CHEMIJA. 2010. Vol. 21. No. 2–4. P. 80–88

Study of electrode kinetics and thermodynamics of zinc complexes with some L-amino acids and vitamin C by voltammetric technique

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A simple and highly sensitive direct polarographic method was developed for determining the stability of ternary complexes of Zn^{2+} employing L-lysine, L-ornithine, L-threonine, L-serine, L-phenylglycine, L-phenylalanine, L-glutamic acid, L-aspartic acid and vitamin C (L-ascorbic acid) at pH = 7.30 ± 0.01, and I = 1.0 M $NaClO_4$ at 25 and 35 °C. The nature of 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 size, basicity and steric hindrance of ligands. The values of the stability constant (log β) varied from 2.45 to11.45 and confirmed that these drugs, i. e. L-amino acids or in combination with vitamin C 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), the degree of irreversibility (λ), the diffusion coefficient (D) and the 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 the solution interface.

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

INTRODUCTION

Vitamin C has long been recognized as an important nutrient in several food products. The reduced form of the vitamin is referred to as L-ascorbic acid, and the oxidized form is referred to as dehydroascorbic acid. On the other hand, vitamin C (L-ascorbic acid) is an important drug used against cancer, scurvy and to reduce the risk of bronchitis or wheezing [1–3]. Vitamin C is also responsible for the functions of various body components and organs and also keeps in order the immune system [4, 5]. The deficiency of vitamin C causes anemia, dental cavities and thyroid insufficiency. Therefore, its metal complexes are biologically important. On the other hand, amino acids act as chelating agents, form stable complexes with various essential metals *in vivo* [6] and play an important role in biology, pharmacy and industry [7–9]. 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 to minimize the toxic effects of some metal ions [10]. 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 [11, 12]. 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 the above compounds for inhibiting the growth of the malarial parasite, plasmodium falciparum [13]. Therefore, the Zn complexes of these drugs are of great im-

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portance. The concentrations of zinc *in vivo* can be reduced by drug therapy, but the drug specificity and its amount are stability constant dependent [14]. Therefore, the authors have undertaken the present study to determine the stability constants, the thermodynamic and kinetic parameters of ternary complexes with these drugs polarographically and for which no reference had been traced in the literature.

EXPERIMENTAL

Instrumentation

An electrochemical experiment, i. e. simple DC polarography, was carried out using a manual polarograph with a Toshniwal PL-50 polyflex galvanometer. The polarographic cell was of Laitinen and Lingane type in which a polarographic capillary 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 the 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 C (Fluka), and their solutions were prepared in double-distilled water. The purity of L-amino acids was checked by the chromatographic method [15]. 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 C were recorded using a depolarizer and ligands (L-amino acids and vitamin C) in the ratio of 1 : 40 : 40, and the concentration of amino acids varied from 0.5 mM to 30.0 mM at two fixed concentrations of vitamin C, i. e. 0.025 M and 0.050 M. $E_{1/2}$ was observed to shift to the more negative side with increasing the concentration of L-amino acids. 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 compatible with human blood pH [16]. 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 [17]. The nature of the current–voltage curves for the complexes was also quasireversible. The $E_{1/2}$ values became more negative with the addition of vitamin C (0.025 M and 0.050 M) to the [Zn–L-amino acids] system which showed formation of 1 : 1 : 1, 1 : 1 : 2 and 1 : 2 : 1 ternary complexes. Gelling's [18] method was used to determine the values of $E_{1/2}^{\text{reversible}}$ from $E_{1/2}^{\text{quasireversible}}$ by plotting $(E - RT / nF \log i_d - i/i)$ vs. i for all the complexes. To know the values of β_{11} and β_{12}, a study was carried out at two constant concentrations of vitamin C (0.025 M and 0.050 M). The values of the stability constant of the complexes (Table 1) were obtained by using the Schaap and McMaster [19] 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 C, and i and j are their stoichiometric numbers, respectively) for [Zn–L-lysinate–vitamin C] system are given in Table 2 and Fig. 1, respectively.

