Polarographic Study of Mixed-Ligand Complexes of In(III) with L-Histidine and Glutamine/L-Proline

S. L. JAIN* and R. C. KAPOOR

Department of Chemistry, University of Jodhpur, Jodhpur, India

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In the In(III)–L-histidine system, the existence of 1:1 and 1:2 complex species has been established at pH 4.1 while 1:2 complex species predominates at pH ⩾ 5.9. The logarithmic values of overall stability constants for In(L-histidinate)$^{++}$, In(L-histidinate)$_2^+$, In(L-histidinate)(L-glutaminate)$^+$ and In(L-histidinate)(L-prolinate)$^+$ are 10.05, 17.86, 16.37 and 18.14, respectively. The higher-than-statistical value of mixing constant for the mixed-ligand complex species In(L-histidinate)(L-prolinate)$^+$ reveals that it is more stable than the parent bis complexes.

Introduction

Radioactive indium chelates possessing short half-lives have been employed as radiopharmaceuticals since they undergo urinary elimination easily [1–3]. Levin *et al.* [3], on investigating fourteen complexes of radioactive indium, found that complexes with log stability constants > 14 were not accumulated and were excreted through the urine. Clinical uses of In(III)–amino acid compounds such as those formed with histidine [1] and polyaminoacids [2] have been reported.

Numerous metal complexes of histidine have been investigated [4–15] during which polarographic technique has also been employed to determine their formation constants with Cd(II), Zn(II) and Hg(II) [4–6]. The Eu(III)–L-histidine system was investigated by Lal [7] using fast polarography. The stability constants of mixed-ligand complexes of Cu(II)/Ni(II) histidine systems have been determined potentiometrically [4, 11–15].

No work has been reported on the determination of stability constants of simple and mixed-ligand complexes of In(III) with histidine. We report here some polarographic studies made on the In(III)–L-histidine, In(III)–L-proline and In(III)–L-histidine–L-glutamine systems.

Experimental

A manual polarographic set-up (Toshniwal) and an automatic recording (Radelkis OH-105, Hungary) Polarograph were employed to obtain current–potential curves. An ultrathermostat (Type E-149, Hungary) was used to maintain the solution at a constant temperature. The pH values of the test solutions were measured with the help of an NIG 333 digital pH-meter.

All the reagents used were of analar grade. The amino acids, L-histidine, L-proline and L-glutamine were obtained from Sigma Chemical Co., U.S.A. Indium nitrate (Schuchardt Munchen, Germany) was dissolved in dilute perchloric acid and was standardised by titrating against an EDTA solution using PAN indicator [16, 17]. Required amounts of sodium perchlorate (Koch Lab., England) were added to the solutions to maintain the desired ionic strengths. The pH of the solutions was adjusted by adding dilute perchloric acid or CO_2-free sodium hydroxide solution. Pure nitrogen was bubbled through the test solution to remove the oxygen and during experiments an atmosphere of nitrogen was maintained over the solution.

The dropping mercury electrode used during the experiments had the following characteristics at −0.8 volt *vs.* SCE in 0.1 *M* $NaClO_4$ solution at 38.5 cm effective mercury height: m = 2.54 mg/sec, t = 3.21 sec.

Results and Discussion

pH Effect

The pH effect was studied in solutions containing 2×10^{-4} *M* In(III) and 0.01 *M* L-histidine in sodium perchlorate medium (I = 0.1) at 30 ± 0.1 °C. Two

*Present address: Department of Chemistry, Government College, Barmer (India).

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TABLE I. Overall Stability Constants of In(III) Complexes of Amino Acids at 30 °C.

Amino acid	pH	Method	log β_1	log β_2
Histidine	4.1	DeFord–Hume	10.05 ± 0.10	17.96 ± 0.08
Histidine	5.9	Lingane	–	17.76 ± 0.04
Glutamine	3.5	DeFord–Hume	6.65 ± 0.03	14.39 ± 0.08
Glutamine	–	pH-metry (25 °C) [17]	7.45	14.45
Proline	3.5	DeFord–Hume	7.99 ± 0.05	17.00 ± 0.08
Proline	–	pH-metry (24 °C) [25]	9.04	17.68

