Inorganica Chimica Acta, 56 (1981) 135–141

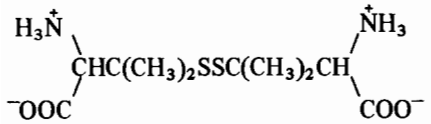
Penicillamine Disulphide Complexation of Copper(II) and Its Biological Relevance

STUART H. LAURIE*, EDWIN S. MOHAMMED and DIANA M. PRIME
School of Chemistry, Leicester Polytechnic, P.O. Box 143, Leicester LE1 9BH, U.K.
Received July 17, 1981

An emf potentiometric study of the complex formation between D-penicillamine disulphide (Pds) and Cu(II) ions (in 0.15 mol dm^{-3} aqueous NaCl, 25 °C) detected the species $Cu_2(Pds)_2$, $Cu_2(HPds)(Pds)^+$, $Cu(HPds)_2$, and $Cu(Pds)_2^{2-}$ with log stability constants 26.87, 31.10, 30.25, and 14.31 respectively. An alternative set, comprising monomeric species only, provided as good a mathematical fit to the potentiometry results but was incompatible with the pH dependence of the uv/vis absorption spectrum and so was rejected. Dilution effects upon the uv/vis and circular dichroism spectra showed the absence of any monomer ⇌ dimer equilibrium. $Cu_2(Pds)_2$, the major species at neutral pH is shown to be structurally identical to that previously found in the solid state. From a consideration of concentrations and stabilities it is concluded that Pds, the oxidised metabolite of the drug D-penicillamine, is unlikely to have therapeutic value as a chelating agent in the treatments of Wilson's disease and rheumatoid arthritis.

Introduction

D-penicillamine is used in the treatment of Wilson's disease, rheumatoid arthritis and other human diseases. In the first case it mobilises the excess copper that has accumulated in these patients [1, 2], a link with copper involvement has also been invoked in the treatment of rheumatoid arthritis. As with other thiols D-penicillamine is readily oxidised to its disulphide form, penicillamine disulphide (Pds)



which is one of the major metabolites of D-penicillamine [3]. Pds is potentially a tetradentate ligand and the suggestion has been made that a Pds chelate of Cu(II) could be of importance in penicillamine therapy [4, 5]. There has been one previous report [4] on the complexation between Pds and Cu(II) in aqueous media, however, no details or species distribution were provided. An X-ray diffraction study of a single crystal isolated from a neutral aqueous alcohol solution showed a dimeric structure of composition $[Cu_2(Pds)_2(H_2O)_2]\cdot 7H_2O$ to be present [6].

We now report a detailed potentiometric and spectroscopic study of the complexation between Pds and Cu(II) ions in 0.15 mol dm^{-3} aqueous NaCl at 25.0 °C. This was used to determine the likely extent of Pds complexation of Cu(II) in human blood plasma. This study also reveals the danger in assuming the non-ambiguity of a computer species fit to potentiometric titration data and highlights the advantage of the combined potentiometric-spectroscopic approach of Sarkar and Kruck [7].

Experimental

D-penicillamine disulphide (Aldrich) stored over P_4O_{10} gave satisfactory elemental analyses and optical rotation and was used without further purification. TLC and NMR showed the complete absence of any penicillamine impurity. All other chemicals were of 'AnalaR' or similar grade and were used without further purification.

Potentiometric titrations were carried out semi-automatically using Radiometer's pHM 64 meter, ABU 12 autoburette and G2040C glass and K4040 calomel electrodes. The stock HCl solution, standardised against potassium hydrogen phthalate, was used to periodically check the 1.0 mol dm^{-3} NaOH titrant solution, both solutions were stored under an Ar atmosphere. The stock Cu(II) solution was prepared from 'AnalaR' $CuCl_2\cdot 2H_2O$ and adjusted to pH 3–4 with HCl. The Cu(II) concentration was obtained by the standard EDTA method. The titration solution (starting volume 50 cm^3) was mechanically stirred and continuously swept with Ar.

*Author to whom correspondence should be addressed.

