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Investigation of electrode kinetics and thermodynamics of [Zn–L-amino acidate–vitamin B_7] complexes by voltammetric technique

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The voltammetric reduction of Zn^{2+} using L-lysine, L-ornithine, L-threonine, L-serine, L-phenylglycine, L-phenylalanine, L-glutamic acid, L-aspartic acid and vitamin B_7 (biotin) at pH 7.30 ± 0.01 and I = 1.0 M $NaClO_4$ was reported at 25 and 35 °C. The nature of the current voltage curves was quasireversible and diffusion-controlled. Zn^{2+} formed 1 : 1 : 1, 1 : 1 : 2 and 1 : 2 : 1 complexes with these drugs as confirmed by the Schaap and McMaster method. The sequence of the stability constant of L-lysine < L-ornithine < L-threonine < L-serine < L-phenylglycine < L-phenylalanine < L-glutamic acid < L-aspartic acid complexes can be explained on the basis of the size, basicity and steric hindrance of ligands. The values of the stability constant (log β) varied from 2.13 to 11.37 and confirm that these drugs, i. e. L-amino acids or in combination with vitamin B_7 or their complexes, could be used against Zn^{2+} toxicity. The thermodynamic parameters such as enthalpy (ΔH), free energy (ΔG) and entropy change (ΔS), are also reported. The kinetic parameters viz. the transfer coefficient (α), degree of irreversibility (λ), diffusion coefficient (D) and standard rate constant (k) were calculated. The values of α confirmed the symmetric nature of the 'activated complex' between oxidants and reductants in response to the applied potential between the dropping mercury electrode and solution interface.

Key words: voltammetry, thermodynamic parameters, electrode kinetics, [Zn–L-amino acidate–vitamin B_7] complexes

INTRODUCTION

Most of L-amino acids are blood plasma ligands that form stable complexes with various essential metals *in vivo* [1] and play an important role in biology, pharmacy and industry [2–4]. Complexes of some metal ions with amino acids can be used as models to study the pharmaco-dynamic effects of drugs or for increasing the biocompatibility and minimizing the toxic effects of some metal ions [5]. On the other hand, L-amino acids are also involved in intracellular metabolism and operate specific transport systems of the plasma membrane; they do not affect cardiac function under normal conditions [6, 7]. The invention provides the use of zinc complexes of selected amino acids and other pharmacologically acceptable salts of zinc. The use of the compound comprises administering an effective amount of said compounds for inhibiting the growth of the malarial parasite, plasmodium falciparum [8]. Vitamin B_7 (biotin or vitamin H) [9] is a member of the vitamin B complex and water-soluble. Biotin is involved in energy metabolism and plays a role in enabling the body to use glucose [10]. Biotin is sometimes chemically linked to a molecule or protein for biochemical assays [11] and earned a keen attention towards the studies of their metal complexes. Therefore, Zn complexes of these drugs are of great importance. The concentrations of zinc *in vivo* can be reduced by drug therapy, but the specificity of the drug and its amount are stability-constant-dependent [12]. Therefore, the authors have undertaken the present study to determine the stability constants, thermodynamic and kinetic parameters of ternary complexes with these selected drugs for which no reference has been traced out so far in the literature.

EXPERIMENTAL

Instrumentation

The electrochemical experiment, i. e. a simple DC polarography, was carried out using a manual polarograph with

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a Toshniwal PL-50 polyflex galvanometer. The polarographic cell was of Laitinen and Lingane type in which a polarographic capillary of 5.0 cm in length and 0.04 mm in diameter was used. The $m^{2/3}\ t^{1/6}$ value was 2.40 $mg^{2/3}\ s^{-1/2}$ at 60.02 cm effective height of mercury. A Systronic pH meter 361 was used to measure the pH of the analyte at 7.30 ± 0.01.

Reagents

The following chemicals were used in the experiments: $HClO_4$ (Sigma), NaOH (Sigma), $NaClO_4$ (Fluka), Triton X-100 (Sigma), $ZnCl_2$ (B. D. H.), L-amino acids (Lobachem) and vitamin B_7 (Fluka), and their solutions were prepared in double-distilled water. The purity of L-amino acids was checked by the chromatographic method [13]. Pure nitrogen gas was passed through the analyte for deoxygenation before recording the current voltage data. The pH of the analyte at 7.30 ± 0.01 was adjusted by using dilute solutions of $HClO_4$ or NaOH as required. Potassium dihydrogen phosphate–sodium hydroxide buffer was added to stabilize the pH of the analyte.

