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A potentiometric and spectroscopic study of copper(II) diaminodioxime complexes*

Graham E. Jackson[a] **and Bandile S. Nakani**[b]

[a] *Department of Chemistry, University of Cape Town, Rondebosch 7700, South Africa*
[b] *Department of Chemistry, University of Transkei, Private Bag X1, Unitra, Umtata, 5100, South Africa*

Copper(II) and H^+ complexes of 3,3,8,8-tetramethyl-4,7-diazadecane-2,9-dione dioxime, 3,3,9,9-tetramethyl-4,8-diazaundecane-2,10-dione dioxime and 3,3,10,10-tetramethyl-4,9-diazadodecane-2,11-dione dioxime have been studied at 25 °C and ionic strength 0.15 mol dm^{-3} NaCl, using glass-electrode potentiometry. Electronic spectra have been used to postulate structures for the different species in solution. In each case the metal binds to the four nitrogen atoms of the oxime. In the MLH_{-1} species hydrogen bonding between the terminal oxime groups produces a pseudo-macrocycle. Computer simulation has been used to predict the effect of the ligands on the blood-plasma metal-ion distribution *in vivo*.

Rheumatoid arthritis is a debilitating disease for which there is no cure. Sorenson [2] and Jackson *et al.*,[3] however, have shown that copper complexes can be used to alleviate the inflammation associated with the disease. In a previous study [4] into the design of copper(II)-based anti-inflammatory drugs we synthesised 3,6,9,12-tetraazatetradecanedioic acid (H_2ttda) and 3,6,9-triazaundecanedioic acid (H_2tuda). Potentiometric and computer-simulation results for these two compounds showed that, thermodynamically, they formed stable copper(II) complexes *in vivo*. Animal studies, however, showed that the complexes were not biologically active as they were rapidly excreted in the urine.[5] In an attempt to change the tissue distribution of the copper(II) complexes the compounds 3,3,8,8-tetramethyl-4,7-diazadecane-2,9-dione dioxime (L^1), 3,3,9,9-tetramethyl-4,8-diazaundecane-2,10-dione dioxime (L^2) and 3,3,10,10-tetramethyl-4,9-diazadodecane-2,11-dione dioxime (L^3) have been synthesised and their copper(II) complexes studied. Complexes of this type have been studied before with regard to their solubility and use as analytical reagents particularly for nickel(II).[6] In addition, we have shown that there is substantial liver uptake (31.0% dose) of technetium-99m complexes of bis(oximes).[7] However, with the exception of L^1, no work has been reported on the solution thermodynamics of copper(II) complexes of these three compounds.

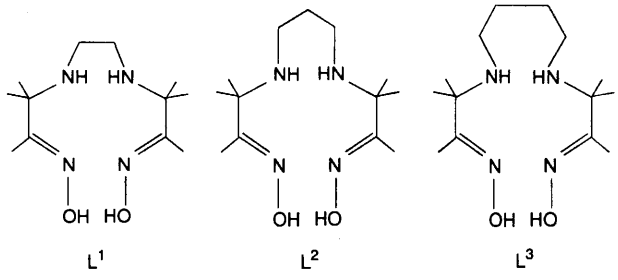
Theoretical

In any solution containing metal ions, M, ligands, L and protons, H, a general equilibrium reaction can take place as described by equation (1), where p, q and r are the

$$pM + qL + rH \rightleftharpoons M_pL_qH_r \qquad (1)$$

stoichiometries of the components in the complex, with $r = -1$ referring to proton removal or hydroxide-ion addition. The equilibrium constant for this reaction is then designated by the symbol β_{pqr}. For convenience the species $M_pL_qH_r$ are denoted by the three stoichiometric coefficients given in the order M, L and H, *e.g.* 21–2 denotes the species M_2LH_{-2}.

For the electronic absorption spectra of solutions containing more than one absorbing species the Beer–Lambert law can be expanded to give a linear combination of terms for each individual species, where c_i is the concentration and ε_i the molar



absorption coefficient of the ith species at the wavelength λ [equation (2)]. If the path length is given in cm, ε_i^{λ} is the molar

$$A^{\lambda} = l(\varepsilon_1^{\lambda}c_1 + \varepsilon_2^{\lambda}c_2 + \ldots + \varepsilon_i^{\lambda}c_i) = l\Sigma\varepsilon_i^{\lambda}c_i \qquad (2)$$

absorption coefficient of the ith species in solution at a wavelength of λ and has the units $dm^3\ mol^{-1}\ cm^{-1}$.