Temperature was maintained at 25.0 ± 0.05 °C. All volumetric glassware was grade 'A' standard. The procedure adopted was that of Sarkar and Kruck's [7] and as used by us previously [8, 9], with the exception that we now prefer to record emf rather than pH as before. The apparatus was calibrated by measuring emf changes of a HCl *v.* NaOH titration over the pH range 2–11 before and after each titration of the Cu/H^+/Pds system. From the calibration curves the standard potential E^θ was obtained, the Nernst slopes obtained from these curves were within 0.02 mV of the theoretical value of 59.16 mV at 25 °C. Titration curves of the Cu–Pds system were repeated until an overall consistency of ±0.01 cm^3 of added titrant was obtained. The concentrations of these solutions are given in Table I. Computation was via the programs [9] PLOT3, GUESS3 and LEASK3 which are modified versions of the original Sarkar and Kruck programs [7] written for our Burroughs B6700 computer.

Uv/vis absorption spectra were recorded with a Perkin Elmer 555 spectrophotometer, the cell block being thermostatted at 25.0 ± 0.1 °C. Circular dichroism spectra were measured on a Cary 61 instrument at room temperature. ESR spectra were recorded at 77 K with a Varian E-3 spectrophotometer calibrated against 2,2-diphenylpicrylhydrazyl.

TABLE I. Reagent Concentrations in the emf Potentiometric Titrations (in 0.15 mol dm^{-3} NaCl, 25.0 °C).

Set	Titration number	Concentrations/10^{-3} mol dm^{-3}		
		Pds	HCl	$CuCl_2$
A	1	0.50	7.80	–
	2	1.00	9.00	–
	3	2.00	10.80	–
B	4	0.45	–	–
	5	0.90	–	–
	6	1.35	–	–
	7	1.57	–	–
C	8	5.04	19.80	0.796
	9	5.04	19.80	0.995
	10	5.04	19.80	1.194
	11	5.04	19.80	1.592
D	12	2.97	15.80	0.995
	13	3.86	17.60	0.995
	14	5.04	19.80	0.995
	15	5.94	21.80	0.995

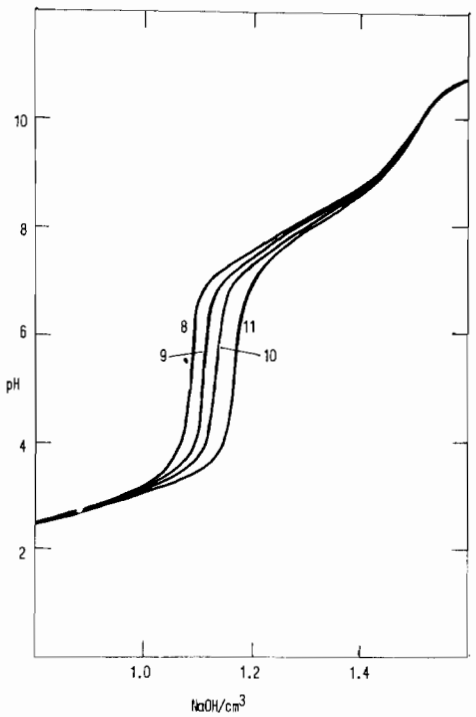


Fig. 1. Titration curves of set C, titration curves 8–11, for details, see Table I.

Results

Proton-penicillamine Disulphide Potentiometry

The proton ionization of Pds consists of two well separated pairs of overlapping ionizations from the two carboxyl and the two amine groups. In order to avoid errors from excessive dilution two sets of titrations were made, set A (see Table I) for the pH* 1–3 region and set B for the pH 6–11 region. In both cases the overlapping ionization constants were resolved as before [8, 9] using program PLOT 3 to obtain the average number of bound protons, $\bar{n}_H$, from the titration curves at each selected pH value, and then solving the equation:

$$\bar{n}_H = \beta_{011}(1 - \bar{n}_H)[H^+] + \beta_{021}(2 - \bar{n}_H)[H^+]^2 \quad (1)$$

The general expression for equilibrium in these systems being given by:

$$pM + qH + rA \rightleftharpoons M_pH_qA_r \quad (2)$$

$$\beta_{pqr} = [M_pH_qA_r]/[M]^p[H]^q[A]^r \quad (3)$$

β_{oqr} therefore refers to the proton ionization equilibria. By using 0.1 pH incremental steps simultaneous sets of eqn. (1) were solved for β_{011} and β_{021} by using LEASK3. The values obtained are given in Table II.

*In this work pH = log[H^+], where [H^+] is the hydrogen ion *concentration* calculated from the measured emf and the Nernst equation.