Voltammetric procedure

Polarographic studies of the ternary complexes of Zn^{2+} with some amino acids and vitamin B_7 were recorded using a depolarizer and ligands (L-amino acids and vitamin B_7) in the ratio 1 : 40 : 40, and the concentration of amino acids varied from 0.5 mM to 30.0 mM at two fixed concentrations of vitamin B_7, i. e. 0.025 M and 0.050 M. $E_{1/2}$ shifted to a more negative side with increasing the concentration of L-amino acids. The current voltage curves were obtained at different pH values. The maximum negative shift of $E_{1/2}$ was obtained within the pH range 7.10–8.50, but pH 7.30 was selected for studying the complexes that are compatible with human blood pH [14]. The concentrations of metal, $NaClO_4$ and Triton X-100 (suppressor) in test solutions were 0.5 mM, 1.0 M and 0.001%, respectively.

RESULTS AND DISCUSSION

Polarographic studies

Zn^{2+} gave two electron quasireversible reduction waves at pH = 7.30 ± 0.01, I = 1.0 M $NaClO_4$ at 25 °C [15]. The nature of current-voltage curves for complexes was also quasireversible. The $E_{1/2}$ values became more negative with addition of vitamin B_7 (0.025 M and 0.050 M) to the [Zn–L-amino acids] system which showed ternary complex formation of 1 : 1 : 1, 1 : 1 : 2 and 1 : 2 : 1 complexes. The Gelling [16] method was used to determine the values of $E_{1/2}^{\text{reversible}}$ from $E_{1/2}^{\text{quasireversible}}$ by plotting ($E–RT / n F \log i_d–i / i$) vs. i for all the complexes. To know the values of β_{11} and β_{12}, the study was carried out at two constant concentrations of vitamin B_7, i. e. 0.025 M and 0.050 M. The values of the stability constant of the complexes, given in Table 1, were obtained by the Schaap and McMaster [17] method. The data and plots of F_{ij} [X, Y] against [X] (where F_{ij} is a Schaap and McMaster function to evaluate the stability constant β_{ij}, X = L-lysine, Y = vitamin B_7, and i and j are their stoichiometric numbers, respectively) for [Zn-L-lysinate-vitamin B_7] system are given in Table 2 and Fig. 1, respectively.

Comparison of the stability of binary and ternary complexes

To compare the stability of the binary and ternary complexes, the values of the mixing constant log K_m were calculated by the following equation [17]:

$$\log K_m = \log \beta_{11} - 1/2\ [\log \beta_{02} + \log \beta_{20}] \qquad (1)$$

The values of log K_m are –0.32, –0.20, –5.18, –0.29, –0.06, 0.08, –0.14, –0.095, respectively, for [Zn-L-lysinate-vitamin B_7], [Zn-L-ornithinate-vitamin B_7], [Zn-L threoninate-vitamin B_7], [Zn-L-serinate-vitamin B_7], [Zn-L-phenylglycinate-vitamin B_7], [Zn-L-phenylalaninate-vitamin B_7], [Zn-L-glutamate-vitamin B_7] and [Zn-L-aspartate-vitamin B_7] complexes. The positive values of log K_m indicate that the ternary complexes are more stable than the binary complexes, while the negative values indicate that the binary complexes are more stable than the ternary ones.

Trend of stability of ternary complexes

The sequence of the stability constants of the complexes with respect to ligands is L-lys < L-orn < L-thr < L-ser < L-phg < L-phe < L-glu < L-asp. As the size of amino acids increased, the stability of its complexes decreased [18]. The stability of L-amino acid complex also depends upon the chelate ring formation and the basicities of ligands [19]. In this study, the stability of the lysinate complex is minimum owing to the lowest pK value of L-lysine, as expected [20]. In case of L-serine and L-threonine, the stability of the latter is less than of the L-serine complex owing to the fact that the electron withdrawing OH^- group is closer to the L-threoninate complex than to the L-serinate complex, causing greater repulsive forces between the metal and the OH^- group in L-threonine complexes than in L-serine complexes. The higher stability of L-aspartate complexes than of L-glutamate ones is obvious from the chelate ring formation; in these amino acids,

Table 1. **Stability constants of ternary complexes, metal ion Zn (II) = 0.5 mM; I = 1.0 M $NaClO_4$; pH 7.30 ± 0.01; temperature = 25 °C**