Results and Discussion

Potentiometry

Of the present compounds, only L^1 has been studied potentiometrically before.[6] At an ionic strength of 0.27 mol dm^{-3}, a temperature of 24.2 °C and in the presence of $Ba(NO_3)_2$ the protonation results shown in Table 1 were reported. Considering the difference in experimental conditions, there is very good agreement between these literature results and our results.

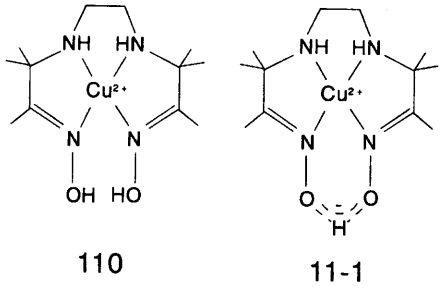
The protonation and complexation results of the three ligands are presented in Table 1. The results show that as the length of the alkyl chain between the two amino groups is increased, so log β_{011} increases from 8.36 (L^1) to 9.29 (L^3). This is expected because of the inductive effect of the alkyl substituents (*viz.* 1,2-diaminoethane, log β_{011} = 9.9; 1,5-diaminopentane, log β_{011} = 10.5).[8] In addition the difference between K_1 (β_{011}) and K_2 (β_{012}/β_{011}) decreases as the alkyl chain length increases (2.75, 1.93, 1.43 log units) indicating that the second protonation is less affected by the first. Similarly, in going from an ethyl to a propyl [9] chain between the amino and oxime groups log β_{011} increases from 8.36 to 9.47.

Copper(II) complexation of the compounds resulted in some interesting changes in colour, from violet in acid medium, to blue-green above pH 7.0. These changes have been used by Murmann [6] to estimate equilibrium constants for the Cu^{2+}–

* Copper Anti-inflammatory Drugs in Rheumatoid Arthritis. Part 4.[1]

L^1–H^+ system. These results, together with our potentiometric results, are given in Table 1. The potentiometric results show the order of stability of the 110 species to be $L^2 > L^1 > L^3$. The stability of this species can be compared with that of the copper(II) complexes of 1,2-diaminoethane (log $\beta_{110} = 10.5$) and 1,3-diaminopropane (log $\beta_{110} = 9.7$).[8] Here, despite the higher protonation constant (*i.e.* increased basicity), 1,3-diaminopropane forms a six-membered chelate ring with Cu^{II} which is less stable than the five-membered ring formed with 1,2-diaminoethane. On the other hand, for the tetraamines, 3,6-diazaoctane-1,8-diamine forms less stable complexes (log $\beta_{110} = 20.1$) than does 3,7-diazanonane-1,9-diamine (log $\beta_{110} = 23.2$)[10] with Cu^{II}. Molecular mechanics calculations[5] have shown that it is the strain introduced into the complex by having three contiguous five-membered chelate rings which destabilises the former complex. A similar situation is likely to exist with CuL^1 and CuL^2, but only if all four nitrogens are co-ordinated. On the other hand, co-ordination by only the two amino groups should, by analogy to 1,2-diaminoethane and 1,3-diaminopropane, give an order of stability of $CuL^1 > CuL^2$. The observed stabilities $\beta_{110}(CuL^1) < \beta_{110}(CuL^2)$ therefore support co-ordination by all four nitrogen atoms. As expected CuL^3 is the least stable of all the complexes. Murmann[6] also proposes four-nitrogen co-ordination for L^1 and the same mode is seen in the crystal structure of bis(μ-4,4,9,9-tetramethyl-5,8-diazadodecane-2,11-dione dioximato)dicopper(II) bromide.[11]

The most likely structure for the 11–1 species involves loss of one of the oxime protons, with the remaining oxime proton being hydrogen bonded between the two terminal groups (Scheme 1). In this way a pseudo-macrocycle is formed which gives the complex added stability. The pK_a values (log β_{110} − log β_{11-1}) of the CuL species are 4.49, 2.63 and 2.74 for L^1, L^2 and L^3 respectively. The complexes are all much more acidic than the free metal ion ($pK_a = 8.0$)[8] indicating that the proton is indeed lost from one of the oxime groups and not from a co-ordinated water molecule. However, the pK_a is also very much less than that of an oxime indicating that metal-ion co-ordination has facilitated the loss of the proton, presumably through hydrogen bonding.



