

# Complex Formation of Thiazolidine-2,4-dicarboxylic Acid with Selected Divalent and Trivalent Metal Ions

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Verification of the stability of (2*RS*,4*R*)-thiazolidine-2,4-dicarboxylic acid (TIDA) and mainly the study of its interaction with Mg(II), Ca(II), Ba(II), Cd(II), Cu(II), Ni(II), Pb(II), Zn(II), Al(III), Ga(III), In(III), and La(III) at ionic strength  $I(\text{NaCl}) = 0.1 \text{ mol dm}^{-3}$  and temperature  $\theta = 25^\circ\text{C}$  using the potentiometric titration method gave the following results: Its aqueous solutions are stable for several weeks at ordinary conditions, at temperatures up to  $70^\circ\text{C}$  at least for 5 h. Secondly, TIDA acts as a tridentate ligand towards the selected metal ions binding them by two chelate rings giving the complexes of  $n(\text{L}):n(\text{M}) = 1:1$  type only. These are of rather low stability and selectivity.

The papers [1–4] are devoted to the study of some properties and behaviour of a series of 2-alkyl or 2-aryl derivatives of (4*R*)-thiazolidine-4-carboxylic acid (TCA) to find out the possibility of their use in pharmacy, mainly as new potential radio-labeled compounds with  $^{99\text{m}}\text{Tc}$ . With the exception of TCA itself and a few of its 2-alkyl derivatives these simple heterocyclic compounds, in most cases, did not enable to perform smoothly evaluable experiments because they are more or less hydrolyzed [2] during the time necessary to carry out planned measurements. Only the (2*RS*,4*R*)-thiazolidine-2,4-dicarboxylic acid (TIDA) was found to be the most stable amongst all the TCA derivatives available to us. Maybe one of the practical uses of TIDA is proposed in a French patent as its (and arginine) magnesium salt in pharmaceutical formulations [5].

Since no deeper information about the TIDA complex equilibria in aqueous solutions was available, we decided to present here the adequately oriented study along with some preceding tests.

## EXPERIMENTAL

TIDA was synthesized from glyoxylic acid and L-cysteine (both Fluka, Germany) with slight modification of that described in [6]. After recrystallization from hot aqueous solution a white crystalline substance with m.p. =  $180\text{--}181^\circ\text{C}$  (Ref. [6] gives m.p. =  $180^\circ\text{C}$ ) and the corresponding elemental analysis and almost identical IR spectrum with that in [6] was obtained. Another proof of identity gave the TIDA titration curve(s) No. 1.

Chemicals used (all anal. grade) were: NaOH,

HCl (Slavus, Slovak Republic),  $\text{NH}_2\text{SO}_3\text{H}$ , NaCl (Chemapol, Czech Republic),  $\text{Mg}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ ,  $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ ,  $\text{Ba}(\text{NO}_3)_2$ ,  $\text{Cd}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ ,  $\text{Cu}(\text{NO}_3)_2 \cdot 3\text{H}_2\text{O}$ ,  $\text{Ni}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ ,  $\text{Pb}(\text{NO}_3)_2$ ,  $\text{Zn}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ ,  $\text{Al}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$ ,  $\text{Ga}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$  (all Lachema, Czech Republic),  $\text{InCl}_3$  (Aldrich, U.S.A.), and  $\text{La}(\text{NO}_3)_3 \cdot 6\text{H}_2\text{O}$  (Apolda, Austria). Concentrations of the metal salts in stock solutions were determined by the complexometric titrations (for details see [7]).

The list of instruments, buffers, and auxiliary material necessary for the potentiometric titrations as well as the description of the titration procedures, is given in [1] with the exception of electrodes: a combined glass electrode OP-0808P (Radelkis, Hungary) was used here.

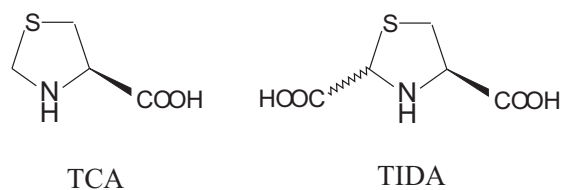
*Procedure:* 1.78 g (0.01 mol) of TIDA was dissolved in  $250 \text{ cm}^3$  of redistilled water.  $25 \text{ cm}^3$  portions of this stock solution were treated as follows: The first portion after dilution (described below) was used for the titrations with NaOH titrant at selected time intervals (start, 1 h, 4 h, 8 h, 24 h, 72 h, 168 h, 336 h, and 1008 h = 6 weeks). Next three portions were heated to  $37^\circ\text{C}$ ,  $50^\circ\text{C}$ , and  $70^\circ\text{C}$ , respectively, and kept at given temperatures for approx. 5 h. Afterwards, all the TIDA portions were filled up to the total volume of  $250 \text{ cm}^3$ . Therefrom  $25 \text{ cm}^3$  volumes have been pipetted into the titration vessel. The rest of stock solution was used for the study of interaction of TIDA with ions of metals analogically as in [1].

