Thermodynamic Considerations in Co-ordination. Part XVII.[1] Glass and Chloride Electrode Studies of Carcinostatic Ternary and Quaternary Complexes of Nickel and Palladium(II) ions with Asparaginate and Chloride Ligands

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Glass and chloride electrode studies at 25° and $I = 3{\cdot}00\text{M}$ $(Na^+)ClO_4^-$ have been used to express the equilibria involved in the Ni^{II}–asparaginate–Cl^-–OH^- and Pd^{II}–asparaginate–Cl^-–OH^- systems in terms of formation constants for the following complexes: $NiCl^+$, $Ni(asn)Cl$, $Ni(asn)_2Cl^-$, $Ni(asn)_2ClOH^{2-}$ and $Pd(asn)H^{2+}$, $Pd(asn)OH$, $Pd(asn)ClOH^-$, $Pd(asn)ClH^+$. The relationship between the compositions of these complexes and carcinostatic activity is discussed.

TERNARY co-ordination complexes are an essential component of all living systems and are becoming more extensively applied in chemotherapy.[2-4] The most famous crystalline carcinostatic metal complex being used as a drug is *cis*-dichlorodiammineplatinum(II) and since it was first reported by Rosenberg *et al.* a large number of related compounds have been screened [5,6] of which several contained bidentate ligands such as amino-acid anions.[4]

Theories concerning the mechanisms of anticancer activity of these metal complexes ought to be based upon the forms adopted by these solid complexes when dissolved in the aqueous environment of the body since some ligand–metal ion bonds are labile and aquation and hydrolysis can markedly modify the compositions of and structures of the complexes administered.

This work reports the formation constants for mixed-ligand complexes of nickel(II) and palladium(II) with asparaginate and chloride. These β values (i) give some indication of the strengths of bonds involved, (ii) permit computer calculations of distribution curves for complexes present around physiological pHs, and (iii) in some instances suggest possible structures for the main complexes present.

Our investigations have been concerned with nickel and palladium ions since they not only have similar chemistries to platinum but also they offer the additional advantage that their kinetics of equilibration are far more rapid than those of platinum, *i.e.* our β studies were possible without having to involve rate constants.

EXPERIMENTAL

Chemicals.—L-Asparagine, perchloric acid, sodium hydroxide, and nickel perchlorate were as previously reported.[7-9] Palladium perchlorate was prepared from palladium nitrate (Johnson Matthey Chemicals Ltd) dissolved in an excess of perchloric acid, evaporated to fuming (care!), cooled, and diluted to 120 mM Pd^{2+}, $2{\cdot}07\text{M}$ in perchloric acid (to suppress hydrolysis). The solution was analysed for palladium using dimethylglyoxime [10] and for acid by the Gran plot method.[11]

Electrodes.—The glass electrodes were Russell pH Ltd. (SF75/B14), the reference electrode was a mercurous sulphate (Russell pH Ltd.). A saturated sodium sulphate salt bridge was used. The silver–silver chloride electrode used for monitoring chloride concentrations was prepared by the method described in ref. 12.

Potentiometric Measurements.—The e.m.f. readings between reference and glass, and between reference and silver–silver chloride, electrodes were measured on millivoltmeters (Radiometer PHM52 Digital pH meters). The titration vessel (Pye Ingold 604) was thermostatted to 25·0° and maintained under an oxygen-free nitrogen atmosphere. All solutions were of ionic strength, $I = 3{\cdot}00\text{M}$ $(Na^+)ClO_4^-$ and electrodes were calibrated against solutions of known $[H^+]$ and $[Cl^-]$ concentrations before, and after, each titration. Volumetric glassware was grade A and calibrated.

Data Treatment.—We have used two new least-squares optimisation computer programs GSCOGS and MINIQUAD. The GSCOGS program is based upon the SCOGS program as published by Sayce [13] and as generalised by Jones, Williams, and Yeo.[8,14] We have further generalised SCOGS to accommodate e.m.f. data from up to three ion selective electrodes. Sets of log β values are varied until the relationship

$$(\text{Titre}_{\text{calc.}} - \text{Titre}_{\text{exp.}}) = \left[\text{Initial volume} \left\{ \frac{(H_{\text{initial in vessel}} - H_{\text{calc.}})}{(H_{\text{calc.}} - H_{\text{titrant}})} \right\} - \text{Experimental volume}\right]$$

is minimised using a least squares approach. H Refers to total analytical concentrations and the expression for $H_{\text{calc.}}$ uses all the measured e.m.f.s.