Drugs	log β_{01}	log β_{02}	log β_{10}	log β_{20}	log β_{30}	log β_{11}	log β_{12}	log β_{21}
L-lysine	–	–	3.80	6.50	9.25	4.43	7.31	10.14
L-ornithine	–	–	3.83	6.58	9.42	4.59	7.53	10.36
L-threonine	–	–	4.25	7.36	9.55	–	7.68	10.47
L-serine	–	–	4.38	7.42	9.68	4.92	7.90	10.69
L-phenylglycine	–	–	4.42	7.58	9.78	5.23	8.10	10.78
L-phenylalanine	–	–	4.50	7.62	9.97	5.39	8.32	11.00
L-glutamic acid	–	–	5.30	8.72	10.00	5.72	9.10	11.15
L-aspartic acid	–	–	5.45	8.95	10.25	5.88	9.32	11.37
Vitamin B_7 (biotin)	2.13	3.00						

Table 2. Polarographic characteristics and F_{ij} [X, Y] values for the [Zn–L-lysinate–vitamin B_7] system, $[Zn^{2+}] = 0.50$ mM, I = 1.0 M $NaClO_4$, pH 7.30 ± 0.01, temperature = 25 °C

	(Vitamin B_7) = 0.025 M							(Vitamin B_7) = 0.050 M					
[lys] × 10^{-3} M	$E_{1/2}$ ′–V vs. SCE	log*Im* / *Ic*	F_{00} [*X*, *Y*]	F_{10} [*X*, *Y*] × 10^4	F_{20} [*X*, *Y*] × 10^7	F_{30} [*X*, *Y*] × 10^7	$E_{1/2}$ ′–V vs. SCE	log*Im* / *Ic*	F_{00} [*X*, *Y*]	F_{10} [*X*, *Y*] × 10^4	F_{20} [*X*, *Y*] × 10^7	F_{30} [*X*, *Y*] × 10^7
0.00	0.986	–	–	–	–	–	0.986	–	–	–	–	–
0.50	1.044	0.0070	102.15	19.43	34.91	177.82	1.053	0.0070	213.15	40.58	69.424	177.81
1.00	1.061	0.0142	374.77	36.97	35.00	177.81	1.070	0.0215	764.07	75.38	69.513	177.80
2.00	1.078	0.0215	1451.74	72.33	35.18	177.80	1.087	0.0289	2915.28	145.25	69.691	177.80
3.00	1.088	0.0289	3246.56	108.05	35.35	177.81	1.097	0.0365	6474.53	215.47	69.868	177.80
4.00	1.096	0.0365	5769.89	144.12	35.53	177.80	1.104	0.0442	11452.50	286.05	70.046	177.81
5.00	1.101	0.0442	9032.41	180.54	35.71	177.79	1.110	0.0520	17859.84	356.99	70.224	177.80
6.00	1.106	0.0520	13044.80	217.33	35.89	177.80	1.115	0.0599	25707.22	428.28	70.402	177.79
8.00	1.114	0.0599	23361.86	291.96	36.24	177.81	1.122	0.0681	45764.85	571.93	70.757	177.80
10.00	1.119	0.0681	36806.26	368.01	36.60	177.80	1.128	0.0763	71710.57	717.00	71.113	177.79
20.00	1.138	0.0763	153927.20	769.61	38.38	177.80	1.146	0.0848	292749.99	1463.69	72.891	177.80
30.00	1.149	0.0848	362033.11	1206.76	40.15	177.79	1.157	0.0848	673798.89	2245.96	74.669	177.81
	log A = 0.69, log B = 4.29, log C = 8.54, log D = 9.25						log A = 1.01, log B = 4.76, log C = 8.84, log D = 9.25					