Comparison of the stability of binary and ternary complexes

To compare the stability of binary and ternary complexes, the values of the mixing constant log K_m were calculated by the following equation [19]:

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

The values of log K_m are 2.97, 3.09, 2.97, 1.08, 3.29, 3.40, 0.52, 3.23, respectively, for [Zn–L-lysinate–vitamin C], [Zn–L-rnithinate–vitamin C], [Zn–L threoninate–vitamin C], [Zn–L-serinate–vitamin C], [Zn–L-phenylglycinate–vitamin C], [Zn–L-phenylalaninate–vitamin C], [Zn–L-glutamate–vitamin C] and [Zn–L-aspartate–vitamin C] complexes.

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	4.53	7.45	–
L-ornithine	–	–	3.93	6.58	9.42	4.69	7.67	10.45
L-threonine	–	–	4.25	7.36	9.55	4.96	7.89	10.67
L-serine	–	–	4.38	7.42	9.68	5.12	–	10.75
L-phenylglycine	–	–	4.42	7.58	9.78	5.36	8.21	10.83
L-phenylalanine	–	–	4.50	7.62	9.97	5.52	8.43	11.05
L-glutamic acid	–	–	5.30	8.72	10.00	5.86	9.21	11.23
L-aspartic acid	–	–	5.45	8.95	10.25	6.02	9.43	11.45
Vitamin C (ascorbic acid)	2.45	3.38						

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

Vitamin C = 0.025 M							Vitamin C = 0.050 M					
[lys] × 10^{-3} M	$E'_{1/2}$ – V vs. SCE	$\log I_m/I_c$	F_{∞} [X, Y]	F_{10} [X, Y] × 10^3	F_{20} [X, Y] × 10^5	F_{30} [X, Y] × 10^7	$E'_{1/2}$ – V vs. SCE	$\log I_m/I_c$	F_{∞} [X, Y]	F_{10} [X, Y] × 10^3	F_{20} [X, Y] × 10^5	F_{30} [X, Y] × 10^7
0.00	0.986	–	–	–	–	–	0.986	–	–	–	–	–
0.50	1.028	0.0142	29.98	26.79	40.51	177.82	1.037	0.0142	61.33	80.48	40.51	177.82
1.00	1.033	0.0215	46.30	29.71	49.40	177.81	1.044	0.0289	104.49	83.40	49.40	177.81
2.00	1.043	0.0289	93.00	38.20	67.18	177.82	1.053	0.0365	204.88	91.89	67.18	177.8
3.00	1.050	0.0365	167.37	50.26	84.96	177.81	1.059	0.0442	332.95	103.95	84.97	177.83
4.00	1.057	0.0520	280.07	65.87	102.75	177.82	1.064	0.0442	499.34	119.56	102.75	177.82
5.00	1.063	0.0599	441.76	85.03	120.52	177.81	1.069	0.0520	714.72	138.72	120.52	177.81
6.00	1.068	0.0520	663.15	107.76	138.31	177.82	1.073	0.0599	989.75	161.44	138.30	177.8
8.00	1.077	0.0599	1 327.53	163.86	173.87	177.81	1.080	0.0681	1761.56	217.55	173.87	177.81
10.00	1.084	0.0763	2 358.73	234.21	209.44	177.82	1.087	0.0763	2 900.15	287.90	209.44	177.82
20.00	1.109	0.0848	16 001.73	799.25	387.24	177.81	1.110	0.0934	17 080.06	852.94	387.24	177.81
30.00	1.124	0.0934	51 617.18	1 720.01	565.08	177.82	1.124	0.0934	53 232.43	1 773.71	565.08	177.82
log A = 1.21, log B = 4.39, log C = 6.50, log D = 9.25							log A = 1.32, log B = 4.89, log C = 6.50, log D = 9.25					

