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# Computer Simulation Models for the Low-molecular-weight Complex Distribution of Cadmium(II) and Nickel(II) in Human Blood Plasma

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## Abstract

A computer simulation investigation into the nature of cadmium(II) and nickel(II) binding by low-molecular-weight ligands in human blood plasma is described. The distribution of these metal ions amongst the complexes formed with nearly 50 ligands has been computed. The most important formation constants required for the calculations have been determined experimentally under biological conditions. The predominant complexes formed by cadmium(II) are binary cysteinate species, whereas nickel(II) exists mainly as a ternary complex involving both cysteinate and histidinate.

## Introduction

Earlier computer models of the low-molecular-weight (LMW) metal ion distribution in human blood plasma have hitherto encompassed only those metals which normally occur in the biofluid at appreciable concentrations [1]. The successful application of these models in understanding the effect of chelating agents on essential trace element metabolism clearly suggests the advantages which could result from the inclusion of polluting metal ions. This paper reports the determination of the metal binding formation constants which such an extended computer model requires and the subsequent calculation of the distribution of cadmium(II) and nickel(II) in normal human blood plasma.

In view of cadmium's widespread industrial applications, it is hardly surprising there have been many cases of serious occupational exposure to this toxic metal. The most acute of these have involved welders exposed to cadmium oxide fumes [2–6]; these men often work in a confined space, sometimes with fatal consequences [2–5]. Chronic cadmium intoxication is also a serious problem and is particularly prevalent amongst workers employed for long periods in industries using cadmium in their manufacturing process, such as in pigment plants [7–9] and in the production of nickel cadmium batteries [10–12]. The symptoms of chronic cadmium poisoning include impaired respiratory function [2–4] and kidney damage characterised by proteinuria [8] and kidney stones [6].

Industrial exposure to nickel falls into two main categories: the ingestion of nickel salts and, more commonly, the inhalation of nickel carbonyl by workers involved in the refinement of the metal [13–15]. Exposure to nickel carbonyl affects the pulmonary system, causing dyspnoea and influenza-type symptoms.

In view of the occurrence of heavy metal intoxication by workers exposed to nickel or cadmium, it is important to monitor those at risk and to establish an effective therapeutic regime for treating patients whose body burdens exceed a critical level. This requires an increased understanding of how metals are absorbed, transported and stored *in vivo* and of how chelating agents can arrest these processes and promote the excretion of the toxic metal. The knowledge provided by computer models of the distribution of such metal ions in blood plasma can play an important part towards achieving these goals.

By analogy with essential metals, the distribution of cadmium(II) and nickel(II) *in vivo* can be represented as four distinct fractions: (i) protein species in which the metal is incorporated in a non-reversible fashion; (ii) protein species in which the metal binding is reversible; (iii) LMW species, and (iv) aquated metal ions [1]. The majority of metal is complexed within the tissues as the inert metalloprotein species resulting from the body's detoxification processes. In plasma the metal ion is distributed in an equilibrium between the labile protein complexes, the LMW species and the aquated metal ions.

Both cadmium and nickel interact with albumin in plasma to form a labile protein complex. The cadmium–albumin complex is thought to be analogous to the zinc species, complexation occurring

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via the sulphydryl groupings [16]. In contrast, nickel(II) has been shown to coordinate to the $NH_2$-terminal region of the molecule [17]. However, neither the cadmium(II)– or the nickel(II)–albumin species are sufficiently well characterised to be included in the computer models. Thus, simulation methods developed to bypass the omission of metal–protein equilibria [18] in earlier models are again utilised in the present work.

Information concerning the nature of the LMW cadmium species in plasma is sparse. *In vitro* experiments have suggested that $^{63}Ni(II)$ in plasma may be complexed to cysteine, histidine and aspartic acid, probably as mixed ligand complexes [19–21]. In the case of cadmium(II), the metal's high affinity for sulphur ligands suggest coordination by cysteinate and cystinate is likely to be significant. Although the concentrations of these LMW nickel(II) and cadmium(II) species are very low, these complexes play an important physiological role by serving as the diffusable species in extracellular transport of the metals.