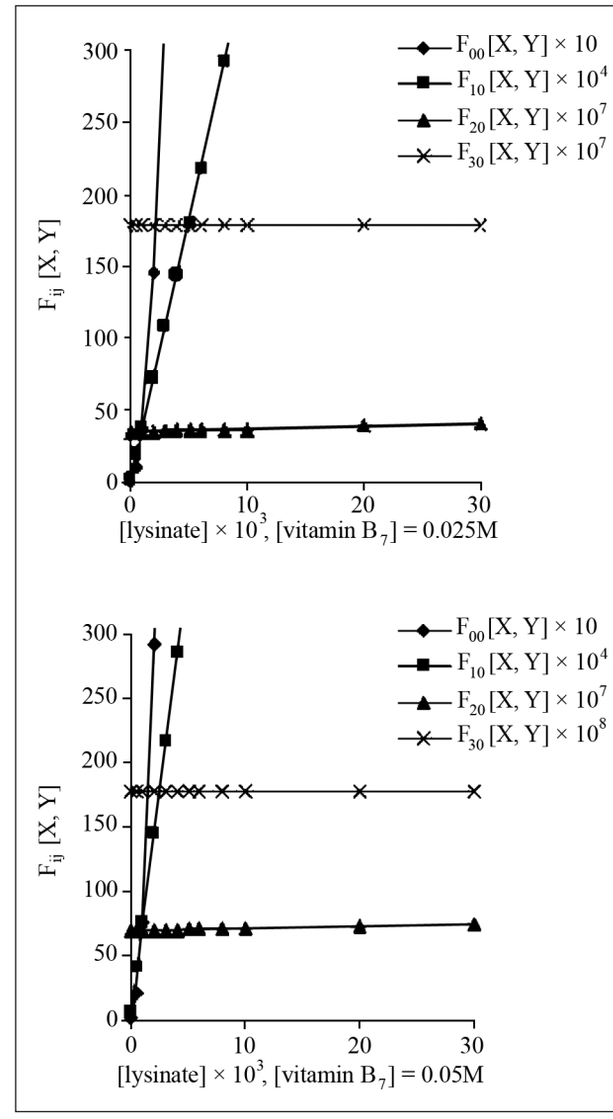

Fig. 1. Plot of F_{ij} [X, Y] vs. [X] for [Zn–L-lysinate–vitamin B_7] system

the aspartate forms one five- and one six-membered ring with the metal, while L-glutamate forms one six- and one seven-membered ring. As the size of the ring in amino acid increases, the stability of a complex decreases [21]. The stability of the L-glutamate and L-aspartate complexes is greater than that of the L-lysinate, L-ornithinate, L-threoninate, L-serinate, L-phenylglycinate, L-phenylalaninate complexes due to the great difference in their basic strength [22]. The same is evident from the pK values of L-amino-acids [23].

In case of vitamin B_7, the N- and O-atoms of the urieodyl group might take part in bond formation with the metal ion [24].

Thermodynamic parameters

The kind of complex species that reduces on a mercury electrode depends on thermodynamic aspects [25]. Thermodynamic parameters such as enthalpy change (ΔH), free energy change (ΔG) and entropy change (ΔS) of the complexes have been calculated by the following equations [26]:

Table 3. Thermodynamic parameters of ternary complexes of [Zn-L-aminoacidate-vitamin B_7] system

System	Stability constants			– ΔH k cal. / mole			– ΔG k cal. / mole			– ΔS cal. / degree / mole		
	$\log\beta_{11}$	$\log\beta_{12}$	$\log\beta_{21}$	$\log\beta_{11}$	$\log\beta_{12}$	$\log\beta_{21}$	$\log\beta_{11}$	$\log\beta_{12}$	$\log\beta_{21}$	$\log\beta_{11}$	$\log\beta_{12}$	$\log\beta_{21}$
	25 °C / 35 °C	25 °C / 35 °C	25 C / 35 C	(35 °C–25 °C) for difference of 10 °C			25 °C / 35 °C	25 °C / 35 °C	25 C / 35 °C	25 °C / 35 °C	25 °C / 35 °C	25 °C / 35 °C
[Zn–L-lys-vit. B_7]	4.43	7.31	10.14	12.6003	13.86033	14.28034	6.0410	9.9683	13.8275	22.0110	13.0603	1.5195
	4.13	6.98	9.80				5.8208	9.8376	13.8121	22.0112	13.0607	1.5201
[Zn–L-orn-vit. B_7]	4.59	7.53	10.36	14.7003	13.1127	14.3727	6.2591	10.2713	14.1305	28.3260	9.5347	0.8128
	4.24	7.22	10.02				5.9758	10.1758	14.1221	28.3262	9.5352	0.8134
[Zn–L-thr-vit. B_7]	–	7.68	10.47	–	12.6003	14.7003	–	10.4729	14.2775	–	7.1388	1.4188
	–	7.38	10.12				–	10.4013	14.2631	–	7.1393	1.4195
[Zn–L-ser-vit. B_7]	4.92	7.90	10.69	14.2803	12.6927	12.6927	6.7092	10.7759	14.5805	25.4064	6.4321	6.3349
	4.58	7.60	10.39				6.4550	10.7114	14.6436	25.4067	6.4326	6.3342
[Zn–L-phg-vit. B_7]	5.23	8.1	10.78	13.8603	12.6003	15.1203	7.1319	11.0456	14.7002	22.5784	5.2169	1.4097
	4.9	7.8	10.42				6.9060	10.9933	14.6859	22.5787	5.2174	1.4104
[Zn–L-phe-vit. B_7]	5.39	8.32	11.00	14.1375	12.6087	16.8928	7.3591	11.3486	15.0032	22.7463	4.2283	6.3407
	5.06	8.02	10.60				7.1315	11.3062	14.9396	22.7466	4.2288	6.3414
[Zn–L-glu-vit. B_7]	5.72	9.10	11.15	12.6003	12.6003	16.8424	7.8001	12.4093	15.2061	16.1079	0.6409	5.4906
	5.42	8.8	10.75				7.6389	12.4027	15.1510	16.1082	0.6414	5.4913
[Zn–L-asp-vit. B_7]	5.88	9.32	11.37	12.6003	14.3727	16.5147	8.0183	12.7123	15.5091	15.3757	5.5719	3.3745
	5.58	8.98	10.98				7.8644	12.6564	15.4752	15.3761	5.5725	3.3752