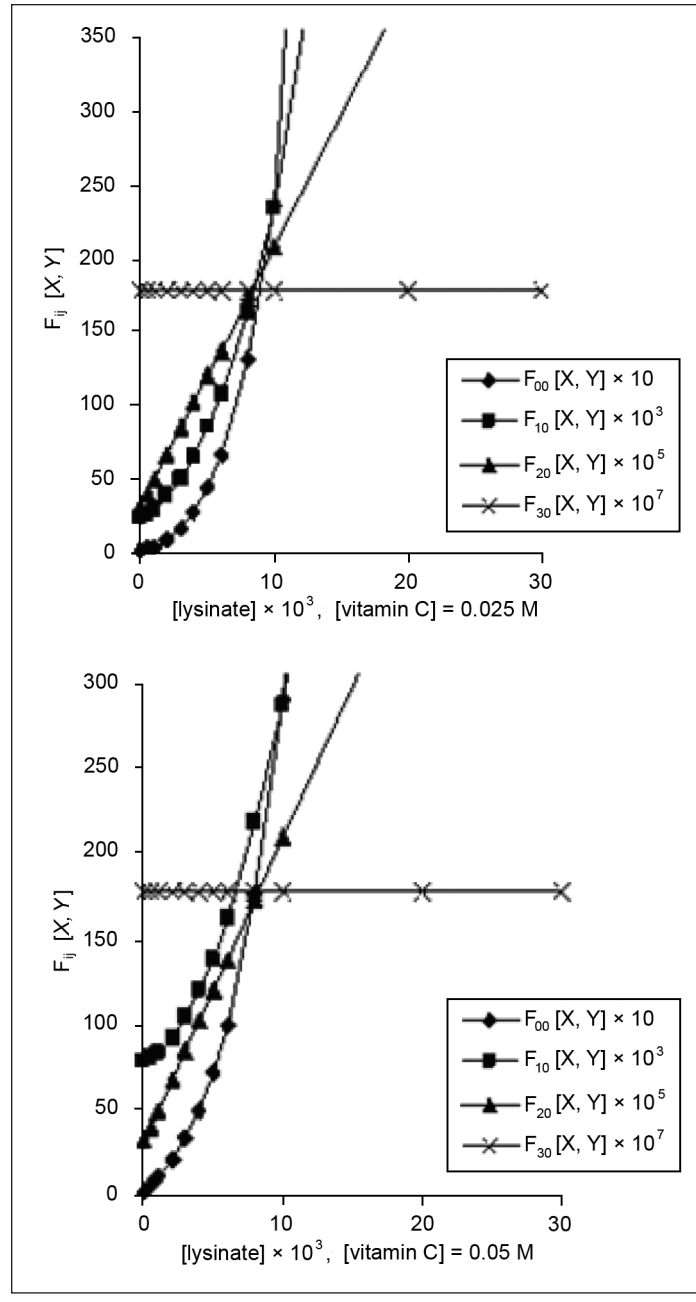

Fig. 1. Plot of F_{ij} [X, Y] vs. [X] for [Zn–L-lysinate–vitamin C] system

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.

The trend of stability of ternary complexes

The sequence of stability constants of complexes with respect to ligands is L-lys < L-orn < L-thr < L-ser < L-phg < L-phe < L-glu < L-asp. As the value of an amino acid increased, the stability of its complexes decreased [20]. The stability of L-amino acid complex also depended upon the chelate ring formation and the basicities of ligands [21]. In this study, the stability of lysinate complex was minimum owing to the lowest pK value of L-lysine, as expected [22]. In case of L-serine and L-threonine, the stability of the latter is less than

