Thermodynamic Considerations in Co-ordination. Part XV.[1] Potentiometric and Calorimetric Study of the Complexes formed between Protons, First Transition Series Metal Ions, and Asparaginate and Glutaminate Anions

By **Allan C. Baxter** and **David R. Williams,*** Department of Chemistry, University of St. Andrews, St. Andrews, Fife, Scotland KY16 9ST

ΔG°, ΔH°, and ΔS° for the asparaginate $-H^{+}$, Mn^{II}, Fe^{II}, Co^{II}, Ni^{II}, and Zn^{II}, and for the glutaminate $-H^{+}$, Ni^{II}, and Cu^{II} systems are reported for aqueous solution studies at 25 °C and $I = 3.00M$ (Na^{+}) ClO_{4}^{-}. The results are used to calculate that quite a large proportion of asparaginate in blood plasma may be complexed to Zn^{II}, Fe^{II}, and Co^{II}, as well as to the expected Cu^{II}.

THE transport of metal ions, for example copper(II), in blood serum occurs through mixed complexes such as human serum albumin–copper(II)–amino-acid anions. These ternary complexes involve the *N*-terminal asparaginyl moiety of albumin and amino-acids chosen from glutamine, histidine, serine, or threonine. Not surprisingly, there are both binary and ternary amino-acid complexes found in serum; for example, histidinate–copper(II)–glutaminate (or asparaginate).[2,3]

Membrane transport phenomena and asparagine are further related in that a supply of the amino-acid is essential for the growth of lymphatic leukaemia cells.[4-7] This unidirectional flow of asparagine from plasma into cancer cells has been suggested as a means of directing anticancer metal complexes into these cells.[6,7] Furthermore, analogous situations arise for the flow of glutamine and serine into myeloid leukaemia cells.[8] Thus, there are pressing reasons for studying the structures formed between asparagine and glutamine with metal ions in aqueous solution.

As a necessary preliminary to the investigation of these structures, we have determined ΔG° (potentiometrically) and ΔH° and ΔS° (calorimetrically) for the

[1] Part XIV, A. M. Corrie, M. L. D. Touche, and D. R. Williams, *J.C.S. Dalton*, 1973, 2561.

[2] B. Sarkar and T. P. A. Kruck, 'The Biochemistry of Copper,' eds. J. Peisach, P. Aisen, and W. E. Blumberg, Academic Press, New York, 1966, p. 183.

[3] B. Sarkar and Y. Wigfield, *Canad. J. Biochem.*, 1968, **46**, 601.

[4] D. R. Williams, *J.C.S. Dalton*, 1973, 1064.

[5] 'Experimental and Clinical Effects of L-Asparaginase, eds. E. Grundmann and H. F. Oettgen, Springer, Berlin, 1970.

[6] D. R. Williams, *Chem. Rev.*, 1972, **72**, 203.

[7] D. R. Williams, *Inorg. Chim. Acta, Rev.*, 1972, **6**, 123.

[8] H. E. Wade and D. A. Rutter, *Sci. J.*, 1970, 62.

TABLE 1

Log β for the species $A_pB_qH_r$ for the present [25 °C and $I = 3M$ (Na)ClO$_4$] and other work. A = asparaginate⁻, B = metal²⁺ ion, H = H⁺, s = standard deviation in log constants, n = number of titration readings for each series

B	p	q	r	Present work	s	n	Other workers				
	1	0	1	9·303	} See						
	1	0	2	11·888	} ref. 10						
Mn	1	1	0	3·102	0·040						
	2	1	0	5·222	0·090	46				4·5 [d]	
Fe	1	1	0	4·366	0·033						
	2	1	0	7·569	0·036						
	3	1	0	10·259	0·054	56				6·5 [d]	
Co	1	1	0	4·903	0·007			4·55 [b]	4·51 [e]		
	2	1	0	9·029	0·009			8·13 [b]	8·01 [e]	8·4 [d]	
	3	1	0	11·855	0·021	60		9·95 [b]			
Ni	1	1	0	6·152	0·007		5·58 [a]		5·68 [e]		
	2	1	0	11·163	0·011		9·96 [a]		10·23 [e]	10·6 [d]	
	3	1	0	14·545	0·055	206					
Cu	1	1	0	8·677	0·023		7·78 [a]		7·86 [e]		log K_2
	2	1	0	16·052	0·024	128	14·13 [a]		14·42 [e]	14·9 [d]	6·45 [e]
Zn	1	1	0	5·070	0·004						
	2	1	0	9·426	0·004					8·7 [d]	8·5 [f]
	3	1	0	12·300	0·026	228					