$$\Delta H = 2.303\ R\ T_1\ T_2 (\log \beta_2 - \log \beta_1) / T_2 - T_1 \quad (2)$$

$$\Delta G = -2.303\ RT \log \beta \quad (3)$$

$$\Delta G = \Delta H - T\Delta S \quad (4)$$

It is clear from the values of ΔS, ΔG and ΔH in Table 3 that the stability constants ($\log \beta_1$) and ($\log \beta_2$) decreased with increasing the temperature, confirming that the complexes are not stable at a higher temperature [27]. The values of ΔS are more negative and of ΔG less negative at a higher temperature, confirming that the complexes are not stable at a higher temperature [28]. The negative values of ΔH show that these reactions are exothermic in nature [29].

Kinetic parameters

The kinetic parameters, viz. the transfer coefficient (α), degree of irreversibility (λ), and standard rate constant (k), determined by the Tamamushi and Tanaka method [30, 31] by plotting ($E - RT / nF \log i_d - i / i$) against i and $\log (Z-1)$ against ($E^r_{1/2} - E$) for the [Zn–L-lysinate–vitamin B_7] system are given in Figs. 2 and 3 (a, b), respectively.

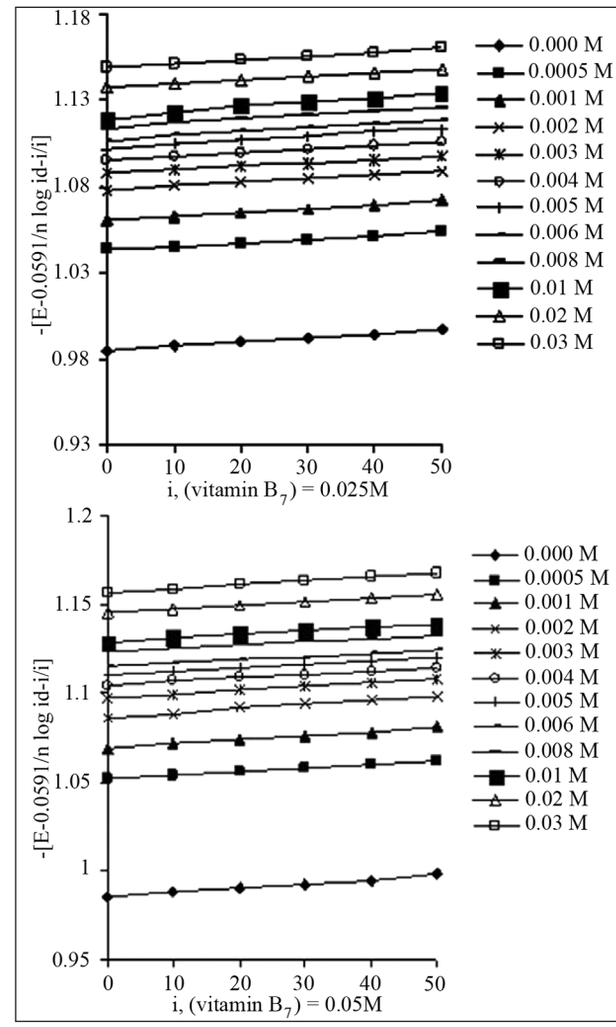

Fig. 2. Plots between -[E-RT / nF log (id–i) / i] vs. i for [Zn–L-lysinate–vitamin B_7] system