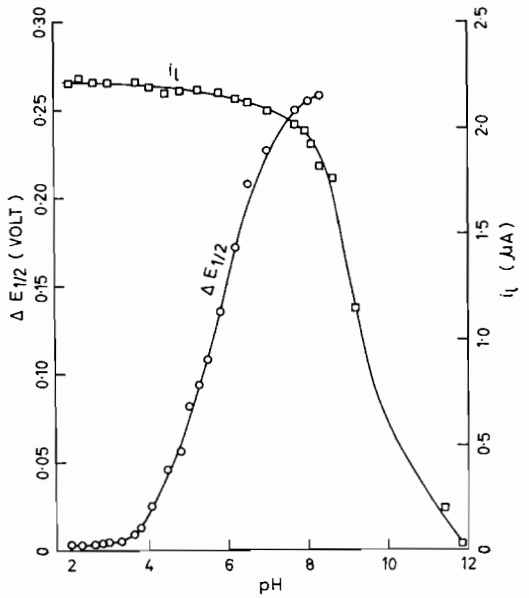

Fig. 1. Plots of $\Delta E_{1/2}$ and limiting current *vs.* pH. Conc. of In(III) = 2×10^{-4} *M*. Conc. of L-histidine = 1×10^{-2} *M*.

irreversible waves were obtained at pH 0.9. The limiting current of the first wave was low (0.43 μA at –0.6 V *vs.* SCE). At pH 1.5, one irreversible wave was observed and the limiting current recorded was much higher than that observed at pH 0.9. The reduction was however found to be reversible (log plot slope = 21 ± 1 mv) at pH ≥ 2.

The shifts in half-wave potential and variation in limiting current of 2×10^{-4} *M* In(III) in presence of 1×10^{-2} *M* L-histidine are shown in Fig. 1. A pronounced shift in $E_{1/2}$ was observed at pH > 4. Slight precipitation of In(III) occurred at pH > 8, which appeared nearly complete at pH ≈ 12.0. The behaviour of In(III) in presence of L-histidine was thus different from that in the presence of other amino acids like L-valine, L-leucine, L-proline, L-glutamine, L-methionine and L-glutamic acid [16–18]. Complete precipitation occurred in the presence of these acids in the pH range 4.6–5.2.

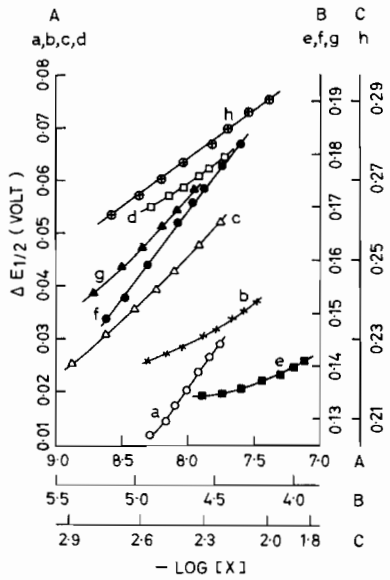

Fig. 2. Plots of $\Delta E_{1/2}$ *vs.* –log [X]. (A) a, L-prolinate; b, L-histidinate at pH 4.10; c, L-prolinate at [L-histidinate] = 1.05×10^{-9} *M*; d, L-histidinate at [L-prolinate] = 4.65×10^{-8} *M*; (B) e, L-glutaminate at [L-histidinate] = 2.21×10^{-6} *M*; f, L-histidinate at pH 5.90; g, L-histidinate at [L-glutaminate] = 1.67×10^{-4} *M*; (C) h, L-histidinate at pH 8.0.

Effect of Ligand Concentration

Investigations were carried out at pH 4.10, 5.90 and 8.00. On increasing the ligand concentration, the half-wave potentials became more negative at all three studied pH values (Fig. 2).

The free ligand ion concentrations were calculated from pK values at 30 °C which were obtained from the reported pK and $-\Delta H$ values of amino acids at 25 °C [18–22]. The system behaved reversibly (log plot slope = 21 ± 1 mv) at pH values 4.1, 5.9 and 8.0 and the limiting current was diffusion controlled in all cases since the plots of limiting current *vs.* $\sqrt{h}$ (corrected) were straight lines passing through the origin. The plot (Fig. 2) of $\Delta E_{1/2}$ *vs.* – log[L-histidinate] appeared as a smooth curve at pH 4.1, while

at pH 5.9 and 8.0, straight lines with a slope of 0.040 volt were obtained. The appearance of a smooth curve at pH 4.1 indicated the simultaneous existence of more than one complex species in solution. The values of the ligand number at pH 5.90 and 8.00 were found to be 2 in both cases which suggested that only 1:2 complex species existed at pH 5.9 and 8.0 under the experimental conditions.