Table 3. **Thermodynamic parameters of ternary complexes of the Zn-L-aminoacidate–vitamin C 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. C]	4.53	7.45	–	12.6003	12.6003	–	6.1773	10.1592	–	21.5534	8.1913	–
	4.23	7.15	–				5.9617	10.0772	–	21.5536	8.1918	–
[Zn–L-orn–vit. C]	4.69	7.67	10.45	12.6003	12.6003	14.7003	6.3955	10.4622	14.2502	20.8212	7.1745	1.5104
	4.39	7.37	10.10				6.1872	10.3903	14.2349	20.8215	7.1750	1.5110
[Zn–L-thr–vit. C]	4.96	7.89	10.67	12.6003	12.6003	15.1287	6.7637	10.7652	14.5532	19.5857	6.1577	1.9312
	4.66	7.59	10.31				6.5678	10.7035	14.5337	19.5860	6.1582	1.9319
[Zn–L-ser–vit. C]	5.12	–	10.75	13.4403	–	14.8851	6.9819	–	14.6653	21.6724	–	0.7376
	4.8	–	10.4				6.7651	–	14.6577	21.6727	–	0.7382
[Zn–L-phg–vit. C]	5.36	8.21	10.83	12.6003	13.0203	15.1203	7.3092	11.1956	14.7684	17.7553	6.1230	1.1809
	5.06	7.9	10.47				7.1315	11.1342	14.7564	17.7556	6.1235	1.1816
[Zn–L-phe–vit. C]	5.52	8.43	11.05	12.6003	13.9527	15.6327	7.5274	11.4986	15.0714	17.0231	8.2351	1.8836
	5.22	8.1	10.68				7.3570	11.4161	15.0523	17.0234	8.2356	1.8843
[Zn–L-glu–vit. C]	5.86	9.21	11.23	13.0203	13.0203	18.1024	7.9910	12.5593	15.3152	16.8767	1.5469	9.3529
	5.55	8.90	10.80				7.8221	12.5436	15.2215	16.8770	1.5475	9.3536
[Zn–L-asp–vit. C]	6.02	9.43	11.45	13.4403	14.7927	15.6747	8.2092	12.8623	15.6182	17.5539	6.4779	0.1896
	5.7	9.08	11.08				8.0335	12.7973	15.6161	17.5543	6.4785	0.1903

of the L-serine complex owing to the fact that the electron withdrawing OH^- group is nearer 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, 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 ring size in amino acid increases, the stability of the complex decreases [23]. The stability of L-glutamate and L-aspartate complexes is higher than that of the L-lysinate, L-ornithinate, L-threoninate, L-serinate, L-phenylglycinate, L-phenylalaninate complexes due to a great difference in their basic strength [24]. The same is evident from pK values of L-amino-acids [25].

In case of vitamin C, oxygen of the enediol group may take part in bond formation with Zn^{2+}, forming a five-membered ring [26].

It is clear from the values of the stability constant of the complexes that vitamin C and L-amino acids alone or in combination could be used to reduce toxicity Zn^{2+} *in vivo*. One should also consider the quantity of drugs that should not be complexed to the other essential metals present *in vivo* and could be excreted easily from the body. On the other hand, for this reason a person who suffers from AIDS has a low concentration of vitamin C; his resistance can be increased by ascorbic acid therapy.

Thermodynamic parameters

The kind of a complex species that reduces on a mercury electrode depends on thermodynamic aspects [27]. Thermodynamic parameters such as enthalpy change (ΔH), free energy change (ΔG) and entropy change (ΔS) of the complexe were calculated by the following equation [28]:

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

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

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

It is clear from the values of ΔS, ΔG and ΔH in Table 3 that the stability constants log β_1 and log β_2 decreased with increasing the temperature, confirming that the complexes are not stable at a higher temperature [29]. The values of ΔS are more negative and ΔG are less negative at a higher temperature, confirming that complexes are not stable at a higher temperature [30]. The negative values of ΔH show that these reactions are exothermic in nature [31].

Kinetic parameters

The kinetic parameters, viz. the transfer coefficient (α), the degree of irreversibility (λ) and standard rate constant (k), determined by the Tamamushi and Tanaka method by

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

[L-lys] × 10^{-3} M	Vitamin C = 0.025 M						Vitamin C = 0.05 M					
	$(E_{1/2})^{qr}$ – V vs. SCE	Slope, mV	α	λ	$D^{1/2} \times 10^3$ $cm^2 s^{-1}$	k × 10^3 cm s^{-1}	$(E_{1/2})^{qr}$ – V vs. SCE	Slope, mV	α	λ	$D^{1/2} \times 10^3$ $cm^2 s^{-1}$	k × 10^3 cm s^{-1}
0.00	1.000	33.00	0.474	1.352	4.085	5.524	1.000	33.00	0.474	1.352	4.085	5.524
0.50	1.049	33.50	0.428	1.517	3.953	5.998	1.062	33.50	0.518	0.957	3.953	3.785
1.00	1.065	34.00	0.486	1.352	3.887	5.257	1.079	34.00	0.508	1.074	3.822	4.105
2.00	1.086	33.50	0.477	1.205	3.822	4.606	1.096	33.50	0.470	1.074	3.756	4.034
3.00	1.095	34.50	0.443	1.205	3.756	4.526	1.108	34.50	0.528	2.404	3.690	8.873
4.00	1.102	33.50	0.500	1.205	3.624	4.367	1.115	33.50	0.443	1.517	3.690	5.598
5.00	1.109	34.50	0.495	0.957	3.558	3.406	1.120	34.50	0.458	1.517	3.624	5.498
6.00	1.112	33.50	0.458	1.352	3.624	4.900	1.124	33.50	0.518	0.853	3.558	3.036
8.00	1.119	34.00	0.495	0.957	3.558	3.406	1.130	34.00	0.526	0.760	3.492	2.655
10.00	1.125	33.50	0.571	0.957	3.426	3.280	1.133	33.50	0.535	0.957	3.426	3.280
20.00	1.146	34.50	0.508	1.074	3.360	3.609	1.154	34.50	0.403	1.517	3.294	4.999
30.00	1.157	35.00	0.403	1.702	3.294	5.609	1.163	35.00	0.403	1.517	3.294	4.999