TABLE II. Log Stability Constants (β_{pqr}) of species ($M_pH_qA_r$) in Cu(II)–H^+–Pds Solutions Containing 0.15 mol dm^{-3} NaCl, and at 25.0 °C.

Species p q r	Log β_{pqr}	
	This work	Literature[a]
0 1 1	8.72(±0.01)[b]	8.94
0 2 1	16.49	16.78
0 3 1	18.47	18.88
0 4 1	19.48	20.70
1 0 1	NF	7.51
1 1 1	NF	16.13
1 0 2	14.31(±0.02)[b]	NF
1 2 2	30.25	(28.0)[c]
2 0 2	26.87	28.50
2 1 2	31.10	NF

[a]From reference 4, data at 37 °C recalculated to 25 °C. [b]Estimated errors. [c]Estimated value from reference 11. NF = not found.

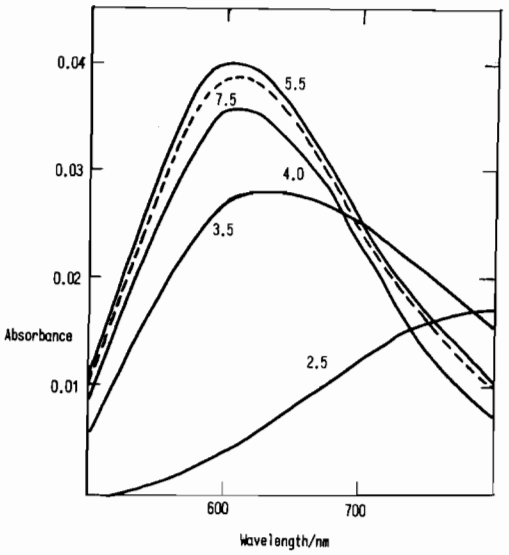


Fig. 3. pH dependence of the d–d absorption spectrum at 25 °C, Cu(II), 1 × 10^{-3} mol dm^{-3}; Pds, 5 × 10^{-3} mol dm^{-3}; NaCl, 0.15 mol dm^{-3}; 25 °C.

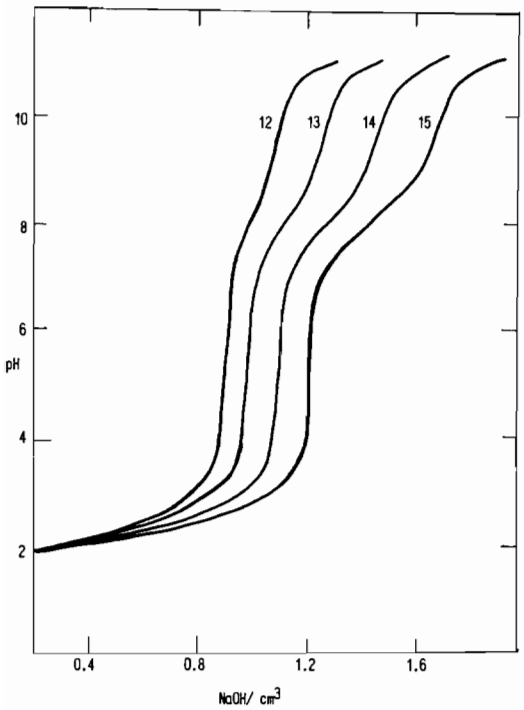


Fig. 2. Titration curves of set D, titration curves 12–15, for details see Table I.

Copper(II)–proton-penicillamine Disulphide Potentiometry

Two sets of titrations are required in the Sarkar-Kruck method, these are the metal variation set (Set C, Table I) and the ligand variation set (set D). The titration curves are shown in Figs. 1 and 2. From these were calculated, via PLOT 3, the free metal and free ligand concentrations for reactant concentrations as used in the 'common titration curve' (titrations 9 and 14) in the two sets (Cu:Pds ~ 1:5). The guessed species input to program GUESS 3 covered the range of coefficients p = 1, 2; q = 3, 2, 1, 0, −1, −2; r = 1, 2. GUESS 3 calculated the concentration term $[M]^p[H]^q[A]^r$ for each species at 0.1 pH steps over the pH range 4–9. These terms and guessed β values for each of the species were then fed into the LEASK 3 programs (two, using different minimisation routines [9]). The β values were 'refined' by LEASK 3 to produce a minimum in the sum of the squares of the differences between the calculated and experimental bound metal concentrations, calculated at each 0.1 pH interval.