The DeFord and Hume method [23] was used to evaluate the stability constants of complex species of In(III) with L-histidine at pH 4.1. The calculated values of $\log\beta_1$ and $\log\beta_2$ are given in Table I. The value of $\log\beta_2$ (Table I) at pH 5.9 was calculated by the Lingane method [24].

The higher values of $\log\beta_1$ and $\log\beta_2$ obtained with L-histidine compared with other bidentate amino acids (Table I) suggests that L-histidine possibly acts as a tridentate ligand. The tridentate behaviour of L-histidine has been observed with complexes of Mn(II), Co(II), and Cu(II) by various workers [4, 11, 12].

Mixed-Ligand Systems

The mixed-ligand systems of In(III) with amino acids have been investigated generally at pH $\leqslant$ 3.6 [16–18]. However the presently studied systems, In(III)–L-histidinate–L-prolinate and In(III)–L-histidinate–L-glutaminate, were studied at pH 4.0–4.15 and 5.95, respectively. This was possible because In(III) did not precipitate even up to pH 8 when L-proline or L-glutamine was added to its solution in the presence of L-histidine. The polarograms showed that the system behaved reversibly (log plot slope = 21 ± 1 mv) and appeared to be diffusion controlled in all cases. On increasing the concentration of the variable ligand L-histidinate [X] at a fixed concentration of L-glutaminate or L-prolinate ion [Y], the half-wave potential shifted towards more negative values. The shift was more marked than in the simple In(III)–L-histidinate system (Fig. 2). Similar results were obtained when the concentration of L-glutaminate or L-prolinate was varied while the concentration of L-histidinate was kept constant. These observations confirm the formation of mixed-ligand complexes.

While investigating different mixed ligand systems smooth curves (Fig. 2) were obtained when $\Delta E_{1/2}$ was plotted against $-\log[X]$ (X = variable ligand) thus indicating the formation of multiple complex species in each system. The method used by Schaap and McMasters [26] was employed to calculate the stability constants of different complex species. The details have already been described [16, 17].

Comparison of Stability of Complexes

The higher values of $\log\beta_{InXY}$ (Table II) for In(L-histidinate)(L-prolinate)$^+$ than for In(L-histidinate)(L-glutaminate)$^+$ may be due to the higher basicity of proline ($pK_1 + pK_2 = 12.47$) than glutamine ($pK_1 + pK_2 = 11.02$).

The relative stability of the mixed complex in solution compared with the parent bis-complexes can be expressed through the mixing constant, K_m [16]. The value of log K_m for the complex species In(L-histidinate)(L-glutaminate)$^+$ is nearly equal to the statistical value of 0.3 (Table II). On the other hand a higher-than-statistical value of log K_m (0.7) has been observed for complex species In(L-histidinate)(L-prolinate)$^+$, which indicates that this mixed-ligand complex species is more stable than the parent bis-complexes. Siegel [27] has reported that the value of logX (=2 log K_m) depends upon the basicity of the variable ligand. The higher basicity of proline compared with glutamine may be held responsible for the higher value of log K_m for In(L-histidinate)(L-prolinate)$^+$ compared with In(L-histidinate)(L-glutaminate)$^+$. Earlier, some workers [8, 12] reported stabilisation of mixed-ligand complexes of the type MAB (where M = Cu(II) or Ni(II), A = histidinate and B = amino acid anion).

The value of logK_m for In(L-glutaminate)(L-histidinate)$^+$ is higher than In(L-glutaminate)$(L_1)^+$ (where L_1 = L-methioninate, L-leucinate or L-prolinate) [17, 18]. Similarly the value of log K_m for In(L-prolinate)(L-histidinate)$^+$ is higher than In(L-prolinate)$(L')^+$ (where L′ = L-glutaminate, L-valinate or L-leucinate) [18]. The higher values of log K_m for In(L-glutaminate)(L-histidinate)$^+$ and In(L-prolinate)(L-histidinate)$^+$ than the corresponding mixed-ligand complex species of other amino acids appear to be due to the tridentate nature of histidinate. Similarly the tridentate behaviour of

TABLE II. Complex Formation Constants for the Mixed-Ligand Complexes at 30 °C and I = 0.1 ($NaClO_4$).

