3 (1984).
5. Radomska, C., Tatarowski, T., Morawiec, J. P and Kozlowski, M., *Inorg. Chim. Acta 106*, 29 (1985).
6. Vinod, K. B., Stayanargan, R. M., and Ram, R. H. G., *Orient. J. Chem. 7*, 117 (1991), cited from *Chem. Abstr. 116*, 114528r (1992).
7. Catrina, E., *Rev. Roum. Chim. 21*, 81 (1976).
8. Catrina, E., Sinon, Z., Szabaday, Z., and Catrina, G., *Rev. Roum. Chim. 21*, 1037 (1976).
9. Owunwanne, A., Patel, M., and Sadek, S., *The Handbook of Radiopharmaceuticals.* Chapman & Hall Medical, London, 1995.
10. Magni, A., Signorelli, G., and Bocchiola, G., *Arzneim.-Forsch. 44*, 1402 (1994).
11. Crimella, T., Orlandi, R., Bocchiola, G., Anders, U. and Stradi, R., *Arzneim.-Forsch. 44,* 1405 (1994).
12. Carroll, M. P., Zera, R. T., Roberts, J. C., Schlafmann, S. E., Feeney, D. A., Johnston, G. R., West, M. A., and Bubrick, M. P., *Dis. Colon. Rectum. 38*, 716 (1995).
13. Rathbun, W. B., Killen, C. E., Hollesachau, A. M., and Magasawa, H. T. *Biochem. Pharmacol. 51*, 1111 (1996).
14. Soloway, H., Kipnis, F., Ornfelt, J., and Spoerri, P. E., *J. Am. Chem. Soc. 70,* 1667 (1948).
15. Přibil, R., *Komplexometrie.* (Complexometry.) Nakla-

datelství technické literatury (Publishers of Technical Literature), Prague, 1977.
16. Martell, A. E. and Motekaitis, R. J., *Determination and Use of Stability Constants*. Second Edition, VCH Publishers, Weinheim, 1992.
17. Reimschneider, R. and Hoyer, G. A., *Z. Naturforsch. 17*, 765 (1962).
18. Cook, A. H. and Heilbron, I. M., *Chemistry of Penicillin*, p. 921. Princeton Press, Princeton, 1949.
19. Majer, J., Butvin, P., Novák, V., Svičeková, M., Füleová, E., Valášková, I., and Novák, J., *Chem. Zvesti 33*, 742 (1979).
20. Martell, A. E. and Smith, R. M., *Critical Stability Constants* (*Amino Acids*), p. 47. Plenum Press, New York, 1974.
21. Luhowy, R. and Meneghini, F., *J. Am. Chem. Soc. 101*, 420 (1979).
22. Pesek, J. J. and Frost, J. H., *Tetrahedron 31*, 907 (1973).
23. Green, M. A., Welch, M. J., Mathias, C. J., Taylor, P., and Martell, A. E., *Int. J. Nucl. Med. Biol. 12*, 381 (1985).
24. Havránek, E., Bumbálová, A., Beláková, M., Kettmann, V., and Světlík, J. *J. Radioanal. Nucl. Chem., Lett. 212*, 69 (1996).

Translated by P. Butvin