After each titration the experimental data ( $\text{pH} = f(V_{\text{NaOH}})$ ) were automatically saved as data files for treatment by the PKAS, BEST, and SPE programs [8]. The respective results are given in Table 1 and

Figs. 1—5. The  $x$ -axis in Figs. 1—3 is scaled in  $a$  units ( $n(\text{base})/n(\text{ligand})$ ) gained by recalculation of the corresponding titrant consumptions and saved again as  $\text{pH} = f(a_{\text{NaOH}})$  under changed names. The correctness of experimental procedures and use of the programs was verified as before [1].

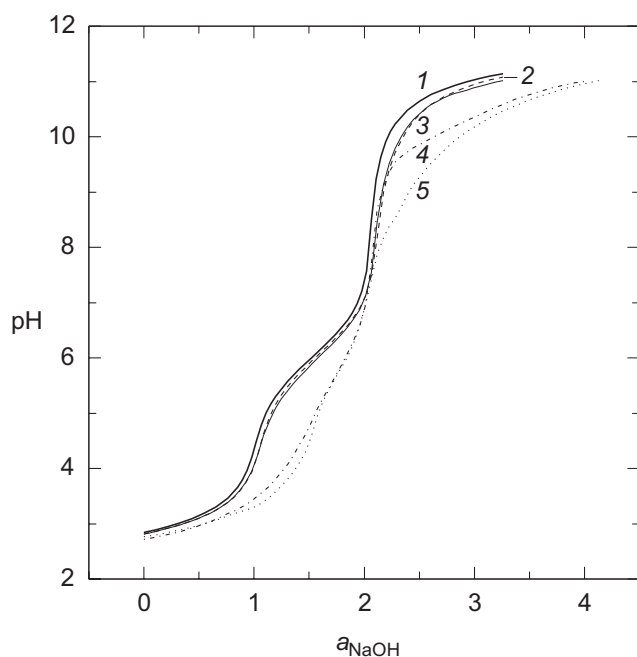
## RESULTS AND DISCUSSION

The TIDA synthesis using free L-cysteine (which yielded 83 % of crude product) was equally effective as that using cysteinium chloride [6]. The structures of TCA and its derivative TIDA are as follows:

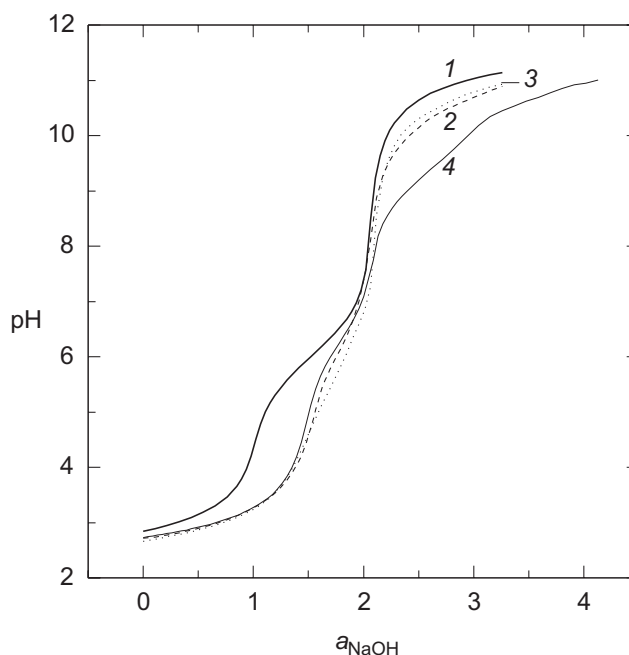


The Gran test [8] has shown that the NaOH titrant contained 0.23—0.55 % of carbonates (at the beginning and end of all titrations) and the combined glass electrode OP-0808P measured pH with sufficient sensitivity and accuracy within the range 2—10.