*Acquisition of Formation Constants*

Following a comprehensive literature search, a critical selection of formation constants for the interaction of cadmium(II) and nickel(II) ions with each of the predominant naturally-occurring amino acids and inorganic anions in plasma was compiled. Corrections for temperature and ionic strength were made to obtain estimates for constants measured under biological conditions as previously described [1]. In the absence of any value from the literature, an estimate was made based on the corresponding constants for copper(II) and zinc(II) complexation.

These constants were then used in computer simulations to give percentage distributions of nickel(II) and cadmium(II) ions amongst the LMW complexes in blood plasma. From the results of these simulations, the species of major importance were identified and the formation constants for these complexes were experimentally determined by potentiometric titration. Formation constants for the interaction of the ligands considered with Cu(II) and Zn(II) ions were also measured in order to update the blood plasma models for these essential metals.

## Experimental

Potentiometric titrations were performed under blood plasma conditions – 37 °C, $I = 150$ mmol $dm^{-3}$ NaCl following our usual approach [22]. All solutions were prepared using distilled and degassed doubly deionised water, the ionic product of which was taken as $\log K_w = -13.31$. Total ligand concentrations ranging from 5 to 20 mmol $dm^{-3}$ were used in the protonation studies and absolute metal and ligand concentrations in the binary and ternary metal amino acid studies were varied as much as possible within the solubility limits.

*Materials*

Analytical reagents were used throughout. Metal ion solutions were prepared from their chlorides (BDH Analar) and analysed by EDTA complexometric titrations for metal ion concentration [23] and by Gran plot [24] for mineral acid content. Ligands studied were as follows:

*(i) Alanine (L-2-aminopropanoic acid)*

*Anal.* Found: C, 40.4; H, 7.9; N, 15.8. Calc. for $C_3H_7NO_2$: C, 40.4; H, 7.9; N, 15.7%. Supplied by BDH Chemicals Ltd.

*(ii) Cysteine (L-2-amino-3-mercaptopropanoic acid)*

*Anal.* Found: C, 29.5; H, 5.7; N, 11.3. Calc. for $C_3H_7O_2NS$: C, 29.7; H, 5.8; N, 11.5%. Supplied by Merck.

*(iii) Cystine (dithiobis(2-amino-3-propanoic acid))*

*Anal.* Found: C, 29.8; H, 4.9; N, 11.6. Calc. for $C_6H_{12}O_4N_2S_2$: C, 30.0; H, 5.0; N, 11.7. Supplied by Merck.

*(iv) Glutamine (L-2-aminopentanedioic acid 5-amide)*

*Anal.* Found: C, 41.0; H, 7.0; N, 19.0. Calc. for $C_5H_{10}N_2O_3$: C, 41.1; H, 6.9; N, 19.2%. Supplied by BDH Chemicals Ltd.

*(v) Glutamic acid (L-2-aminopentanedioic acid)*

*Anal.* Found: C, 40.8; H, 6.1; N, 9.4. Calc. for $C_5H_9O_4N$: C, 40.8; H, 6.2; N, 9.5%. Supplied by BDH Chemicals Ltd.

*(vi) Glycine (aminoacetic acid)*

*Anal.* Found: C, 32.1; H, 6.5; N, 18.5. Calc. for $C_2H_5O_2N$: C, 32.0; H, 6.7; N, 18.6%. Supplied by BDH Chemicals Ltd.

*(vii) Histidine (2-amino-3-(4'-imidazolyl)propanoic acid)*

*Anal.* Found: C, 46.6; H, 5.8; N, 27.0. Calc. for $C_6H_9N_3O_2$: C, 46.6; H, 5.8; N, 27.1%. Supplied by BDH Chemicals Ltd.

*(viii) Lysine (2,6-diaminohexanoic acid) used as the monochloride*

*Anal.* Found: C, 39.6; H, 8.4; N, 15.5. Calc. for $C_6H_{15}O_2N_2Cl$: C, 39.7; H, 7.8; N, 15.4%. Supplied by BDH Chemicals Ltd.