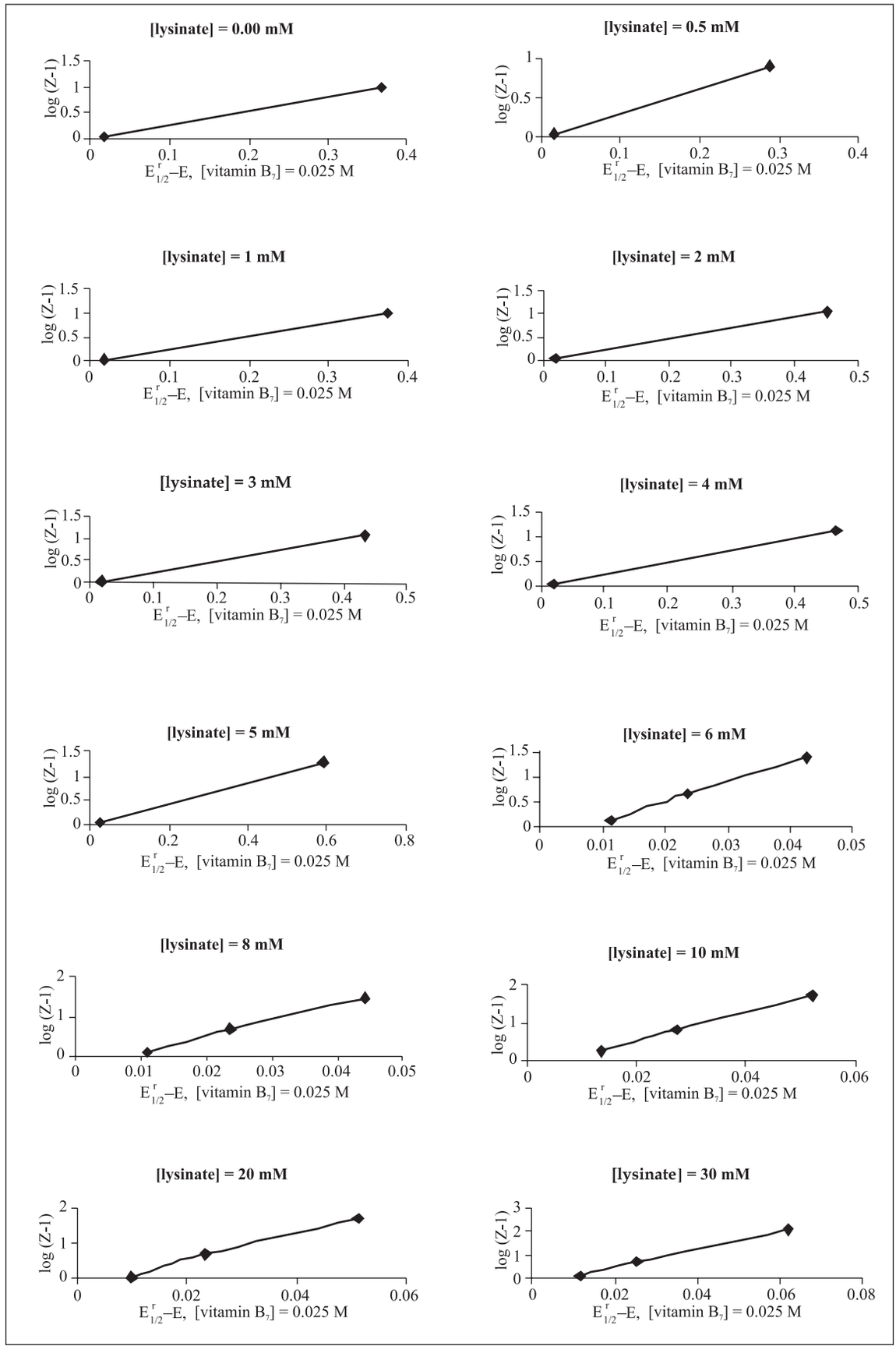

Fig. 3(a). [Zn–L-lysinate–vitamin B_7] system, (vitamin B_7) = 0.025 M.
Plot of ($E^r_{1/2}$–E) vs. log (Z–1), Y-axis = log (Z–1), X-axis = ($E^r_{1/2}$–E)