[a] At 25 °C, 0·16M, N. C. Li, E. Doody, and J. M. White, *J. Amer. Chem. Soc.*, 1958, **80**, 5901. [b] At 25·1 °C, 0·15M, C. Tanford and W. S. Shore, *J. Amer. Chem. Soc.*, 1953, **75**, 816. [e] At 25 °C, 0·1M, J. H. Ritsma, G. A. Wiegers, and F. Jellinek, *Rec. Trav. chim.*, 1965, **84**, 1577. [d] At 20 °C, 0·01M, A. Albert, *Biochem. J.*, 1950, **47**, 531. [e] At 25 °C, 0·1M, W. E. Bennett, *J. Amer. Chem. Soc.*, 1959, **81**, 246. [f] At 15 °C, 0·005M, D. J. Perkins, *Biochem. J.*, 1953, **55**, 649.
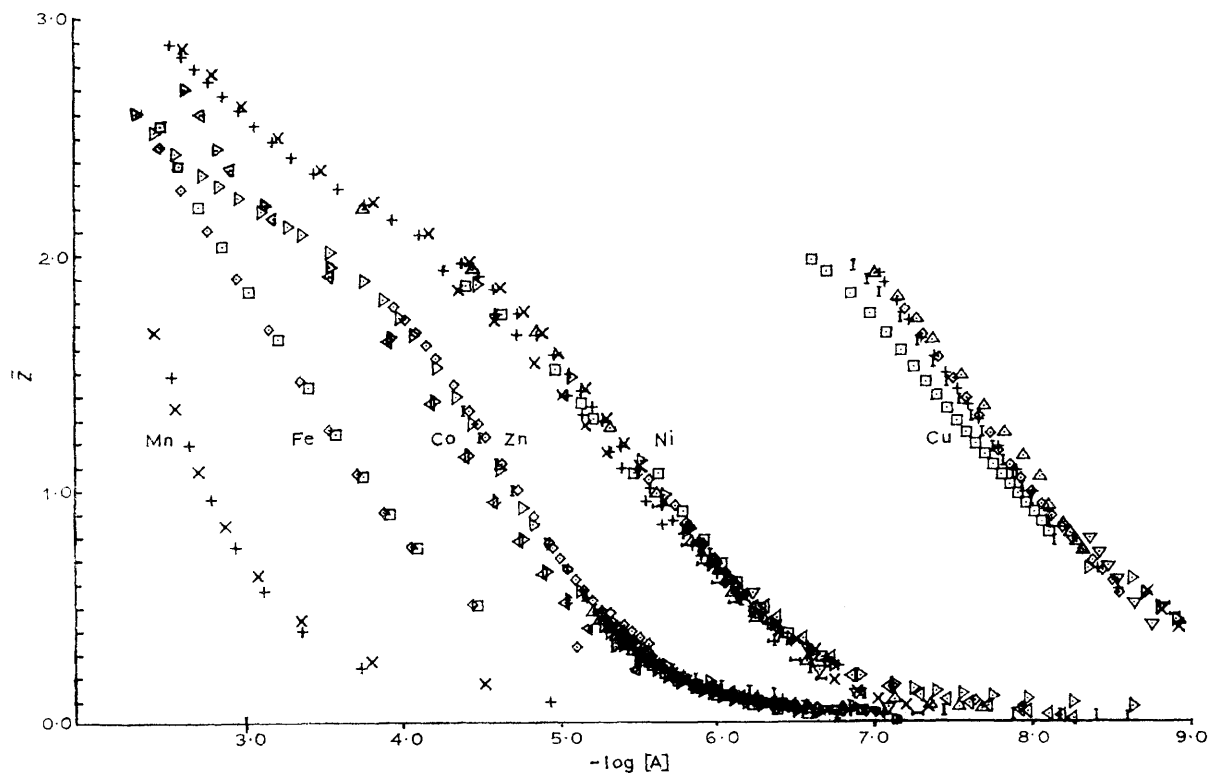