Many of the proposed species were rejected in the first runs, finally two equally viable model fits were obtained whose overall residuals were very close. Model I comprised the species M_2A_2, M_2HA_2, MH_2A_2 and MA_2. Model II comprised the species MA, MA_2, MHA and MH_2A_2. No viable model could be found in which both the monomer MA and dimer M_2A_2 co-existed.

Copper(II)–proton-penicillamine Disulphide Spectroscopy

Sarkar and Kruck [7] advocated that the mathematical speciation model obtained from the computer treatment of the titration data should be verified for its chemical reality by examining the pH dependence of the uv/vis absorption spectrum of the same system and obtaining the spectra of the individual species. The only assumption that need be made is

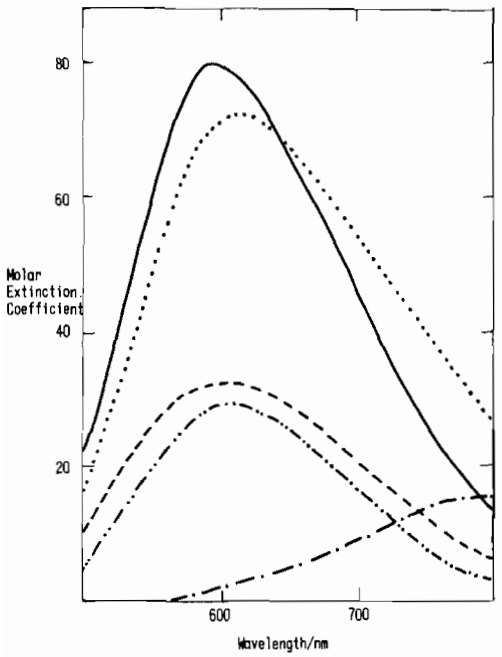


Fig. 4. d–d absorption spectra of the Cu(II)/H$^+$/Pds species. M_2A_2 (——), M_2HA_2 (·····), MA_2 (·–·–·), MH_2A_2 (– – –); and M(– · –); NaCl 0.15 mol dm^{-3}, 25 °C.

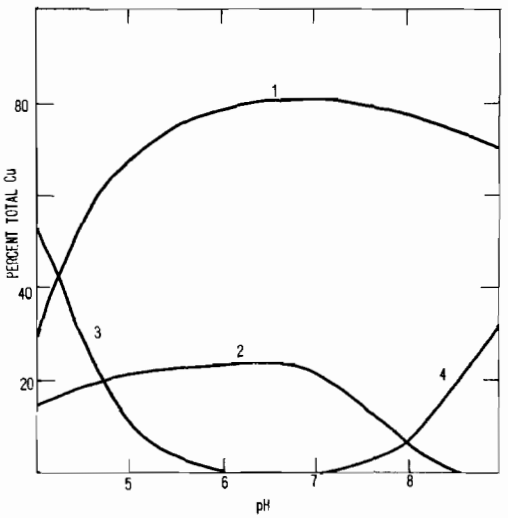


Fig. 5. Species distribution as a function of pH for the Cu(II)–Pds solution of Cu, 0.995 × 10^{-3} mol dm^{-3}; Pds, 5.04 × 10^{-3} mol dm^{-3}; NaCl, 0.15 mol dm^{-3}; 25 °C. *1*, M_2A_2; *2*, MH_2A_2; *3*, M_2HA_2; *4*, MA_2.

that the Beer-Lambert law is obeyed *i.e.* at any wavelength λ and for a solution of pH_j, the measured absorbance (per unit path length)A_j^λ is given by:

$$A_j^\lambda = \sum_i \epsilon_{\lambda,i}[M_pH_qA_r]_i \quad (4)$$

where, $\epsilon_{\lambda,i}$ is the molar extinction coefficient of the ith species at wavelength λ. The concentrations of the species at each pH value were obtained from the β_{pqr} values obtained from the potentiometric analysis.