A fundamental titration curve of TIDA is denoted as the curve 1 (Figs. 1—3). According to the weighed amount, dilutions, and the titrant consumptions at the two end points it was calculated that TIDA contained



**Fig. 1.** Titration curves of TIDA.  $c_{\text{NaOH}} = 0.1045 \text{ mol dm}^{-3}$ ,  $I(\text{NaCl}) = 0.1 \text{ mol dm}^{-3}$ ,  $\theta = 25^\circ\text{C}$ . 1. TIDA alone, 2.  $n(\text{TIDA}):n(\text{Ca}) = 2:1$ , 3.  $n(\text{TIDA}):n(\text{Mg}) = 2:1$ , 4.  $n(\text{TIDA}):n(\text{Cd}) = 2:1$ , 5.  $n(\text{TIDA}):n(\text{Zn}) = 2:1$ .

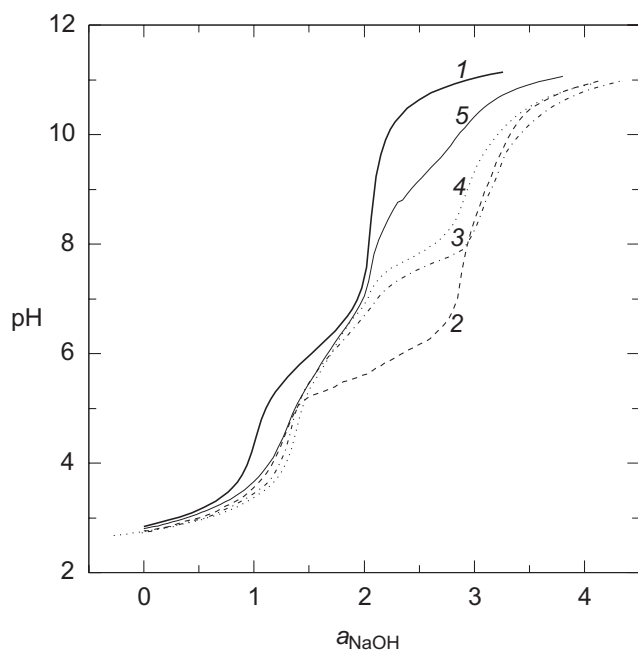


**Fig. 2.** Titration curves of TIDA.  $c_{\text{NaOH}} = 0.1045 \text{ mol dm}^{-3}$ ,  $I(\text{NaCl}) = 0.1 \text{ mol dm}^{-3}$ ,  $\theta = 25^\circ\text{C}$ . 1. TIDA alone, 2.  $n(\text{TIDA}):n(\text{Cu}) = 2:1$ , 3.  $n(\text{TIDA}):n(\text{Ni}) = 2:1$ , 4.  $n(\text{TIDA}):n(\text{Pb}) = 2:1$ .

98.8 % of the declared compound. Only unreacted cysteine should form the impurities, but due to its low amount and remarkably higher  $\text{p}K_{\text{a}2}$  and  $\text{p}K_{\text{a}3}$  [9] it should not affect the calculated results for TIDA. All titration curves from the aging measurements or after the thermal treatment did not differ more than 1.3 % from the fundamental one (*i.e.* shifts of their two end points) pointing at really high stability of TIDA in aqueous solutions at given conditions. Its resistance against action of oxidizing, reducing or otherwise attacking agents has been probably investigated (as in [6] or elsewhere), but with regard to the aim and conditions of our experiments it is redundant to review it here.

Knowledge of dissociation constants of a ligand should precede the study of its complex formation by the potentiometric pH measurements. Thus the values for TIDA are:  $\text{p}K_{\text{a}1} = 5.86 \pm 0.05$  (heterocyclic —NH— group),  $\text{p}K_{\text{a}2} = 2.31 \pm 0.03$  (carboxylic group at the C-4), and  $\text{p}K_{\text{a}3} = 1.70 \pm 0.02$  (carboxylic group at the C-2). Assignment of these  $\text{p}K_{\text{a}}$ 's and mainly their order was established with regard to  $\text{p}K_{\text{a}}$  ( $\approx 1$ ) of —COOH in thiazolidine-2-carboxylic acid [10], values of chemical shifts of the methine proton signals at the C-2 and C-4 of TIDA [6], and the subsequent complex formation experiments (the order in [2] is not fully correct). The  $\text{p}K_{\text{a}}$ 's calculated from the aging measurements or thermal treatment were close ( $\text{SD}'\text{s} \leq 0.025$ ) to those listed above.