FIGURE 1 Metal complex formation curves for the asparaginate system at 25·0 °C, $I = 3·00M$ (Na)ClO$_4$. The symbols represent different combinations of total metal and total ligand concentrations

formation of H⁺, Mn^{II}, Fe^{II}, Co^{II}, Ni^{II}, Cu^{II}, and Zn^{II} asparaginate complexes.

The experimental measurements were performed using an ionic background of $I = 3·00M$ (sodium) perchlorate, this having been shown to be a suitable medium for minimising variations in activity coefficients arising

⁹ G. Biedermann, *Svensk. kem. Tidsk.*, 1964, **76**, 1.
¹⁰ R. D. Graham, D. R. Williams, and P. A. Yeo, *J.C.S. Perkin II*, 1972, 1876.
¹¹ D. R. Williams and P. A. Yeo, *J.C.S. Dalton*, 1972, 1988.

from opposite charges being neutralised during protonation or complexation.⁹

EXPERIMENTAL

L-Asparagine,H$_2$O and L-glutamine were as previously reported.⁴,¹⁰ Other reagents were as described in ref. 11.

Potentiometric Measurements and Data Treatment.— These were as described in ref. 4. The protonation constants necessary for calculating formation constants were as previously reported.⁴,¹⁰ Formation curves are shown in Figure 1 and the SCOGS β values are listed in Table 1.

Calorimetric Measurements.—These were as described in refs. 11 and 12. Enthalpic curves are shown in Figure 2 and the RWCALCOR and RWSOLV derived thermo-dynamic parameters are as listed in Table 2. The iron(II)
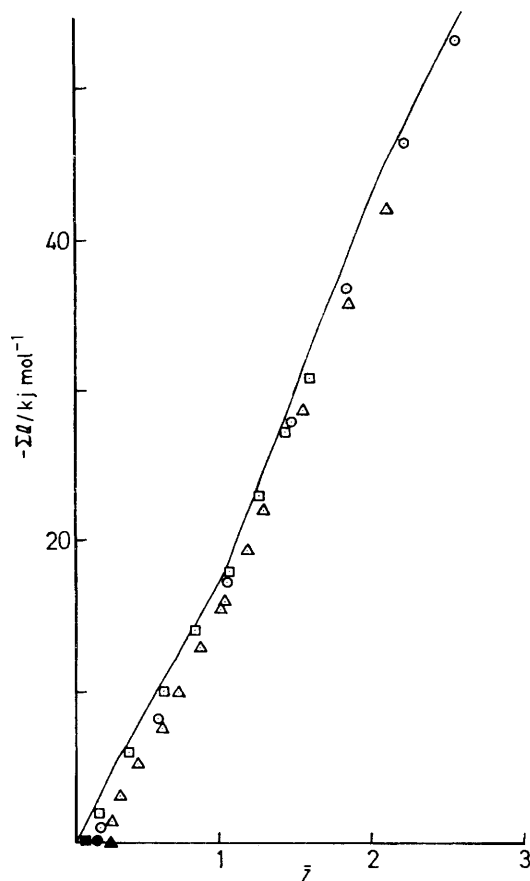

FIGURE 2 Enthalpic curve for the nickel(II) asparaginate system at 25 °C, $I = 3\cdot00\text{M}$ (Na)ClO$_4$. The different symbols refer to different titrations (varying in the amount of reactants present in the reaction vessel at the commencement of a titration). The theoretical enthalpic curve has been calculated using the thermodynamic parameters in Table 2. Since the three titrations have differing initial $\bar{Z}$ values ($\bullet$, $\blacksquare$, and $\blacktriangle$) the experimental curves are parallel to the calculated curve (ref. 13)

and to a small extent the manganese(II) systems hydrolysed slowly during the interval of a calorimetric titration and so accurate ΔH° were unobtainable.