The pH dependence of the spectrum of a solution of the same concentrations as that of the common titration curve is shown in Fig. 3. Only small shifts occurred in the spectrum above pH 5.5. Fitting of the computer model speciation to the spectra over the pH range 4–9 showed that model I gave a reasonable fit with acceptable spectra of the individual species (Fig. 4). However, with model II

TABLE III. Computer Evaluation of Species Concentrations in the System Cu(II) (1 × 10^{-6} mol dm^{-3}), His (85 × 10^{-6} mol dm^{-3}), Pds (Z mol dm^{-3}), NaCl (0.15 mol dm^{-3}), 25 °C.

Species	Logβ		Percent species at Pds concentrations Z/10^{-5} mol dm^{-3}				
		Z =	1.0	3.0	5.0	7.0	10.0
HHis	9.11[a]		91.6	91.6	91.6	91.7	91.7e
H_2His^+	15.17[a]		4.2	4.2	4.2	4.2	4.2e
$HPds^-$	8.72[b]		29.3	29.4	29.4	29.2	29.3f
H_2Pds	16.49[b]		69.2	69.2	69.2	69.1	69.2f
$Cu(His)_2$	18.45[a]		99.2	97.6	96.0	94.5	92.3g
$Cu_2(Pds)_2$	26.87[b]		*In*	*In*	*In*	*In*	*In* g
$Cu(Pds)_2(H^+)_2$	30.25[b]		*In*	*In*	*In*	*In*	*In* g
$Cu(His)(Pds)^-$	16.7 [c]	(17.59[d])	*In*	0.5	0.8	1.1	1.5g
$Cu_2(His)_2(Pds)$	32.3 [c]		*In*	*In*	*In*	*In*	*In* g
$Cu(His)(Pds)(H^+)$	24.7 [c]	(25.35[d])	0.7	1.9	3.1	4.3	6.1g

[a]From reference 14. [b]This work. [c]Estimated values (see text). [d]Estimated values (at 37 °C) from reference 4. [e]Based on total histidine. [f]Based on total Pds. [g]Based on total Cu. *In* = insignificant.

a poor fit resulted with totally unrealistic spectra for the species MA_2 and MH_2A_2. Model I therefore is the more chemically viable; the species distribution from this model, calculated from the stability constants given in Table II, is given in Fig. 5.

A plot of the absorbance at 600 nm against Cu concentration was found to be linear over the range 10^{-1} to 10^{-5} mol dm^{-3} and extrapolated to the origin. The remaining conditions of the solutions used were identical, *i.e.* Pds/Cu ratio 5/1, pH 7.0, 0.15 mol dm^{-3} NaCl. Likewise the circular dichroism spectra (broad positive Cotton effect at 600 nm, negative above 700 nm) of the same solutions showed only dilution effects.

The esr spectrum of the 10^{-1} mol dm^{-3} solution (77 K) was consistent with the presence of a single major Cu(II) species with no magnetic exchange interactions. The signal gave parameters of A_{11} 0.0181 cm^{-1} and g_{11} 2.26.

The Ternary Cu–histidine–Pds System

The ability of Pds to compete with histidine (His) for Cu(II) present in blood plasma was examined by computer simulation using the program ECCLES*.

The concentrations used in evaluating the species (see Table III) were those that approximate to blood plasma conditions. The Pds concentration range was evaluated from information on penicillamine levels [12, 13]. Assuming that all the penicillamine is oxidised to its disulphide form would then give a likely range from zero to a maximum of *ca.* 1×10^{-4} mol dm^{-3} for patients receiving a daily dosage of 1.0–1.5 g. At pH 7.4 the major binary species are $Cu(His)_2$ [14], $Cu_2(Pds)_2$ and $Cu(Pds)_2(H^+)_2$ [this work]; these were the only binary species considered and likely ternary Cu–His–Pds complexes were considered to be formed from these and the ligands present in excess amounts (see Table III). The stability constants for the ternary species were estimated from the corresponding binary species with allowance for a favourable statistical factor. Thus, for the complex $Cu(His)(Pds)(H^+)$ we may consider the equilibrium:

$$Cu(Pds)_2(H^+)_2 + Cu(His)_2 \rightleftharpoons 2Cu(His)(Pds)(H^+) \quad (5)$$

If,

$$\beta\{\text{ternary}\} = \frac{[Cu(His)(Pds)(H^+)]}{[Cu][His][Pds][H^+]} \quad (6)$$

*Program ECCLES (evaluation of constituent concentrations in large equilibrium systems), developed at the University of Wales Institute of Science and Technology, was kindly supplied by Dr. P. M. May. For further details see references 10 and 11.