TIDA ( $\approx 0.1 \text{ mmol}$ ) has been left to react with the selected metal ions at mutual mole ratios  $n(\text{M}):n(\text{L})$



**Fig. 3.** Titration curves of TIDA.  $c_{\text{NaOH}} = 0.1045 \text{ mol dm}^{-3}$ ,  $I(\text{NaCl}) = 0.1 \text{ mol dm}^{-3}$ ,  $\theta = 25^\circ\text{C}$ . 1. TIDA alone, 2.  $n(\text{TIDA}):n(\text{Al}) = 3:1$ , 3.  $n(\text{TIDA}):n(\text{Ga}) = 3:1$ , 4.  $n(\text{TIDA}):n(\text{In}) = 3:1$ , 5.  $n(\text{TIDA}):n(\text{La}) = 3:1$ .

= 1:2, 1:3, 1:4, and 1:6 (equimolar mixtures have led to frequent formation of precipitates and/or irregularities upon the titration curves). Afterwards, the

complex formation has been evaluated by the BEST program (for more details refer to [1]). All  $\log \beta$  (Table 1) were estimated with  $\sigma_{\text{pH fit}} < 0.026$ . The sixth column in Table 1 contains available data for TCA (comparison purpose), the seventh appropriate reference numbers. All the TIDA titration curves used for the final computations are plotted in Figs. 1–3.

Figs. 1–3 show that TIDA forms the complexes with all the metal ions studied (? Ba(II) – its curves (not drawn) practically overlap the curve 1). A negative part of the  $a$  values (Fig. 3 – for In(III) it is visible) means that there was firstly titrated an excess of mineral acid contained in the In or Ga salt solutions (to prevent their hydrolysis).

$\log \beta$  of the TIDA metal complexes (ML type) are, in general, higher than those of TCA. Thus, in the order of Ni(II), Zn(II), and Cd(II) the latter  $\log \beta$  has increased the most (+2.74 units, *i.e.* nearly twice). These findings may be assigned to the TIDA ability to form the second chelate ring (concluded at the labeling of TIDA with  $^{99\text{m}}\text{Tc}$  [3, 4], too). Only a bit higher are  $\log \beta$  of TIDA with Ca(II) and Mg(II). The compared values of TIDA and TCA with Cu(II), Al(III), and In(III) are almost the same (without an additional testing it cannot be seriously discussed). In any case, the formation of higher successive complexes was not disclosed by the BEST mathematical modeling even for the ratio  $n(\text{M}):n(\text{L}) = 1:6$ . At certain circumstances this could be advantageous as in the case of EDTA (see [7]), but entirely low  $\log \beta$  and se-

**Table 1.** Overall Complex Stability Constants  $\log \beta$  of TIDA and TCA with Selected M(II) and M(III) Ions

Cation	$p$	$q$	$r$	TIDA	TCA	Ref.
Mg(II)	1	1	0	2.26	1.68	[10]
Ca(II)	1	1	0	1.92	1.65	[10]
Ba(II)	1	1	0	?	–	
Cd(II)	1	1	0	5.52	2.78	[10]
Cu(II)	1	1	0	5.96	6.02	[11]
Ni(II)	1	1	0	6.35	4.04	[10, 11]
	1	1	–1	–5.29	–	
Pb(II)	1	1	0	6.11	–	
Zn(II)	1	1	0	5.65	3.21	[10, 11]
	1	1	–1	–3.45	–5.79 <sup>a</sup>	[10]
Al(III)	1	1	0	5.65	5.55 <sup>b</sup>	[1]
Ga(III)	1	1	0	6.04	11.33 <sup>b</sup>	[1]
			–1	–1.47	–	
In(III)	1	1	0	5.88	6.14	[1]
	1	1	–2	–9.72	–3.60	
La(III)	1	1	0	4.63	–	
	1	1	–1	–4.53		

$p$  = number of metal ions,  $q$  = number of ligands,  $r$  = number of protons ( $-r$  means number of hydroxo groups).

<sup>a</sup>) Value belonging to a thiazolidine-2-carboxylate complex, <sup>b</sup>) values belonging to the TCA (ML<sub>2</sub>) complexes.