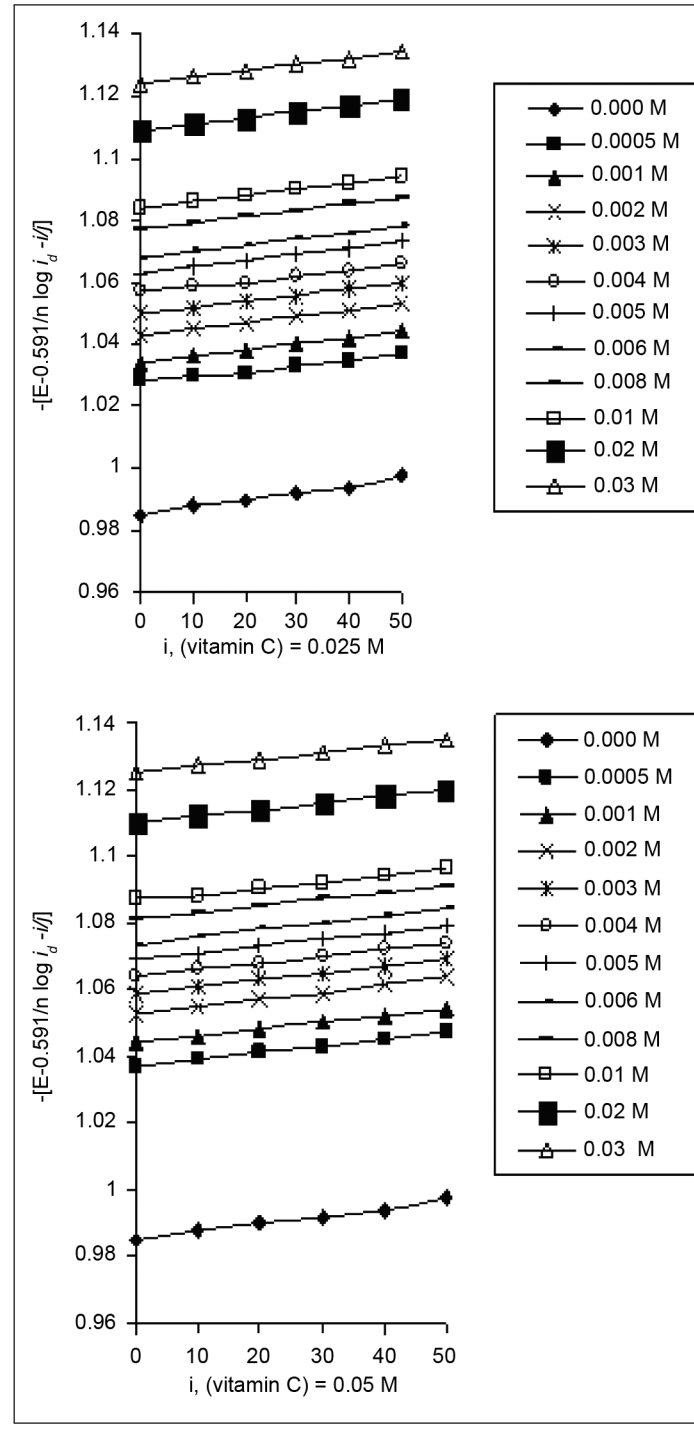

Fig. 2. Plots between [*E-RT / nF* log (i_d – *i*) / *i*] vs. *i* for [Zn–L-lysinate–vitamin C] system