Scheme 1 Proposed structures for the ML and MLH_{-1} complexes of L^1

The loss of the second proton from the complex, *i.e.* formation of species 11–2, is typical of hydrolysis of axially co-ordinated water, occurring with pK_a 10.28, 11.19 and 11.62 for CuL^1, CuL^2 and CuL^3 respectively. Similar hydrolysis is postulated to occur with the complexes of Fe^{2+}, Co^{2+} and Zn^{2+} with 4,4,9,9-tetramethyl-5,8-diazadodecane-2,11-dione dioxime (H_2tmdddo).[9]

Spectroscopy

The Cu–L^1 system shows interesting colour changes from pale violet in an acidic medium to blue green in basic solution. This is indicative of changes in the absorption maxima of the dominant species in solution at a particular pH. The shift is from red through violet to blue-green. Fig. 1 shows how the speciation and hence colour of the solution changes with pH.

Analysis of the spectroscopic titration data yielded spectra (Fig. 2) for the individual metal species. The smoothness of the deconvoluted spectra lends confidence to the results as we have found in the past that the use of an incorrect potentiometric model in the analysis of the data gives rise to disjointed electronic spectra.[5] The spectra of the individual complex species can be used to infer the co-ordination geometry of the metal ion. Each metal species shows one absorption band corresponding to the three spin-allowed transitions $^2A_{1g} \longleftarrow {}^2B_{1g}$, $^2B_{2g} \longleftarrow {}^2B_{1g}$ and $^2E_g \longleftarrow {}^2B_{1g}$, characteristic of a d^9 tetragonally distorted copper(II) complex.[10]

Billo[12] has proposed a method for calculating λ_{max} for a square-planar copper complex (with axially co-ordinated water molecules). This is given in equation (3), where ν_i (10^3 cm^{-1}) is

$$\nu_{calc} = \Sigma\nu_i \qquad (3)$$

the contribution each of the four co-ordinating groups makes to the calculated absorption frequency. For an amino group $\nu_N = (4.53 \pm 0.07) \times 10^3$ cm^{-1}, while for H_2O and OH^-, $\nu_O = (3.01 \pm 0.03) \times 10^3$ cm^{-1}. Billo does not report a value for ν_N(oxime H) but from the visible spectrum of $[Cu(H_2dmg)Cl_2]$ (H_2dmg = dimethylglyoxime) ($\lambda_{max} = 770$ nm)[13] and assuming $\nu_{Cl} = \nu_O$, a value of 3.48×10^3 cm^{-1} can be estimated. This is for an oxime without hydrogen bonding. Using this figure a λ_{max} of 624 nm is calculated for CuN_2(amine)N_2(oxime), the structure (Scheme 1) proposed for the ML species from

Table 1 Logarithms of the overall stability constants, β_{pqr}, of copper(II)–L^1, –L^2 and –L^3 complexes at 25 °C $I = 150$ mmol dm^{-3} (Na)Cl^-; n is the number of experimental observations used as data in the least-squares calculations, R the Hamilton R factor. The general formula of the complexes is $M_pL_qH_r$

L	p	q	r	log β_{pqr}	Lit.[6]	n	pH range	R
L^1	0	1	1	8.36(1)	8.21	347	2.1–10.8	0.009
	0	1	2	13.97(1)	13.66			
	1	1	0	12.49(3)	13.0	193	2.2–11.0	0.009
	1	1	−1	8.00(3)	8.8			
	1	1	−2	−2.28(3)	−1.1			
L^2	0	1	1	8.97(1)		359	2.0–10.5	0.006
	0	1	2	16.01(1)				
	1	1	0	13.94(3)		176	2.0–11.0	0.004
	1	1	−1	11.31(3)				
	1	1	−2	0.12(4)				
L^3	0	1	1	9.29(1)		178	2.0–10.8	0.006
	0	1	2	17.16(1)				
	1	1	0	11.14(7)		172	3.7–11.0	0.008
	1	1	−1	8.40(1)				
	1	1	−2	−3.22(3)				