The MINIQUAD program is based upon LETAGROP and has been developed by Sabatini, Vacca, and Gans.[15] Compared to SCOGS the program varies sets of β (rather

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[1] Part XVI, G. K. R. Makar and D. R. Williams, preceding paper.

[2] 'An Introduction to Bio-inorganic Chemistry,' ed. D. R. Williams, 1975 (at press).

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

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

[5] B. Rosenberg, L. van Camp, J. E. Trosko, and V. H. Mansour, *Nature*, 1969, **222**, 385.

[6] T. A. Connors, M. Jones, W. C. J. Ross, P. D. Braddock, A. R. Khokhar, and M. L. Tobe, *Chem. Biol. Interactions*, 1972, **5**, 415.

[7] R. D. Graham, D. R. Williams, and P. A. Yeo, *J.C.S. Perkin II*, 1972, 1876.

[8] A. D. Jones and D. R. Williams, *J. Chem. Soc.* (*A*), 1970, 3138.

[9] D. R. Williams and P. A. Yeo, *J.C.S. Dalton*, 1972, 1988.

[10] A. Vogel, 'A Textbook of Quantitative Inorganic Analysis,' 3rd edn., Longmans, London, 1968, p. 511.

[11] F. J. C. Rossotti and H. Rossotti, *J. Chem. Educ.*, 1965, **42**, 375.

[12] D. J. G. Ives and G. J. Janz, 'Reference Electrodes: Theory and Practice,' Academic Press, New York, 1961, 205.

[13] I. G. Sayce, *Talanta*, 1968, **15**, 1397.

[14] P. A. Yeo, Ph.D. thesis, University of St. Andrews, 1972.

[15] A. Sabatini, A. Vacca, and P. Gans, to be published.

than log β), minimises the sum of the squares in analytical concentrations rather than titre volumes, can handle several rather than three ion-selective electrodes simultaneously, and approaches the least-squares minima using the Fletcher and Powell steepest descent method rather than a Jacobian matrix. Reference 15 lists nine advantages of MINIQUAD over existing programs. However, being a new program MINIQUAD has been less extensively tested with experimental data than SCOGS. It is hoped that there are sufficient differences between these programs to highlight spurious β values that may have occurred had just one program have been used alone. The β values produced by these two programs agreed to within the standard deviations quoted in the Table.

DISCUSSION

The double electrode approach has been checked as rigorously as possible at each stage. The E° calibrations and electrode response times were checked daily. The original use of least-squares computer programs in solution co-ordination chemistry research was as a supplement to graphical methods of β calculation. These latter methods are not feasible for ternary complexes expressed as e.m.f. data from more than one electrode. Thus, the GSCOGS and MINIQUAD programs had to be applied with extreme care and frequent checks. For example, (i) the β values derived for the Ni^{2+}–asn–Cl^- system were used in the HALTAFALL program to produce simulated titration e.m.f.s. which were then introduced into the GSCOGS program to establish that the original βs were reobtained; (ii) a very wide range of combinations of β values were offered to the least squares programs, these sets of β_{pqrs} being chosen from $p = 0$—3, $q = 0$—4, $r = 0$—2, $s = -6$—2.

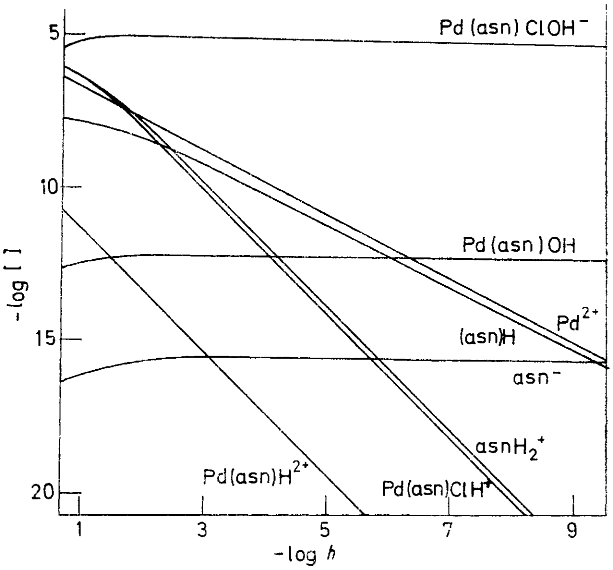
Log β for the species $B_p(asn^-)_qCl_r^-H_s^+$ where B = metal²⁺ ion. Temp. = 25 °C, $I = 3\text{M}\ ClO_4^-$, σ = standard deviation in log constants, n = number of titration readings for each series

B	p	q	r	s	log β_{pqrs}	σ	n
Ni^{II}	1	0	1	0	0·687	0·04	
	1	1	1	0	6·1	0·010	40
	1	2	1	0	11·7	0·4	
	1	1	1	−1	11·64	0·09	74
Pd^{II}	1	1	0	1	12·11	0·04	
	1	1	0	−1	9·1	0·5	107
	1	1	1	1	18·29	0·01	
	1	1	1	−1	17·0	0·2	64

The least-squares ' best ' constants that we report in the Table are remarkable not so much because of the existence of hydroxy complexes at low −log h (Pd^{2+} itself forms hydroxy species above −log h = 1) but rather (i) because so few complexes are present, and (ii) because the intermediate species Pd(asn)Cl is not found between $Pd(asn)ClH^+$ and $Pd(asn)ClOH^-$.