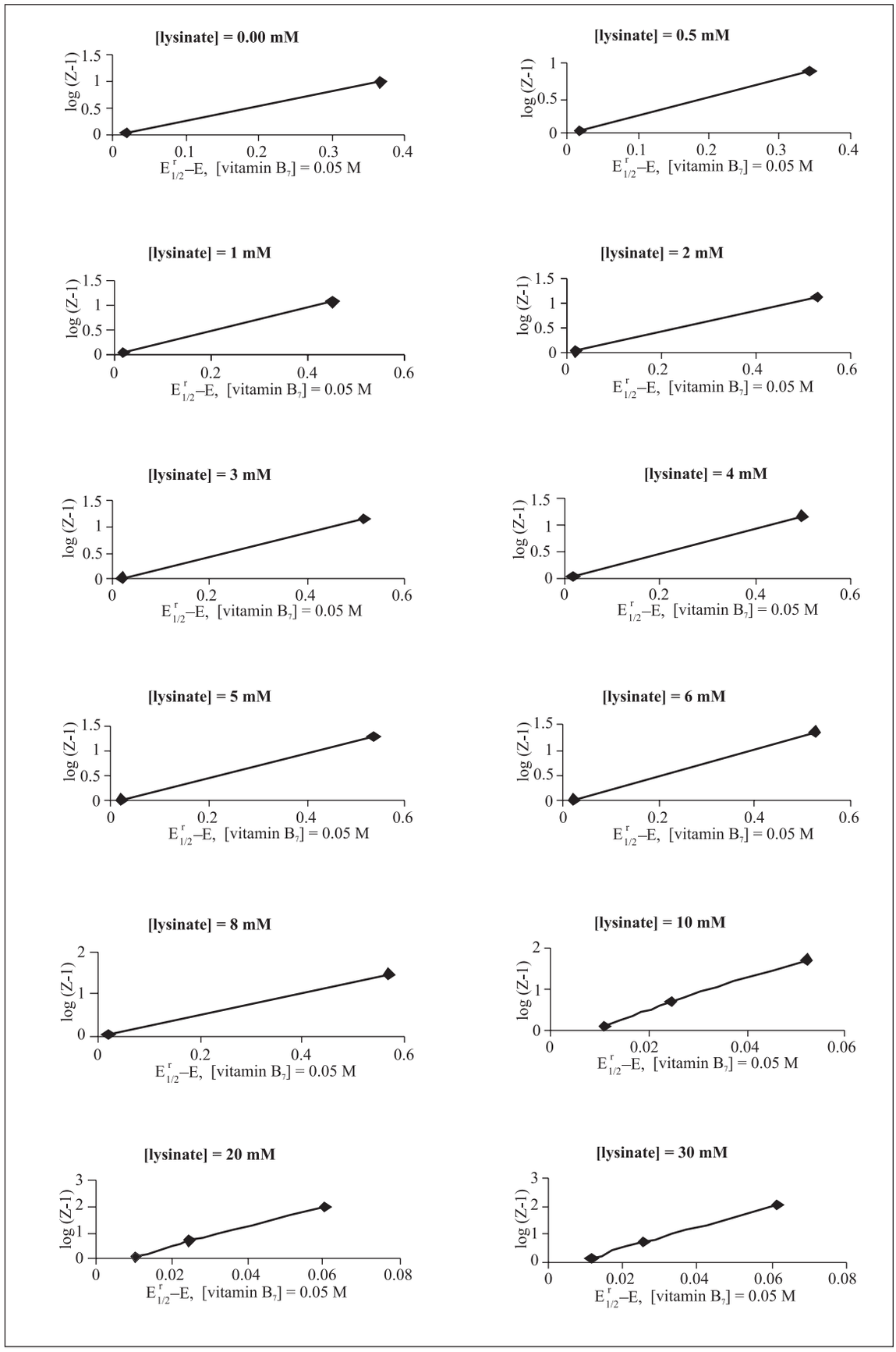

Fig. 3(b). [Zn–L-lysinate–vitamin B_7] system, (vitamin B_7) = 0.05 M.
Plot of ($E^r_{1/2}$–E) vs. log (Z–1), Y-axis = log (Z–1), X-axis = ($E^r_{1/2}$–E)

Table 4. Kinetic parameters of [Zn–L-lysinate–vitamin B_7] system, $[Zn^{2+}]$ = 0.50 mM, I = 1.0 M $NaClO_4$, pH 7.30 ± 0.01, temperature = 25 °C

