Thermodynamic Considerations in Co-ordination. Part XIII.[1] Formation Constants for the Glutaminate– and Serinate–Proton, –Manganese(II), –Iron(II), –Cobalt(II), –Nickel(II), –Copper(II), and –Zinc(II) Systems

By **David R. Williams**, Department of Chemistry, The University, St. Andrews, KY16 9ST, Scotland

Formation constants for AH, AH_2, AB, A_2B, and A_3B (A = glutaminate or serinate, H = H^+, B = Mn^{II}, Fe^{II}, Co^{II}, Ni^{II}, Cu^{II}, and Zn^{II}) are reported for I = 3·00M $(Na^+)ClO_4^-$ at 25 °C. The accuracy of our usual potentiometric approach has been increased by improved instrumentation.

BOTH L-glutamine and L-serine and also the transition metal ions manganese(II), iron(II), cobalt(II), copper(II), and zinc(II) are indispensable components of living systems. The two amino-acids in question are important to our anticancer programme [2,3] as myeloid leukaemia cells require an external source of glutamine and serine whereas normal cells synthesise their own supplies.[4] Clearly, this fundamental, qualitative, difference between normal and malignant cells ought to be capitalised upon to the detriment of the cancer and, as a preliminary, we report formation constants for the L-glutaminate– and L-serinate–proton, –manganese(II), –iron(II), –cobalt(II), –nickel(II), –copper(II), and –zinc(II) systems at 25·0 °C and I = 3·00M $(Na)ClO_4$. (The nickel studies were included because of their Periodic Table relationship to recently discovered Group VIII inorganic anticancer therapeuticals.[3,5]) These constants are essential for computer simulation studies of the solution equilibria involved in anticancer therapy by use of metal ion–amino-acid complexes.[3]

This paper contains two improvements to our usual techniques: (*a*) the potentiometric procedure was modified to be capable of reading glass electrodes to ±0·01 mV,[6] and (*b*) all formation-curve points were plotted by a C.I.L. plotter rather than manually.[7]

EXPERIMENTAL

Ligands.—L-Glutamine (B.D.H. biochemical grade), m.p. 180—185 °C [lit., 185—186 °C (decomp.)] (Found: C, 41·0; H, 6·8; N, 19·2. Calc. for $C_5H_{10}N_2O_3$: C, 41·1; H, 6·9; N, 19·2%) and L-serine (B.D.H., biochemical grade), m.p. 210—220 °C [lit., 228 °C (decomp.)] (Found: C, 34·2;

[1] Part XII, D. R. Williams and P. A. Yeo, *J.C.S. Dalton*, 1972, 1988.
[2] D. R. Williams, *Chem. Rev.*, 1972, **72**, 203.
[3] D. R. Williams, *Inorg. Chim. Acta Rev.*, 1973, **6**, 123.
[4] H. E. Wade and D. A. Rutter, *Sci. J.*, 1970, **6**: **3**, 62.
[5] R. D. Graham and D. R. Williams, unpublished work.
[6] R. P. Henry, J. E. Prue, F. J. C. Rossotti, and R. J. Whewell, *Chem. Comm.*, 1971, 868.
[7] R. D. Graham, Ph.D. Thesis, University of St. Andrews, 1973.

H, 6·7; N, 13·3. Calc. for $C_3H_7NO_3$: C, 34·3; H, 6·7; N, 13·3%) were dried and used without further purification.

Other Reagents.—These were as described in ref. 1.

Potentiometric Measurements.—We have modified our apparatus to that suggested by Henry *et al.*[6] by introducing an impedance converter and a digital voltmeter. Our equipment was as laid out as in the Chart. The electrodes and solutions were thermostatted at 25·00 °C and the titration cell was maintained under an atmosphere of purified, thermostatted, presaturated, nitrogen. E.m.f. values for standard perchloric acid solutions were reproducible to ±0·01 mV. The potentiometric approach was as described in ref. 8.