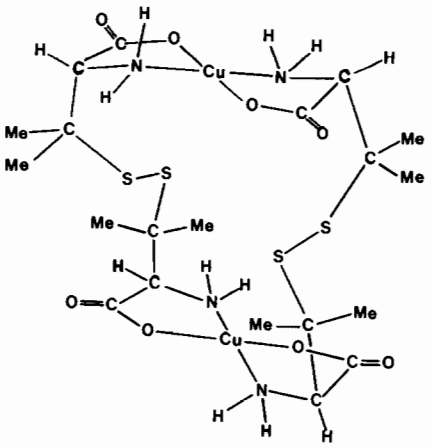


Fig. 6. Structure of the dimer $Cu_2(Pds)_2$ observed in the solid state, from reference 6.

and

$$K_D = \frac{[Cu(His)(Pds)(H^+)]^2}{[Cu(Pds)_2(H^+)_2][Cu(His)_2]} \quad (7)$$

then,

$$\log\beta\{\text{ternary}\} = 0.5\,[\log\beta\{Cu(Pds)_2(H^+)_2\} + \log\beta\{Cu(His)_2\}] + 0.5 \log K_D \quad (8)$$

Assuming no special stabilisation or destabilisation factors influence the ternary species formation then K_D is simply a statistical factor of 4.0. It can be seen that the major contributing factors to the stability of the ternary species are the stabilities of the parent binary complexes.

The results in Table III clearly show that Pds is very ineffective in competing against histidine under the conditions specified. This finding was re-inforced by the observation that the addition of Pds to our plasma model solution [2, 15] (L-alanine/histidine/Zn(II)/Cu(II) ratio 3000/85/16/1, Cu(II) = 1×10^{-4} mol dm^{-3}, Pds = 50×10^{-4} mol dm^{-3}, 37 °C) had no effect on the uv/vis absorption spectrum. Variation of the available Cu level from 10^{-9} to 10^{-5} mol dm^{-3} in the computer simulation had a negligible effect on the relative species concentrations.

Discussion

Over the concentration ranges examined by both emf potentiometry and uv/vis spectroscopy the main species in neutral aqueous 0.15 mol dm^{-3} NaCl solution is the dimer $Cu_2(Pds)_2$. The absorption maxi-

mum at 600 nm and the esr parameters [16, 17] are consistent with the two Cu(II) centres having a CuN_2O_2 chromophore environment. Its structure is therefore likely to be that observed for the dimer in the solid state [6], as depicted in Fig. 6. The stability constant of $Cu_2(Pds)_2$ and of the other copper complexed and protonated forms of Pds are in good agreement with those reported by Perrin and Agarwal [4]. The latter workers, however, also reported the presence of the monomer CuPds with a log stability constant 7.22 (at 37 °C). In actual fact, examination of the species distribution using these authors data shows the monomeric species to be insignificant. This is also apparent from consideration of the dimerisation equilibrium:

$$2\ CuPds \overset{K_2}{\rightleftharpoons} Cu_2(Pds)_2 \qquad (9)$$

Hence $K_2 = \beta(\text{dimer})/\beta^2(\text{monomer}) \cong 10^{13}$. The absence of this monomer was also confirmed from the uv/vis and circular dichroism spectral changes with successive dilutions.

The species MA_2 and MH_2A_2 from the computed titration results are both seen to have absorption maxima at 600 nm (Fig. 4) consistent with their having CuN_2O_2 chromophores. MH_2A_2 may therefore be written as $Cu(HPds)_2$ in which protonation of the ligands occurs at the non-coordinated amine groups remote from the Cu(II) centre.

The formation of MA_2 species, *i.e.* $Cu(Pds)_2^{2-}$ at higher pH is to be expected on purely statistical grounds as the amount of fully deprotonated ligand increases in this region. M_2HA_2 or $Cu_2(Pds)(HPds)^+$ requires protonation at one of the amine groups and hence contains both CuN_2O_2 and $CuNO_3$ chromophores. Consistent with this is the observed shift in absorption maximum from 600 nm for the CuN_2O_2 chromophore species to 620 nm for the M_2HA_2 species.