$[L\text{-}lys] \times 10^{-3}$ M	(Vitamin B_7) = 0.025 M						(Vitamin B_7) = 0.050 M					
	$(E_{1/2})^{red}$–V vs. SCE	Slope (mV)	α	λ	$D^{1/2} \times 10^3$ $cm^2 s^{-1}$	$k \times 10^3$ $cm\ s^{-1}$	$(E_{1/2})^{red}$–V vs. SCE	Slope (mV)	α	λ	$D^{1/2} \times 10^3$ $cm^2 s^{-1}$	$k \times 10^3$ $cm\ s^{-1}$
0.00	1.000	33.00	0.495	1.352	4.085	5.524	1.000	33.00	0.464	0.957	4.085	3.911
0.50	1.045	33.50	0.486	1.352	4.019	5.435	1.057	33.50	0.469	1.352	4.019	5.435
1.00	1.062	34.00	0.518	1.074	3.953	4.246	1.074	34.00	0.535	0.853	3.887	3.317
2.00	1.079	33.50	0.500	1.074	3.887	4.176	1.090	33.50	0.486	1.205	3.822	4.606
3.00	1.090	34.50	0.464	1.352	3.822	5.168	1.100	34.50	0.477	1.352	3.756	5.079
4.00	1.099	33.50	0.500	1.074	3.756	4.034	1.109	33.50	0.500	1.352	3.690	4.990
5.00	1.104	34.50	0.582	1.702	3.690	6.282	1.113	34.50	0.470	1.352	3.624	4.900
6.00	1.109	33.50	0.500	1.205	3.624	4.367	1.118	33.50	0.535	1.074	3.558	3.822
8.00	1.116	34.00	0.546	1.074	3.558	3.822	1.126	34.00	0.528	1.074	3.492	3.751
10.00	1.121	33.50	0.535	1.074	3.492	3.751	1.130	33.50	0.560	0.853	3.426	2.923
20.00	1.141	34.50	0.500	0.957	3.426	3.280	1.150	34.50	0.508	0.957	3.360	3.217
30.00	1.151	35.00	0.518	1.205	3.360	4.050	1.160	35.00	0.518	1.074	3.360	3.609

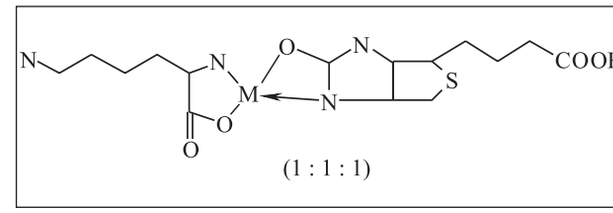

Fig. 4. The probable structure of [Zn–L-lysinate–vitamin B_7] system

Parameter Z is calculated by the following equation [30, 31]:

$$Z = \text{anti log}\ \{n\ F / 2.303\ RT\ (E^{r}_{1/2} - E)\} + \log i_d - i / i \qquad (5)$$

The values of the kinetic parmeters are given in Table 4. It is obvious that the α values varied from [Zn–L-lysinate–vitamin B_7] 0.464 to 0.582 (about 0.50), and the values of α for the other systems were also about 0.50, confirming that the 'transition state' lies midway between the dropping mercury electrode and the solution interface. The values of the rate constant (k), varying from 3.28 to 6.28 cm. sec^{-1}, confirm that the electrode processes are quasireversible. The values of the diffusion coefficient (D), as determined by Ilkovic equation [32], are as expected.

CONCLUSIONS

In the present paper, ternary complexes of Zn with L-amino acids and vitamin B_7 at pH 7.30 ± 0.01 were investigated using simple DC polarography. The results have indicated that current voltage curves are quasireversible and diffusion-controlled in 1.0 M $NaClO_4$ at pH = 7.30 ± 0.01 and at 25 and 35 °C. It is clear from the stability constant values of the complexes that vitamin B_7 and amino acids, used either singly or simultaneously, might be effective to reduce the toxicity of metal *in vivo*. The negative values of ΔH indicated the exothermic nature of the metal–ligand interaction. The complexes were not stable at a higher temperature, which was confirmed by the values of ΔG and ΔS. The values of the transfer coefficient (α) varied from 0.464 to 0.582 (0.50), showing that the 'transition state' behaves between the oxidant and the reductant response to the applied potential and lies in the midway between the dropping mercury electrode and solution interface. The values of the rate constant (k) varied from 3.28 to 6.28 cm. s^{-1}, confirming the quasireversible nature of electrode processes.

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ZN–L-AMINORŪGŠČIŲ–VITAMINO B_7 KOMPLEKSŲ ELEKTROCHEMINĖS KINETIKOS IR TERMODINAMIKOS TYRIMAS VOLTAMPEROMETRIJOS METODU

Santrauka

Ištirta Zn^{2+} kompleksų su L-lizinu, L-ornitinu, L-treoninu, L-serinu, L-fenilglicinu, L-fenilalaninu, L-glutamino rūgštimi, L-asparto rūgštimi ir vitaminu B_7 elektrocheminė redukcija. Nustatytos kompleksų stabilumo konstantos ir termodinaminiai bei kinetiniai parametrai.