Mixed-ligand Complex	$\log\beta_{InXY}$	log K_m
In(L-Histidinate)(L-Glutaminate)$^+$	16.37 ± 0.06	0.26 ± 0.02
In(L-Histidinate)(L-Prolinate)$^+$	18.14 ± 0.03	0.70 ± 0.01

glutamate was also found to be responsible for the higher-than-statistical value of log K_m of mixed complex species In(L-glutamate)(L-leucinate) [16] and In(L-glutamate)(L-methioninate) [28]. Gergely *et al.* [19] and Ramamoorthi and Manning [29] have reported that there is a small but essentially larger stabilisation when one of the ligands contains two and the other three donor groups.

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References

1 M. Popov and I. Uzunov, *Med. Radiol., 20,* 39 (1975); *C.A., 84,* 71033h (1975).
2 J. Servian and A. Robies, *C.A., 85,* 68261w (1976).
3 V. I. Levin, L. V. Starovoitova, G. E. Kodine, N. F. Taru and M. D. Kozlova, *Med. Radiol., 21,* 38 (1976); *C.A., 85,* 168422x (1976).
4 R. J. Sundberg and R. B. Martin, *Chem. Rev., 74,* 471 (1974).
5 L. G. Sillen and A. E. Martell, 'Stability Constants of Metal-ion Complexes', Special Pub. No.s 17 and 25, The Chemical Soc. London (1964) and (1971).
6 S. C. Saraya and A. K. Sundaram, 'A Review on the Study of Complexes by Polarography', Bhabha Atomic Research Centre, Bombay, India (1969).
7 S. Lal, *Monatsh. Chem., 105,* 974 (1974).
8 J. R. Blackburn and M. M. Jones, *J. Inorg. Nucl. Chem., 35,* 1605 (1973).
9 D. H. Brown and D. Neumann, *J. Inorg. Nucl. Chem., 37,* 330 (1975).
10 L. D. Pettit and J. L. M. Swash, *J. Chem. Soc., Dalton Trans.,* 588 (1976).
11 A. C. Baxter and D. R. Williams, *J. Chem. Soc., Dalton Trans.,* 1797 (1975).
12 G. Brookes and L. D. Pettit, *J. Chem. Soc. Dalton Trans.,* 42 (1976); 1918 (1977).
13 I. Sovago, T. Kiss and A. Gergely, *Magy. Kem. Foly., 84,* 130 (1978).
14 T. P. A. Kruck and B. Sarkar, *Can. J. Chem., 51,* 3549 (1973).
15 P. G. Daniele and G. Ostacoli, *J. Inorg. Nucl. Chem., 40,* 1273 (1978).
16 S. L. Jain and R. C. Kapoor, *Proc. Indian Nat. Sc. Academy, 46A,* 53 (1980).
17 S. L. Jain and R. C. Kapoor, *Indian J. Chem., 19A,* 351 (1980).
18 S. L. Jain, *Ph.D. Thesis,* Jodhpur University (1981).
19 A. Gergely, I. Nagypal and E. Farkas, *J. Inorg. Nucl. Chem., 37,* 551 (1975).
20 R. J. F. Nivard and G. I. Tesser, 'Comprehensive Biochemistry', Ed. M. Florkin and E. H. Stotz, Elsevier (1965).
21 J. J. Christensen, R. M. Izatt, D. P. Wrathall and L. D. Hansen, *J. Chem. Soc. A,* 1212 (1969).
22 D. S. Barnes and L. D. Pettit, *J. Inorg. Nucl. Chem., 33,* 2177 (1971).
23 D. D. DeFord and D. N. Hume, *J. Am. Chem. Soc., 73,* 5321 (1951).
24 J. J. Lingane, *Chem. Rev., 29,* 1 (1941).
25 B. Khan, O. Farooq and N. Ahmad, *J. Electroan. Chem., 74,* 239 (1976).
26 W. B.Schaap and D. L. McMasters, *J. Am. Chem. Soc., 83,* 4699 (1961).
27 H. Siegel, *J. Inorg. Nucl. Chem., 37,* 507 (1975).
28 S. L. Jain, M. L. Soni and R. C. Kapoor, *Proc. Indian Sc. Cong. Part III, Abstracts,* 22 (1979).
29 S. Ramamoorthi and P. G. Manning, *J. Inorg. Nucl. Chem., 35,* 1571 (1973).