Having established the mode and strength of the Cu(II)–Pds complexation the question then arises as to the significance of this complexation during penicillamine therapy. Our results clearly show (Table III) that Pds is ineffective in competing against histidine for the available Cu (assumed to be Cu(II)) in the blood plasma low molecular weight fraction. The only species found to be of any significance were the ternary $Cu(His)(Pds)^-$ and $Cu(His)(Pds)(H^+)$ complexes, the latter probably being Cu(His)(HPds). The same two species according to Perrin and Agarwal's model [4] would, contrary to our results, account for some 60% of the low molecular weight Cu fraction. Micheloni *et al.* [11] came to similar conclusions as Perrin and Agarwal using their [4] stability constants. These authors [11] found only *ca.* 23% of the Cu to be in the $Cu(His)_2$ form, using similar His and Pds concentrations. These directly contradictory results can be related to the different stability constant data set used. However, since no special factors were assumed for formation of the ternary species it is surprising that these authors should find so little $Cu(His)_2$ in the ternary Cu–His–Pds system. Considering, for example the equilibrium:

$$2\ Cu(His)_2 + 2Pds \overset{K_3}{\rightleftharpoons} Cu_2(Pds)_2 + 4His \qquad (10)$$

then the formation constant

$$K_3 = \beta_{Cu_2(Pds)_2}/\beta^2_{Cu(His)_2}$$

Hence, $K = 10^{-10}$ (our data) or $10^{-7.6}$ (Perrin and Agarwal's data), *i.e.* the equilibrium lies strongly to the left. In support of this conclusion and our computational results we found that the addition of Pds to our plasma model solution (using L-alanine to represent the other amino acids) had no effect on the Cu speciation, whereas at least 70% of the Cu would be expected to be in ternary species form under the conditions used if the previous calculations [4, 11] were correct. In this case a substantial spectral change would have been anticipated.

We therefore conclude that even if all the administered penicillamine was oxidised to the disulphide, this latter form has no influence on the Cu mobilisation in Wilson's disease; a result also reached by Micheloni *et al.* [11] using their plasma mobilising index criterion. This also means that a claim [5] for the involvement of a Cu(II)–Pds complex in the anti-inflammatory action of D-penicillamine should be discounted.

Acknowledgements

Special appreciation is made to Dr. B. Raynor for esr measurements, to Dr. M. Scopes for the circular dichroism spectra, and to the MRC for a grant to purchase the uv/vis spectrophotometer. ESM and DMP were recipients of Leicestershire LEA awards.

References

1 J. M. Walshe, *Am. J. Med., 21,* 487 (1956).
2 S. H. Laurie and D. M. Prime, *J. Inorg. Biochem., 11,* 229 (1979).
3 D. Perrett, *Proc. Roy. Soc. Med., 70, Suppl. 3,* 61 (1977).
4 D. D. Perrin and R. P. Agarwal, in 'Metal Ions in Biological Systems', Editor H. Sigel, Marcel Dekker, New York, vol. 2, 168 (1973).

5 D. A. Gerber, *Biochem. Pharmacol., 27,* 469 (1978).
6 J. A. Thich, D. Mastropaolo, J. Potenza and H. J. Schugar, *J. Am. Chem. Soc., 96,* 726 (1974).
7 B. Sarkar and T. P. A. Kruck, *Canad. J. Chem., 51,* 3541 (1973).
8 S. H. Laurie and B. Sarkar, *J. Chem. Soc. Dalton,* 1822 (1977).
9 S. H. Laurie, D. M. Prime and B. Sarkar, *Canad. J. Chem., 57,* 1411 (1979).
10 P. M. May, P. W. Linder and D. R. Williams, *J. Chem. Soc. Dalton,* 588 (1977).
11 M. Micheloni, P. M. May and D. R. Williams, *J. Inorg. Nucl. Chem., 40,* 1209 (1978).
12 K. Gibbs and J. M. Walshe, *Quart. J. Med., 40,* 275 (1971).
13 A. Sass-Kortsak, private communication.
14 T. P. A. Kruck and B. Sarkar, *Canad. J. Chem., 51,* 3549 (1973).
15 S. H. Laurie and E. S. Mohammed, *Inorg. Chim. Acta, 55,* L63 (1981).
16 S. H. Laurie, T. Lund and B. Raynor, *J. Chem. Soc. Dalton,* 1389 (1975).
17 J. Peisach and W. E. Blumberg, *Mol. Pharmacol., 5,* 200 (1969).