CHART

Solartron digital voltmeter LM1867 with impedance converter (Analog Devices operational amplifier 311K; Coutant power supply OA10)	Russell pH Ltd. glass electrode SF75/B14	Equilibrium solution in Pye Ingold titration vessel cat. no. 604	3M-$NaClO_4$ salt bridge	Two Russell pH Ltd., saturated sodium chloride calomel reference electrodes CR4/5/Na/B14

Data Treatment.—The modified SCOGS programme [1,8-10] has been further enlarged to include a ZPLOT programme which plots $\bar{Z}$ against pH or pA for formation curves using the same input data as SCOGS. Such curves, when plotted for a range of concentrations and pH, if superimposable for a system, signify that hydroxo-complexes, polynuclear species, and protonated complexes are all absent. The SCOGS programme was then able to produce least-squares 'best' constants and standard deviations in the usual manner. It is noteworthy that the introduction of the improved potentiometric equipment produced better data having smaller standard deviations without requiring an increase in the number of titration points in the data.

RESULTS AND DISCUSSION

The reactions between ligand, metal ion, and protons (A, B, and H respectively) can be represented by equation (1), the formation constant for this generalised reaction

$$pA + qB + rH \rightleftharpoons A_pB_qH_r \text{ (charges omitted)} \quad (1)$$

being β_{pqr}. From protonation titrations for which B was absent, ZPLOT calculated and plotted $\bar{Z}$ against $-\log[H^+]$ for glutaminate and serinate protonation. SCOGS Gave the constants shown in the Table and these were then used to produce metal complexing formation curves from titrations involving all three components, A, B, and H (Figures 1 and 2; $\bar{Z}$ is the average number of A per B). SCOGS Values calculated for the metal complex formation reactions are also listed in the Table.

Protonations.—Values of $p\beta_{101}$ and $p\beta_{102}$, as reported by other workers, are listed in the Table, serine having received far more attention than glutamine. As we have previously noted, our $I = 3{\cdot}00$M $(Na)ClO_4$ formation constants are consistently larger than those measured in systems of lower I.[1,11] From the reasoning given in Part XI it may be assumed that **9·640** and **9·574** refer to protonating each primary amine nitrogen whereas the **12·361** and **12·133** are overall log formation constants for protonating both the carboxylate oxygen and the amine nitrogen. The potentiometric data for the pH range 2—10 showed no disposition towards glutamine amide nitrogen protonation or serine hydroxyl (de)protonation.

Metal Complexing Reactions.—Previous results are recorded in the Table and, the 3M-ClO_4^- influence apart, our results correlate well (except ref. *k*). This log β^{3M}—log $\beta°$ effect becomes more noticeable as log β increases. These log constants are of magnitudes in accordance with glycine-type co-ordination and obey the Irving–Williams series. The insolubility of the manganese(II) system prevented our obtaining β_{310} but

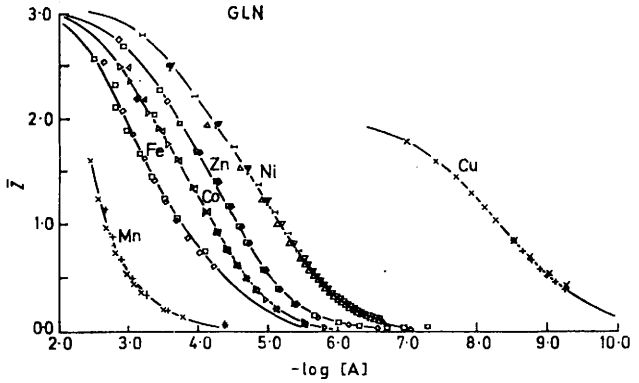

FIGURE 1 Metal complex formation curves for the glutamine system at 25·0 °C, $I = 3{\cdot}00$M $(Na)ClO_4$. The symbols represent different combinations of total metal and total ligand concentrations