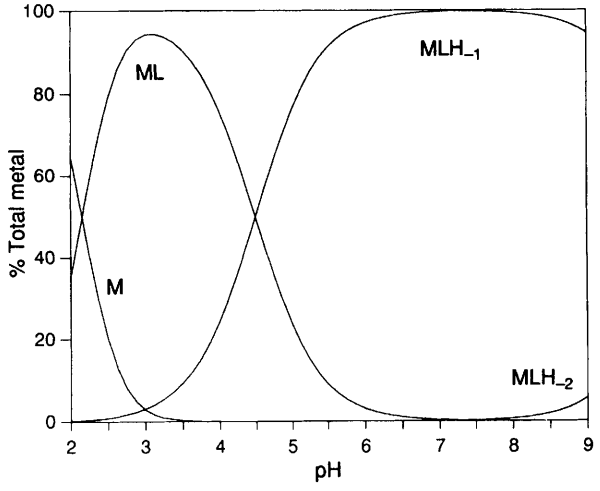


Fig. 1 Calculated speciation of a Cu^{II} (0.1 mol dm^{-3})–L^1 (0.1 mol dm^{-3}) solution as a function of pH

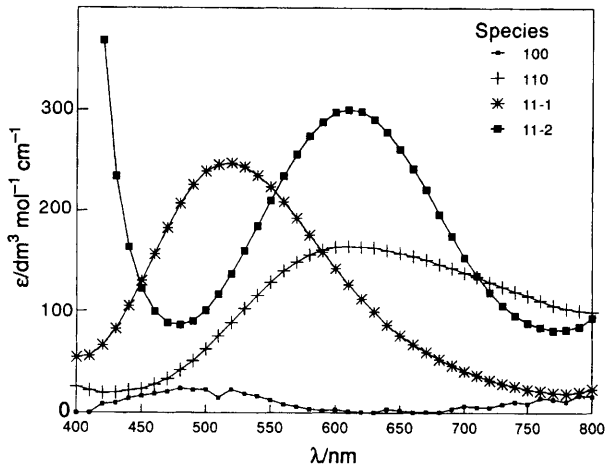


Fig. 2 Calculated absorption spectra of individual Cu–L^1 species in aqueous solution. Literature[6] results reported for these complexes are: λ_{max} (ε_{max}) 587 (183) for ML, 518 (306) for MLH_{-1} and 622 nm (314 dm^3 mol^{-1} cm^{-1}) for MLH_{-2}

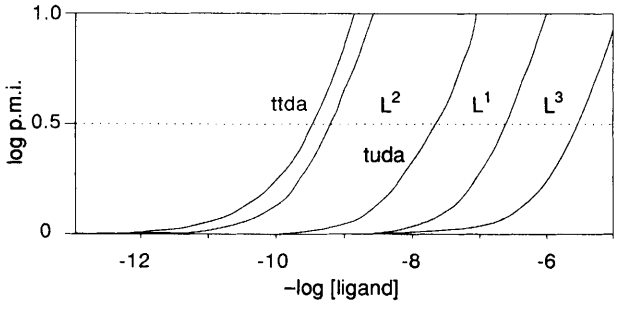


Fig. 3 Plasma mobilising index (p.m.i.) (ratio of low molecular mass metal concentration in the presence and absence of ligand) as a function of ligand concentration

potentiometric results. This calculated result is in good agreement with the λ_{max} of 625 nm observed for $[CuL^1]^{2+}$ and lends support to the proposed structure. The calculated λ_{max} for two other possible structures, CuN_2(amine)$O_2(H_2O)$ (663 nm) and CuN_2(amine)N(oxime H)O(H_2O) (643 nm), does not agree with the observed wavelength.

The complex $[CuL^1H_{-1}]^+$ has a λ_{max} of 520 nm. $[Cu(H_2dmg)_2]$ has a λ_{max} of 567 nm from which a value of 4.41×10^3 cm^{-1} can be estimated for ν_N(oxime), the shift parameter for an oxime with hydrogen bonding. Using this value a λ_{max} of 560 nm is calculated for CuN_2(amine)N_2(oxime). This is the same structure as proposed from the potentiometric results and no other gives as good agreement with the observed λ_{max}.

Finally the complex $[CuL^1H_{-2}]$ has a λ_{max} of 610 nm. From the potentiometric results we have proposed that this complex is derived from $[CuL^1H_{-1}]^+$ by hydrolysis of an axially co-ordinated water molecule. It is well known that increased axial co-ordination decreases the energy of the three spin-allowed copper(II) d–d transitions.[14] A shift of 50 nm in λ_{max} is observed[12] in going from $[Cu(en)_2]^{2+}$ to $[Cu(en)_2(OH)]^+$ (en = 1,2-diaminoethane). The observed difference in λ_{max} for $[CuL^1H_{-2}]$ and $[CuL^1H_{-1}]^+$ is 90 nm which, while larger than expected, is still consistent with hydrolysis of axially co-ordinated water.