DISCUSSION

Protonations.—In Table 2, the first parameters for the first protonations, ΔG°_1, ΔH°_1, and ΔS°_1 refer to protons being added to the primary amine sites, and the second protonations to the carboxylate sites.[4,10,14] The effect of adding a CH$_2$ to asparaginate (giving glutaminate) is reflected in glutaminate's slightly larger $-\Delta G^\circ$, $-\Delta H^\circ$ remaining essentially the same for both ligands. This increase in $-\Delta G^\circ$ arises from an approximately 20% increase in the entropies of protonating glutaminate as compared to asparaginate. This probably occurs be-

cause the glutaminate ligand possesses more water of aquation which is shed upon protonation. A consequence of $\Delta H^\circ_{\text{glutaminate}} \doteq \Delta H^\circ_{\text{asparaginate}}$ is that ΔG° and ΔS° (glutaminate) fit the $\Delta G^\circ_{\text{-coo-}}$ *versus* $\Delta S^\circ_{\text{-coo-}}$ plot shown in Figure 3 of ref. 10.

The large standard deviations reported for carboxylate protonation occur because the correction terms for heats of dilution necessary when handling calorimetric solutions of $-\log h \doteq 2$ are comparable in magnitude with the very low $\Delta H^\circ_{\text{-coo-}}$ being determined.

Complex Formation.—Figure 1 and Table 1 show that the order of complexing obeys the Irving–Williams series which in turn suggests octahedral co-ordination. It is also clear from the Table that, having accepted the principle that concentration constants for 3M-(Na)ClO$_4$ are larger than those for other ionic strengths,[10,11] our results are in agreement with other published results. It is remarkable that so few constants have been published for a ligand that is as biologically important as asparaginate.

With the exceptions of copper and manganese, all systems form tris complexes, the zinc system being marginally more stable than the cobalt (*cf.* histidinate in ref. 15). The formation curves for zinc and cobalt
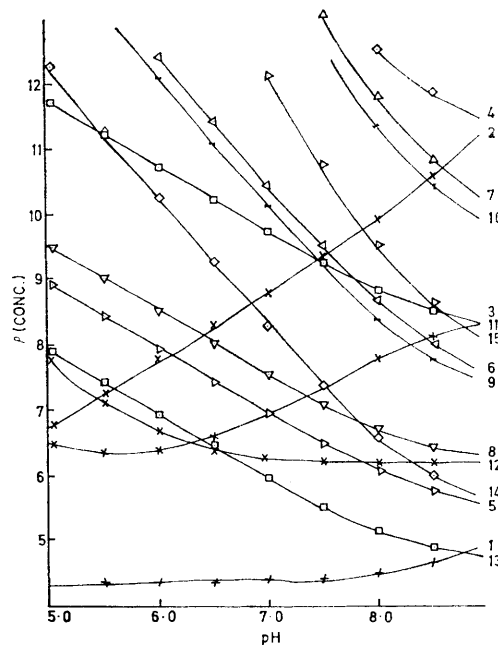

FIGURE 3 COMPLOT model of asparaginate–metal ion complexes occurring at a range of pH. Total concentrations used were asparagine 43·9 µM, Mn^{II} 0·73 µM, Fe^{II+III} 23·3 µM, Co^{II} 1·73 µM, Cu^{II} 0·65 µM, and Zn^{II} 45·9 µM; 1, Hasn; 2 H$_2$asn$^+$; 3, Mn(asn)$^+$; 4, Mn(asn)$_2$; 5, Fe(asn)$^+$; 6, Fe(asn)$_2$; 7, Fe(asn)$_3$$^-$; 8, Co(asn)$^+$; 9, Co(asn)$_2$; 10, Co(asn)$_3$$^-$; 11, Cu(asn)$^+$; 12, Cu(asn)$_2$; 13, Zn(asn)$^+$; 14, Zn(asn)$_2$; 15, Zn(asn)$_3$$^-$

coalesce at $\bar{Z} \doteq 2\cdot2$ because of a lower than expected $-\Delta H^\circ_{2\cdot3}$ for zinc(asparaginate)$_3$. The maximum $\bar{Z}$ measurable for all systems was determined by the insolubility of the hydrolysis products of the complexes