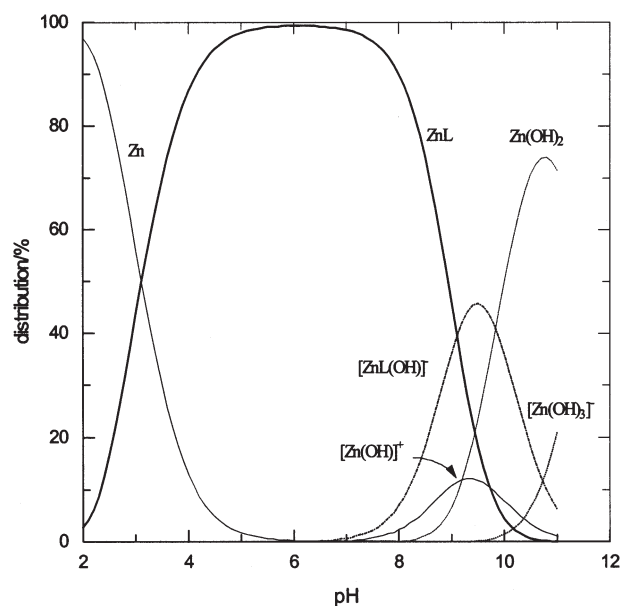


Fig. 4. Species distribution plot of the  $n(\text{TIDA}):n(\text{Zn}) = 2:1$ .

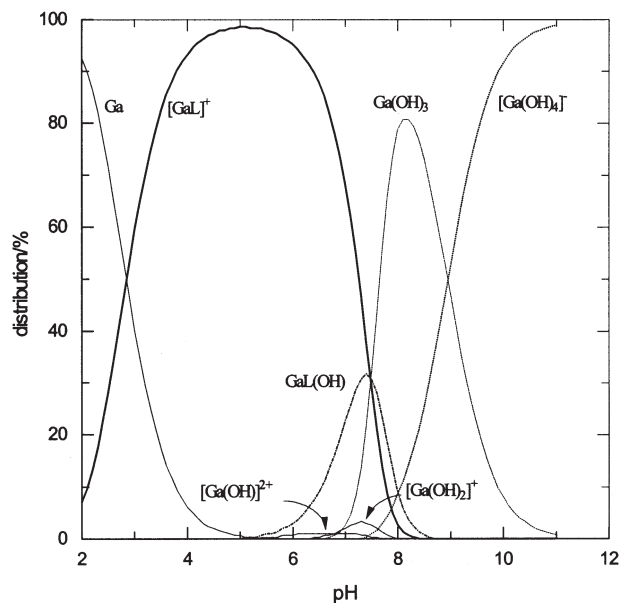


Fig. 5. Species distribution plot of the  $n(\text{TIDA}):n(\text{Ga}) = 3:1$ .

lectivity (small differences among the individual  $\log \beta$ ) degrade the “complexing power” of TIDA. Tendency to form the hydroxo complexes is very close to that of TCA and its derivatives [1, 9]. It may be summarized that TIDA acts as tridentate ligand binding a metal ion by the two five-membered chelate rings, heterocyclic S atom is not therein involved (agreement with earlier observations [10, 11]). The mutual ratio of the TIDA two diastereoisomers as interpreted in [6] could play a role at the metal complex formation (less effective *trans* isomer prevails in acidic medium), which probably contributes to the above-mentioned characterization of the TIDA complex formation properties.

The typical distributions of various species of TIDA in the presence of M(II) and M(III) in dependence on pH (obtained by the SPE program) are plotted in Fig. 4 and 5, respectively. Therefrom it may be deduced that the highest relative amounts of the ML species exist in narrow intervals of pH, in which for M(III) there are present at least the traces of other species (free metal ions, hydrolytic products or both). The paper electrophoresis experiments have shown that TIDA complexes with  $^{99\text{m}}\text{Tc}$  are only of the ML type, too, with ions of  $\text{Cl}^-$  ( $\text{pH} < 7$ ) or  $[\text{OH}]^-$  incorporated [3], which supports our findings and assumptions.

When thinking about potential use of the TIDA metal complexes for a pharmaceutical purpose according to their existence at physiological pH, those of Zn(II) and Mg(II) could be utilized, the latter was already proposed [5]. One cannot, however, exclude that at suitable experimental conditions (preparation, isolation) and/or use of auxiliary agents (*e.g.* to support the formation of ternary complexes as in [10])

there can be obtained some other useful TIDA metal complexes.

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