Suggested Structures for the Complex Species Present.—Nickel. In $NiCl^+$, the chloride has a choice of six octahedrally arranged co-ordination positions and in Ni(asn)Cl there is a choice of four, two being *cis* to the asparaginate and two being *trans*. $Ni(asn)_2Cl^-$ very probably has the asparaginates *trans* to each other about the Ni^{2+} as a centre of symmetry and a chloride and a water molecule occupying the remaining apical positions. In $Ni(asn)_2ClOH^{2-}$ the water molecule may be presumed to have lost a proton to form the apical hydroxide ligand.

Palladium. $Pd(asn)H^{2+}$ can either be glycinate-type asparaginate bonding, the proton being attached to the amide grouping, or, if chelation is through the amide and amine groups, the carboxylate group may be protonated. Pd(asn)OH can have glycinate type bonding and the hydroxide has a choice of the remaining two square planar co-ordination positions. $Pd(asn)ClOH^-$ may also be presumed square planar with glycinate type chelation and *cis* chloride and hydroxide anions. $Pd(asn)ClH^+$ is probably square planar, the fourth position being occupied by a co-ordinated water molecule and the proton being either attached to the asparaginate amide group (assuming glycinate type co-ordination) or to the carboxylate group (assuming amide + amine co-ordination).



HALTAFALL[*] computation of the distribution of complexes formed between Pd^{II}, asparaginate, Cl^-, and OH^- at various −log h. The conditions refer to total concentrations of palladium = 10 μM, asparaginate = 10 μM, chloride = 150 mM

[*] N. Ingri, W. Kakołowicz, L. G. Sillén, and B. Warnqvist, *Talanta*, 1967, **14**, 1261.

One may enquire why Pd^{II} does not have the same series of complexes as Ni^{II}. The answer lies in palladium's preference for planar bonding whereas nickel prefers octahedral and so can accept an additional chelate [*cf.* Pd(asn)ClOH and $Ni(asn)_2ClOH$].

Anticancer Activity.—Palladium complexes are considerably more active against carcinoma than are nickel complexes.[16] It is very important to establish the form in which such complexes exist in aqueous solution. The Figure shows a plot of the species present at various −log h and it is iconoclastic to find that the only complex species present at physiological pHs is $Pd(asn)ClOH^-$—a

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[16] R. D. Graham, D. C. Tipney, and D. R. Williams, unpublished information.

quaternary complex. How does such a complex fit existing theories of anticancer activity?

Howle, Gale, *et al.*[4,17,18] have found that the carcinostatic activity of *cis*-$Pt(NH_3)_2Cl_2$ is time dependent and they have suggested that the complex dissociates to form an anticancer agent *via* an intermediate stage such as $Pt(NH_3)_2Cl_2 \longrightarrow Pt(NH_3)_2Cl^+ \longrightarrow Pt(NH_3)_2^{2+}$. (The reactant being devoid of activity, the intermediate complex preventing DNA, RNA, and protein synthesis, and the final product being specific against DNA synthesis.) Our equivalent scheme would be $Pd(asn)Cl_2^- \longrightarrow Pd(asn)Cl(OH)^- \longrightarrow$?. By analogy, this suggests that Howle, Gale, *et al.*'s intermediate may actually be $Pt(NH_3)_2Cl(OH)$. Exchange of one of these monodentate anions for a DNA donor group could produce the primary effect observed (a general non-selective inhibition) and then when the second anion is exchanged the secondary effect is observed, a more stable bifunctional inhibition.[4,17,18]

We thank Dr. Peter Gans for giving us a copy of MINIQUAD prior to publication.

[3/2185 *Received, 24th October,* 1973]

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[17] J. A. Howle, H. S. Thompson, A. E. Stone, and G. R. Gale, *Proc. Soc. Exp. Biol. Med.*, 1971, **135**, 820.

[18] J. A. Howle and G. R. Gale, *Biochem. Pharmacol.*, 1970, **19**, 2757.