[12] A. D. Jones and D. R. Williams, *J. Chem. Soc. (A)*, 1970, 3138.
[13] F. C. Davidson, J. P. Sloan, and D. R. Williams, *J. Appl. Chem. Biotechnol.*, 1971, **21**, 300.
[14] A. D. Jones and D. R. Williams, *Inorg. Nuclear Chem. Letters*, 1972, **8**, 1009.
[15] D. R. Williams, *J.C.S. Dalton*, 1972, 790.

formed. However, in general, solubility was not a major problem in this study, asparagine being soluble up to 160 mM and its complexes not being markedly less soluble. Hydrolysis of these complexes gave an immediate visible precipitation; however, neither these hydrolysed, nor protonated, complexes could be detected potentiometrically as β values for the equilibrated

entropies than the rest of the group studied (and lower $\Delta H°$ than Co, Ni, and Cu). One ponders whether this might be another ramification of the preference of zinc(II) for tetrahedral co-ordination (N–Zn–N and weak $-COO^-$–Zn interactions).

Simple Model of Asparaginate in Plasma.—Our modified version of the computer program COMICS [16]

TABLE 2

Gibbs free energy changes, enthalpies, and entropies for the formation of asparaginate and glutaminate complexes at 25·0 °C, $I = 3·00$M (Na)ClO$_4$. ($\Delta G°$ and $\Delta H°$ are in units of kJ mol^{-1}, $\Delta S°$ are in J mol^{-1} K^{-1}.) [ ] are 3 × standard deviations in computed thermodynamic parameters and n denotes the number of calorimetric measurements