Blood-plasma simulation

The primary objective of this research was to produce a ligand which was able to mobilise copper(II) in blood plasma. Fig. 3 shows the calculated increase in low-molar-mass copper(II) caused by L^1, L^2 and L^3. For comparison the results for ttda and tuda are also shown. These results were calculated using the constants determined in this study, together with the published blood-plasma database of May *et al.*[15,16] Previous studies have shown that one of the main metal-ion competitors *in vivo* is Zn^{2+}. As equilibrium constants for this metal ion and our oximes were not available, constants for the analogue H_2tmdddo were used instead. Since the values for H_2tmdddo are greater than those of L^1 with Cu^{2+} this is likely to be an overestimate.

The results show that, while all three oximes should mobilise copper in blood plasma, L^2 is some three orders of magnitude better than the other two. Compared to ttda and tuda, L^1 is similar to ttda in its ability to mobilise copper(II). This may seem surprising as Cu^{II} forms much more stable complexes with ttda than with L^2. The reason for this result is the high concentration of zinc(II) in blood plasma and the relative stability of the copper and zinc complexes of these two ligands.

Animal studies have shown that the complex $[Cu(ttda)H_{-2}]$ does not decompose *in vivo*,[5] and hence we predict that neither will the $[CuL^2H_{-1}]^+$ complex, which appears to be the dominant species in the range pH 5–9. Also the $[CuL^2H_{-1}]^+$ complex should have a different hydrophobicity to that of $[Cu(ttda)H_{-2}]$ and may therefore undergo slower urinary excretion. Based on these results we feel that it is justified to proceed with animal experiments and to test $[CuL^2H_{-1}]^+$ as a potential copper-based anti-inflammatory drug.

Experimental

The compounds L^1 (m.p. 180–181 °C; lit.,[6] 181–182) and L^2 (m.p. 185–186 °C; lit.,[17] 180–181) were synthesised according to the method of Vassian and Murmann[17] and characterised by NMR and elemental (C,H,N) analysis. The compound L^3 was synthesised using a similar method. To a cooled (0 °C) solution of freshly distilled 1,4-diaminobutane (0.74 g, 0.01 mol) in anhydrous methanol (20 cm^3) was added 2-chloro-2-methylbutan-3-one oxime (2.98 g, 0.022 mol). The temperature of the mixture was allowed to rise to room temperature (20 °C) over a period of 2 h and the mixture was then refluxed for 15 h. The solvent was removed under reduced pressure and the residue dissolved in water. After filtration the solution was neutralised with $NaHCO_3$, the product collected and recrystallised from methanol, m.p. 193–194 °C (Found: C, 55.1; H, 10.3; N, 18.4. $C_{14}H_{30}N_4O_2 \cdot H_2O$ requires C, 55.2; H, 10.5; N, 18.4%); $\delta_H(D_2O)$ 1.38 (12 H, s, 3,3,10,10-CH_3), 1.62 (4 H, t, 6,7-CH_2), 1.80 (6 H, s, CH_3) and 2.87 (4 H, t, 5,8-CH_2). The purity of all the oximes was checked by acid–base titration and found to be >98%. All other reagents were commercially available and of analytical grade.

Solutions were prepared in glass-distilled, deionised water which had been boiled to remove dissolved CO_2. Solutions of

Table 2 Summary of experimental and computational details

Potentiometer	Radiometer PHM84
Electrodes	Metrohm 6.0104.100 glass, 6.0726.100 Ag–AgCl reference
Calibration	Strong acid–strong base–oxime titrations: data processed by ESTA[18,19]
Burette	Computer-controlled Metrohm Dosimat 665 fitted with a non-return valve to prevent back diffusion
I/mol dm^{-3}, electrolyte	0.15, NaCl
T/°C	25
Inert atmosphere	High-purity dinitrogen passed successively through 50% w/w KOH, Fieser's solution[20] and thermostatted background electrolyte
Calculation method	ESTA[15,16] with weighing; initial volume 0.05 cm^3, emf 0.10 mV, titre volume 0.005 cm^3
UV/VIS	Philips Scientific SP1700, readings taken at 10 nm intervals in quartz cuvettes thermostatted at 25 °C
Spectral analysis	Local BASIC program which uses Gaussian elimination with partial-row pivoting to solve the extended Beer–Lambert law equation[5]
NMR spectrometer	Varian VXR 200