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 C system are given in Figs. 2 and 3 (a, b), respectively. Parameter Z is calculated by the following equation [32, 33]:

$$Z = \text{anti log}\{n\,F / 2.303\,RT\,(E^r_{1/2} - E)\} + \log i_d - i / i. \quad (5)$$

The values of the kinetic parmeters are given in Table 4. It is obvious that the α values varied from [Zn–L-lysinate–vitamin C] 0.403 to 0.571 (about 0.50), and the values of α for other systems were also

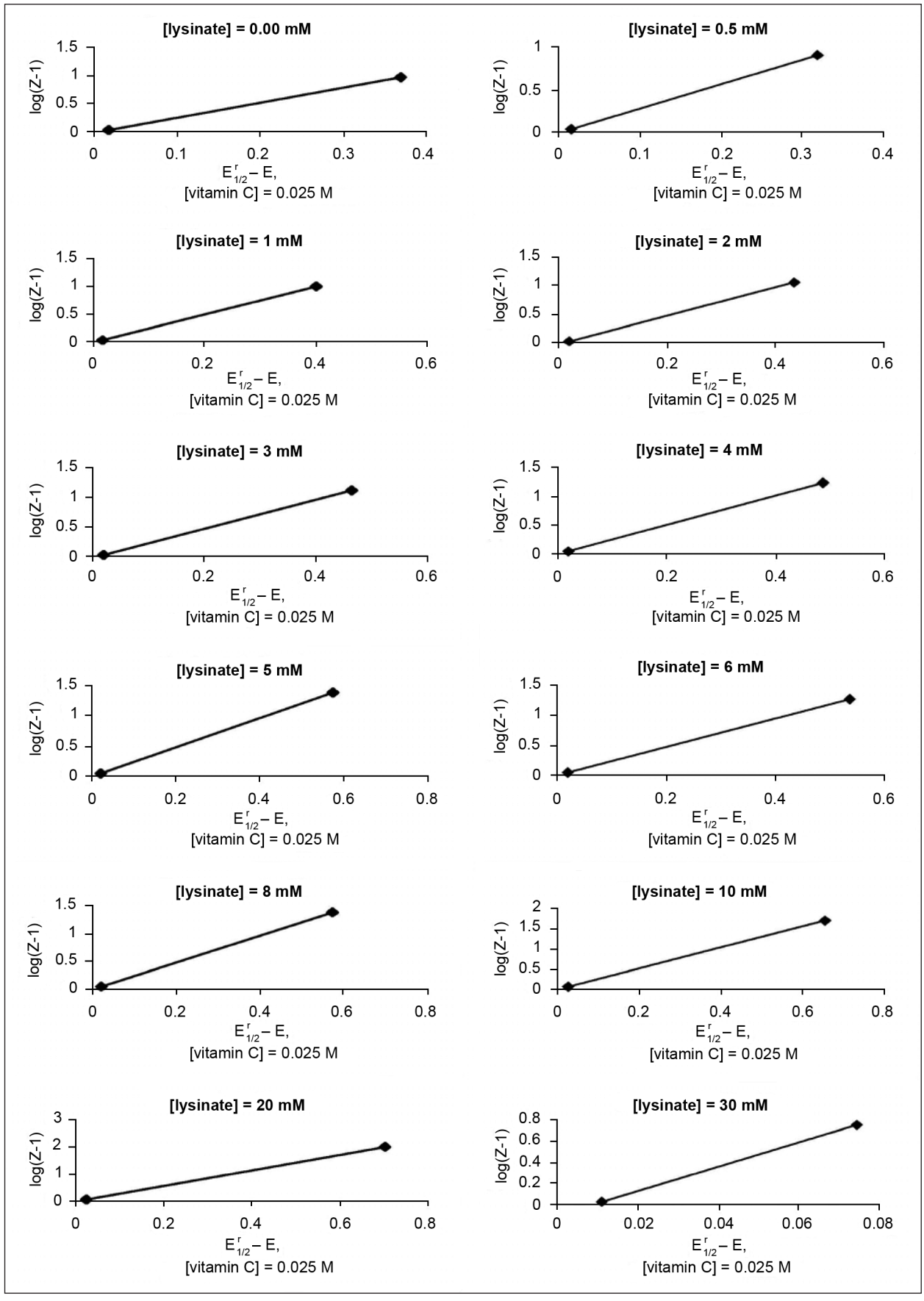

Fig. 3 (a). [Zn–L-lysinate–vitamin C] system, (vitamin C) = 0.025 M.
Plot of ($E^r_{1/2} - E$) vs. log (Z–1), Y-axis = log (Z–1), X-axis = ($E^r_{1/2} - E$)