| | Asparaginate | | | | | | | Glutaminate | | |
Asparaginate	H+	Manganese (II)	Iron(II)	Cobalt(II)	Nickel(II)	Copper(II)	Zinc(II)	H+	Nickel(II)	Copper(II)
$-\Delta G°_1$	53·10 [0·10]	17·70 [0·23]	24·93 [0·19]	28·00 [0·05]	35·12 [0·04]	49·54 [0·13]	28·93 [0·02]	55·03 [0·02]	31·75 [0·05]	51·68 [0·11]
$-\Delta G°_{1.2}$	14·76 [0·13]	12·10 [0·32]	18·28 [0·19]	23·56 [0·05]	28·66 [0·04]	41·10 [0·13]	24·87 [0·02]	15·55 [0·02]	26·96 [0·06]	42·78 [0·34]
$-\Delta G°_2$	67·86 [0·13]	29·80 [0·55]	43·21 [0·21]	51·56 [0·06]	63·78 [0·06]	90·64 [0·14] †	53·82 [0·02]	70·58 [0·02]	58·71 [0·11]	94·46 [0·44]
$-\Delta G°_{2.3}$		*	15·37 [0·21]	18·12 [0·25]	19·26 [0·25]		16·36 [0·13]		20·17 [0·19]	
$-\Delta G°_3$		*	58·58 [0·31]	67·68 [0·31]	83·04 [0·31]		70·18 [0·15]		78·88 [0·30]	
$-\Delta H°_1$	50·5 [0·4]	7·26 [0·8]	*	11·95 [0·50]	17·11 [0·40]	27·5 [1·0]	10·44 [0·40]	50·86 [0·5]	13·28 [0·6]	16·5 [1·0]
$-\Delta H°_{1.2}$	5·10 [0·05]	6·98 [0·8]	*	14·76 [0·50]	26·34 [0·40]	34·0 [1·0]	12·73 [0·40]	4·42 [0·5]	22·81 [0·6]	26·0 [1·0]
$-\Delta H°_2$	55·6 [0·4]	14·23 [1·6]	*	26·71 [1·0]	43·45 [0·80]	61·5 [2·0] †	23·17 [0·79]	55·28 [1·0]	36·09 [1·2]	42·5 [2·0]
$-\Delta H°_{2.3}$		*	*	9·69 [1·0]	20·05 [0·80]		4·38 [0·80]		18·66 [1·2]	
$-\Delta H°_3$		*	*	36·40 [1·5]	63·50 [1·0]		27·55 [1·5]		54·75 [1·8]	
$\Delta S°_1$	8·9 [1·0]	35·0 [1·9]	*	53·8 [1·8]	60·4 [1·2]	73·9 [2·9]	62·1 [1·3]	14·0 [1·6]	61·9 [1·8]	118·0 [3·0]
$\Delta S°_{1.2}$	32·4 [0·6]	17·2 [1·9]	*	19·5 [1·8]	7·5 [1·3]	23·8 [3·3]	40·6 [1·3]	37·4 [1·7]	18·0 [1·9]	56·3 [3·0]
$\Delta S°_2$	41·3 [1·0]	52·2 [3·5]	*	83·3 [3·2]	67·9 [2·5]	97·7 [6·2] †	102·7 [2·6]	51·4 [3·3]	75·9 [3·7]	174·3 [5·2]
$\Delta S°_{2.3}$		*	*	21·6 [3·2]	$-2·4$ [2·5]		40·3 [2·6]		5·0 [3·7]	
$\Delta S°_3$		*	*	104·8 [4·0]	65·5 [2·3]		143·0 [4·5]		80·9 [5·0]	
n	24+18	16		29	28	29	19	35	24	30

Additional data used: pK_w = 14·21; $\Delta H°_w$ = 55·7 [0·07] kJ mol^{-1}.

* = Study prevented by hydrolysis or oxidation. † D. S. Barnes and L. D. Pettit, *J. Inorg. Nuclear Chem.*, 1971, **33**, 2177, reported $-\Delta G°_2$ = 82·4, $-\Delta H°_2$ = 47·3 kJ mol^{-1}; $\Delta S°_2$ = 118 J mol^{-1} K^{-1} at 25 °C, I = 0·10M.

solutions. (The spread of experimental formation points in Figure 1 has been the subject of extensive computational analysis using SCOGS but it could not be described by other than binary complexes.)

The thermodynamics of complex formation follow the principles established in our phenylalanate study $-\Delta H°_1 < -\Delta H°_{1.2}$, and $\Delta S°_1 \gg \Delta S°_{1.2}$.[11]

$\Delta S°_{2.3}$ for the nickel systems are surprisingly low (a pattern not found in our previous amino-acid studies). This conceivably arises since the amide groupings occupy, albeit without formal bonding, a co-ordination position on the metal ion in bis complexes so that when the third ligand is added very little water of solvation needs to be removed from these positions. In this connection, $\Delta S°$(nickel) are higher than expected for comparable systems (*i.e.* three metal bond positions are already desolvated). We have not yet established why this trait is so particular to nickel complexes.

Zinc also has its own characteristics having larger

(COMPLOT) was used to compute the concentrations of metal complexes in a blood plasma model for a range of pH. The total metal concentrations used were blood plasma exchangeable metal ion values (*e.g.* the copper concentration is the albumin and amino-acid bound, not the non-exchangeable ceruloplasmin copper concentration) and the formation constants were those in Table 1. The results shown in Figure 3 suggest that the mono zinc, iron, and cobalt asparaginate complexes may be present in concentrations comparable to the mono and bis copper asparaginate complexes. This situation occurs in spite of the copper complexes having the highest β values because exchangeable copper is found at relatively low concentrations in plasma.

[16] D. D. Perrin and I. G. Sayce, *Talanta*, 1967, **14**, 833.