Table 3 Total proligand and free metal-ion concentrations used as blood-plasma model[15,16]

Component	Concentration/mol dm^{-3}	Component	Concentration/mol dm^{-3}
Serum albumin	7.2×10^{-4}	Tyrosinate	5.8×10^{-5}
Transferrin	2.5×10^{-5}	Valinate	2.3×10^{-4}
Alaninate	3.7×10^{-4}	Carbonate	2.5×10^{-2}
2-Aminobutyrate	2.4×10^{-5}	Phosphate	1.6×10^{-3}
Arginine	9.5×10^{-5}	Thiocyanate	1.4×10^{-5}
Asparaginate	5.5×10^{-5}	Silicate	1.4×10^{-4}
Aspartate	5.0×10^{-6}	Sulfate	2.1×10^{-4}
Cysteinate	2.3×10^{-5}	Ammonia	2.4×10^{-5}
Cystinate	4.0×10^{-5}	Citrate[a]	1.1×10^{-4}
Citrullinate[b]	2.7×10^{-5}	Lactate	1.8×10^{-3}
Glutamate	4.8×10^{-5}	Malate	3.5×10^{-5}
Glutaminate	5.2×10^{-4}	Oxalate	1.2×10^{-5}
Glycinate	2.4×10^{-4}	Pyruvate	9.5×10^{-5}
Histidinate	8.5×10^{-5}	Salicylate	5.0×10^{-6}
Histamine[c]	1.0×10^{-8}	Succinate	4.2×10^{-5}
Hydroxyprolinate	7.0×10^{-6}	Ascorbate	4.3×10^{-5}
Isoleucinate	6.5×10^{-5}	OH^-	1.2×10^{-6}
Leucinate	1.2×10^{-4}	Ca^{2+}	1.1×10^{-3}
Lysinate	1.8×10^{-4}	Mg^{2+}	5.2×10^{-4}
Methionate	2.9×10^{-5}	Cu^{2+}	1.0×10^{-20}
Ornithinate	5.8×10^{-5}	Fe^{2+}	1.0×10^{-11}
Phenylalanate	6.4×10^{-5}	Fe^{3+}	1.0×10^{-23}
Prolinate	2.1×10^{-4}	Pb^{2+}	1.0×10^{-14}
Serinate	1.2×10^{-4}	Mn^{2+}	1.0×10^{-12}
Threoninate	1.5×10^{-4}	Zn^{2+}	1.0×10^{-9}
Tryptophanate	1.0×10^{-5}		

[a] 3-Carboxy-3-hydroxypentane-1,5-dioate. [b] 2-Amino-5-carbamoylaminopentanoate. [c] Imidazole-4-ethanamine.

NaOH were prepared from Merck Titrisol ampoules and stored under an atmosphere of N_2(g) in high-density polyethylene bottles. These solutions were standardised against recrystallised potassium hydrogenphthalate and used within 1 week of preparation or discarded. The HCl solutions were also prepared from ampoules and standardised against recrystallised sodium tetraborate (borax) and the previously standardised NaOH. Copper(II) solutions were standardised against ethylenediamine-*N*,*N*,*N*′,*N*′-tetraacetate using fast sulphon black F as indicator. All solutions were prepared at an ionic strength of 0.15 mol dm^{-3} (Cl^-).

The titration procedure has been described previously[4] except that the oximes were weighed directly into the titration vessel and the electrode system calibrated *in situ*. The data were analysed using the ESTA[18,19] suite of programs. Spectrophotometric measurements were taken manually at 10 nm intervals in the range 400–800 nm. Solutions at seven different pH values were used and the concentration of the various species in solution calculated at each titration point. These data were then imposed upon a linear absorption matrix which was solved for the absorption coefficients of the different species in solution. A simple computer program using a Newton–Raphson iterative procedure was written for this purpose. The results are presented as electronic spectra of the individual species (see Table 2 for details).

Blood-plasma simulation was performed on a VAX mainframe computer using the ECCLES[15] program and the database of May *et al.*[15,16] Table 3 lists the total component concentrations of the blood-plasma model.

Acknowledgements

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