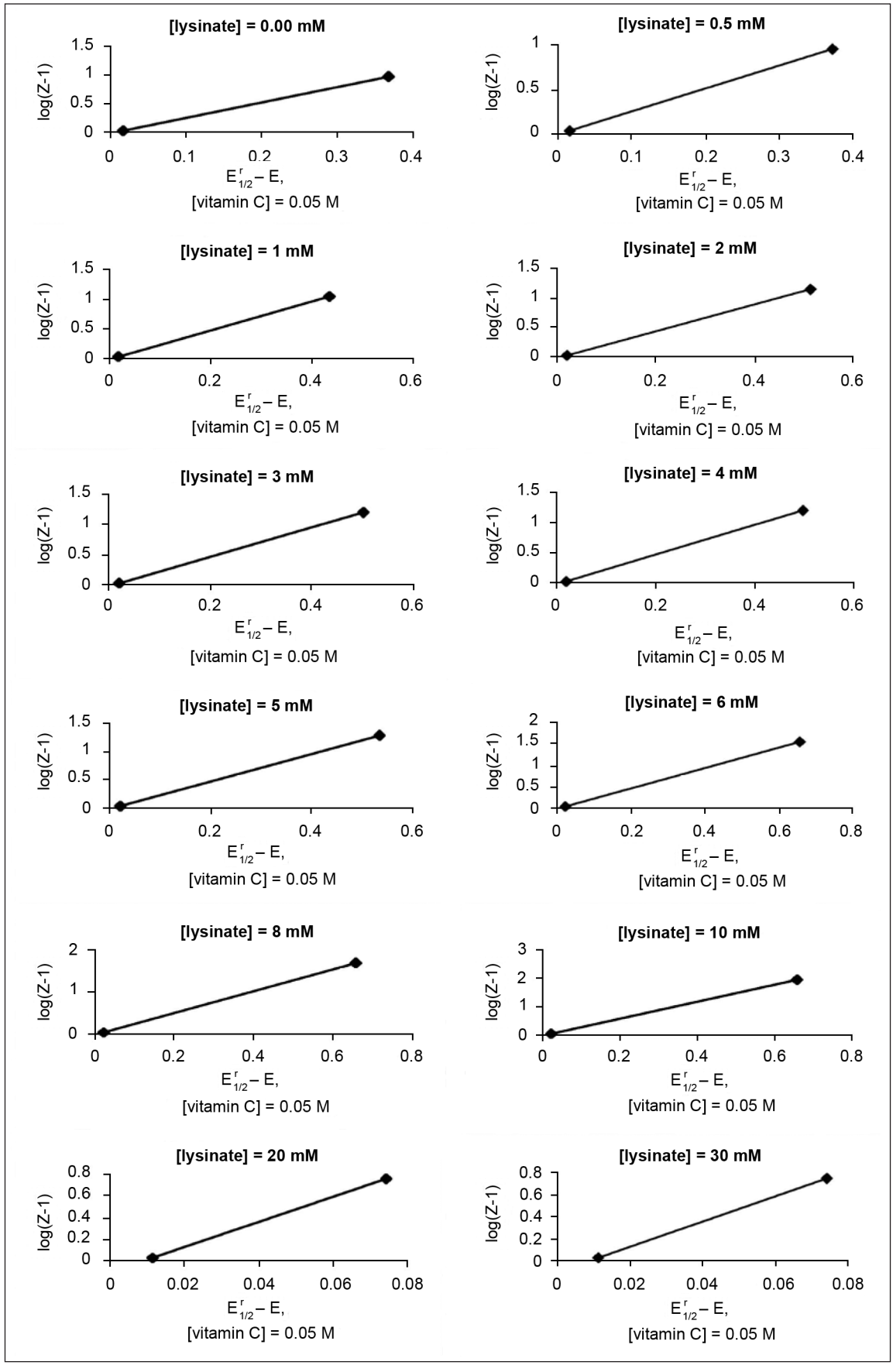

Fig. 3 (b). [Zn–L-lysinate–vitamin C] system, (vitamin C) = 0.05 M.
Plot of $(E^r_{1/2} - E)$ vs. log $(Z-1)$, Y-axis = log $(Z-1)$, X-axis = $(E^r_{1/2} - E)$

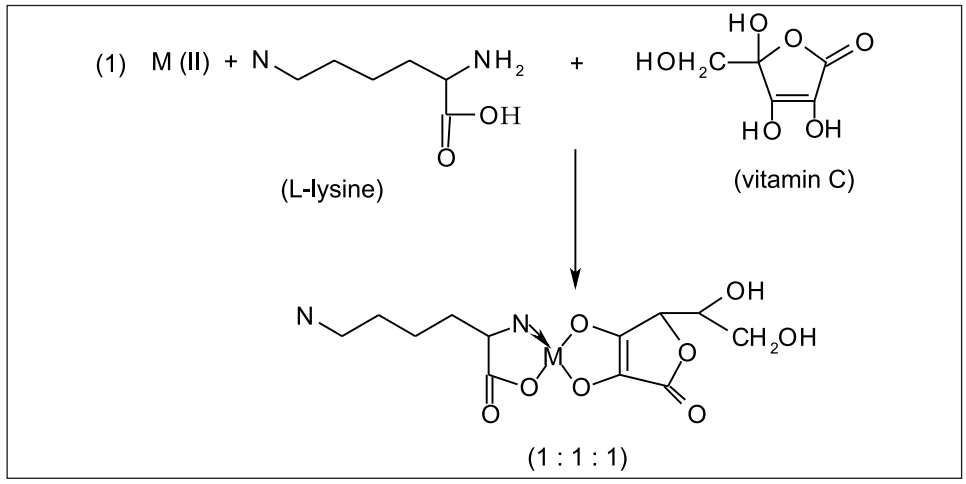

Fig. 4. Probable structure of ternary complex of [Zn–L-lysinate–vitamin C] system

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 5.99 cm sec^{-1}, confirm that the electrode processes are quasireversible. The values of the diffusion coefficient (D), as determined by the Ilkovic equation [34], are as expected.

CONCLUSIONS

In the present paper, the interaction of Zn with L-amino acids and vitamin C at pH 7.30 ± 0.01 was investigated by 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 C and amino acids, used either alone or in complex, might be effective to reduce the toxicity of metal *in vivo* and also be very much useful not only to control the aging process, but also to prevent the HIV replication *in vivo*. The negative values of ΔH indicated the exothermic nature of the metal–ligands 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 0.403 to 0.571 (0.50), showing that the 'transition state' behaves between oxidant and reductant response to the applied potential and lies in the midway between the dropping mercury electrode and the solution interface. The values of the rate constant (k) varied from 3.28 to 5.99 cm sec^{-1}, confirming the quasireversible nature of electrode processes.

ACKNOWLEDGEMENT

The authors are thankful to Head of the Department of Chemistry, Dr. H. S. Gour University, Sagar for providing the laboratory facilities.

Received 1 September 2009
Accepted 15 March 2010

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CINKO KOMPLEKSŲ SU KAI KURIOMIS L-AMINORŪGŠTIMIS IR VITAMINU C KINETIKOS IR TERMODINAMIKOS TYRIMAS VOLTAMPEROMETRIJOS METODU

S a n t r a u k a

Sukurtas paprastas ir jautrus tiesioginis poliarografinis būdas cinko jonų kompleksų su L-lizinu, L-ornitinu, L-treoninu, L-serinu, L-fenilglicinu, L-fenilalaninu, L-glutamino rūgštimi, L-asparto rūgštimi ir vitaminu C stabilumui nustatyti.