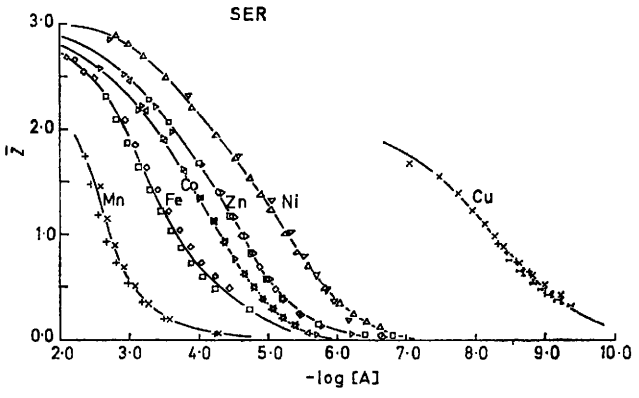

FIGURE 2 Metal complex formation curves for the serine system at 25·0 °C, $I = 3{\cdot}00$M $(Na)ClO_4$. The symbols represent different combinations of total metal and total ligand concentrations

tris-complexes of iron, cobalt, nickel, and zinc were obtained, in spite of previous workers being unable to

[8] D. R. Williams, *J.C.S. Dalton*, 1972, 790.
[9] A. D. Jones and D. R. Williams, *J. Chem. Soc.* (*A*), 1970, 3138.
[10] I. G. Sayce, *Talanta*, 1968, **15**, 1397 (including errata note).
[11] R. D. Graham, D. R. Williams, and P. A. Yeo, *J.C.S. Perkin II*, 1972, 1876.

obtain β_{310} for iron(II) because of hydrolysis. The zinc–glutaminate or serinate formation curves follow those of zinc–tryptophanate in being *more* stable than their corresponding cobalt curves whereas the zinc and cobalt histidinate systems were the other way round (as discussed in ref. 12).

The possibilities of this phenomenon being due to amide involvement are to be investigated thermodynamically.

For serine complexes, however, there are several reports demonstrating the absence of hydroxy-metal bonding (from ^{1}H n.m.r.,[13] thermodynamic,[14] and *X*-ray studies [15]).

log β for the species $A_pB_qH_r$ for the present (25 °C and I = 3M-ClO_4^-) and other work. A = amino-acid anion, B = metal 2+ ion, H = H^+, s = standard deviations in log constants, n = number of titration readings for each series

A = glutaminate

							Other workers, ref., temp., and I	
							a, b	*c, d*
B	*p*	*q*	*r*	Present work	*s*	*n*	~0M	0·1M
	1	0	1	9·640	0·003		9·34	9·01
	1	0	2	12·361	0·004	104		11·18
Mn	1	1	0	2·863	0·04			
	2	1	0	4·62	0·22	36		
Fe	1	1	0	4·432	0·043			
	2	1	0	7·258	0·096			
	3	1	0	10·401	0·064	42		
Co	1	1	0	4·518	0·018			4·03
	2	1	0	8·361	0·022			7·29
	3	1	0	11·405	0·036	66		
Ni	1	1	0	5·561	0·008			5·14
	2	1	0	10·282	0·019			9·38
	3	1	0	13·816	0·053	150		
Cu	1	1	0	9·052	0·019			7·75
	2	1	0	16·544	0·079	34		14·25
Zn	1	1	0	4·826	0·017			
	2	1	0	9·165	0·016		8·4	
	3	1	0	11·843	0·061	66		

A = serinate

				Present			*e, f*	*g, f*	*b, d*	*h, d*	*i, d*	*j, k*	*l, k*	*f, d*	*m, d*	*n, d*	*o, f*	*p, d*	*d,*	*q, f*	*k, d*	*a,*
B	*p*	*q*	*r*	work	*s*	*n*	1M	1M	0·6M	0·2M	0·16M	0·15M	0·15M	0·1M	~0·06M	0·05M	~0·01M	—	—	—	→0M	~0
	1	0	1	9·574	0·005		9·12	9·12	9·24	9·12	9·18		8·84	9·83	9·14	9·10	9·24	9·15				9·3—4
	1	0	2	12·133	0·007	88	—	11·38	—	11·41	—		11·02	—	—	11·25	11·44	11·36				
Mn	1	1	0	2·893	0·04					2·5											3·4	
	2	1	0	4·791	0·15	36				4·0											6·7	
Fe	1	1	0	4·299	0·027		3·43			3·7							—				—	
	2	1	0	7·377	0·044		—			6·4							7·0				7·7	
	3	1	0	10·299	0·048	50	—			—							—				—	
Co	1	1	0	4·584	0·017					4·33		4·20									4·90	
	2	1	0	8·568	0·023					7·66		7·56					8·0				9·10	
	3	1	0	11·554	0·043	62						9·81									—	
Ni	1	1	0	5·626	0·020					5·42	5·45		5·21					5·48	5·44	5·44	6·0	
	2	1	0	10·621	0·029					9·76	9·98		9·59					9·94	9·82	10·06	10·6	
	3	1	0	14·178	0·087	54				—	13·52		12·49					12·97	12·79	13·17	—	
Cu	1	1	0	8·950	0·021					7·89	7·85		7·56		—	7·93	—				8·40	
	2	1	0	16·230	0·132	54				14·40	14·50		14·01		14·54	14·67	14·60				14·50	
Zn	1	1	0	4·898	0·010					4·66		4·47									5·30	—
	2	1	0	9·279	0·010					8·38		8·31									9·75	8·6
	3	1	0	11·909	0·033	86				—		10·56									—	—

a D. J. Perkins, *Biochem. J.*, 1953, **55**, 649. *b* Yu. M. Azizov, A. Kh. Miftakhova, and V. F. Toropova, *Russ. J. Inorg. Chem.*, 1967, **12**, 345. *c* J. H. Ritsma, G. A. Wiegers, and F. Jellinck, *Rec. Trav. chim.*, 1965, **84**, 1577. *d* S. Pelletier, *Compt. rend.*, 1957, **245**, 160. *e* D. D. Perrin, *J. Chem Soc.*, 1959, 290. *f* V. D. Panasynk and A. Golub, *Russ. J. Inorg. Chem.*, 1965, **10**, 2732. *g* D. D. Perrin, *J. Chem. Soc.*, 1958, 3125. *h* E. V. Raju and H. B. Mathur, *J. Inorg. Nuclear Chem.*, 1968, **30**, 2181. *i* J. E. Letter and J. E. Bauman, *J. Amer. Chem. Soc.*, 1970, **92**, 437. *j* D. D. Perrin and V. S. Sharma, *J. Chem. Soc.* (*A*), 1969, 2060. *k* A. Ya. Sychev, *Russ. J. Inorg. Nuclear Chem.*, 1964, **9**, 2343. *l* D. D. Perrin, I. G. Sayce, and V. S. Sharma, *J. Chem. Soc.* (*A*), 1967, 1755; 1968, 446. *m* N. C. Li and E. Doody, *J. Amer. Chem. Soc.*, 1952, **74**, 4184. *n* A. Gergely, I. Nagypál, and I. Sóvágó, *Acta Chim. Acad. Sci. Hung.*, 1971, **67**, 241. *o* A. Albert, *Biochem. J.*, 1950, **47**, 531. *p* S. Pelletier, Thesis, Paris, 1960; *Acad. Sci. Paris*, 1967, **245**, 160. *q* S. Pelletier, J. Curchod, and M. Quintin, *Compt. rend.*, 1956, **243**, 1868.

The pKs and pβs of glutamine and asparagine exhibit an unusual homologue effect ΔpK_1 (glutamine–asparagine) = +0·34 whereas $\Delta p\beta_{110}$ = −0·58 (values for nickel complexes have been used in this example).

[12] D. R. Williams, *J. Chem. Soc.* (*A*), 1970, 1550.

[13] N. C. Li, R. L. Scruggs, and E. D. Becker, *J. Amer. Chem. Soc.*, 1962, **84**, 4650.

[14] J. E. Letter and J. E. Bauman, *J. Amer. Chem. Soc.*, 1970, **92**, 437.

I thank the Royal Society for a grant to purchase potentiometric instrumentation, Mr. R. D. Graham for modifying a computer programme, and the referees for a useful suggestion.

[2/2466 *Received*, 31*st October*, 1972]

[15] D. Van der Helm, W. A. Franks, M. B. Hossain, and C. G. Fisher, *Acta Cryst.*, 1969, **25**, 451, 457; 1970, **26**, 1172.