Protonation and binary metal–ligand potentiometric titration data were processed using the ZPLOT

TABLE I. Formation Constants for Proton–, Cd(II)–, Cu(II)–, Ni(II)–, and Zn(II)–Amino Acid Interaction at 37 °C.[a]

| Interaction | Species | | | $\log \beta_{pqr}$ | Standard deviation | Sum of squared residuals | MINIQUAD R factor | Number of points | Number of curves |
|---|---|---|---|---|---|---|---|---|---|
| | *p* | *q* | *r* | | | | | | |
| Alaninate protonation | 1 | 0 | 1 | 9.367 | 0.001 | $7.2 \times 10^{-7}$ | 0.002 | 394 | 7 |
| | 1 | 0 | 2 | 11.698 | 0.001 | | | | |
| Cd(II)–alaninate | 1 | 1 | 0 | 3.446 | 0.005 | | | | |
| | 2 | 1 | 0 | 6.317 | 0.009 | $1.2 \times 10^{-6}$ | 0.004 | 338 | 6 |
| | 1 | 1 | −1 | −6.63 | 0.06 | | | | |
| Cu(II)–alaninate | 1 | 1 | 0 | 7.876 | 0.003 | | | | |
| | 2 | 1 | 0 | 14.265 | 0.006 | $9.1 \times 10^{-7}$ | 0.004 | 382 | 6 |
| | 1 | 1 | −1 | −0.02 | 0.04 | | | | |
| Ni(II)–alaninate | 1 | 1 | 0 | 5.261 | 0.003 | | | | |
| | 2 | 1 | 0 | 9.567 | 0.005 | $4.7 \times 10^{-7}$ | 0.003 | 250 | 5 |
| | 3 | 1 | 0 | 12.36 | 0.02 | | | | |
| Zn(II)–alaninate | 1 | 1 | 0 | 4.440 | 0.008 | $3.1 \times 10^{-6}$ | 0.006 | 317 | 6 |
| | 1 | 1 | −1 | −3.17 | 0.01 | | | | |
| Cysteinate protonation | 1 | 0 | 1 | 10.102 | 0.002 | | | | |
| | 1 | 0 | 2 | 18.030 | 0.003 | $4.5 \times 10^{-7}$ | 0.002 | 348 | 5 |
| | 1 | 0 | 3 | 19.992 | 0.004 | | | | |
| Cd(II)–cysteinate | 1 | 1 | 0 | 10.3 | 0.1 | | | | |
| | 1 | 1 | −1 | 2.42 | 0.06 | | | | |
| | 2 | 1 | 0 | 16.92 | 0.04 | | | | |
| | 2 | 1 | 1 | 24.97 | 0.04 | $1.40 \times 10^{-6}$ | 0.004 | 454 | 13 |
| | 2 | 1 | 2 | 30.93 | 0.05 | | | | |
| | 3 | 1 | 0 | 19.78 | 0.04 | | | | |
| | 3 | 1 | 1 | 29.21 | 0.06 | | | | |
| Ni(II)–cysteinate | 1 | 1 | 0 | 9.603 | 0.008 | | | | |
| | 2 | 1 | 0 | 19.219 | 0.008 | $7.9 \times 10^{-7}$ | 0.002 | 367 | 8 |
| | 3 | 2 | 0 | 31.49 | 0.02 | | | | |
| Zn(II)–cysteinate | 2 | 1 | 0 | 17.77 | 0.01 | | | | |
| | 1 | 1 | 1 | 14.67 | 0.02 | | | | |
| | 3 | 2 | 0 | 30.26 | 0.04 | $9.4 \times 10^{-7}$ | 0.002 | 333 | 7 |
| | 3 | 2 | 1 | 36.14 | 0.04 | | | | |
| | 3 | 2 | 2 | 41.73 | 0.03 | | | | |
| Cystinate protonation | 1 | 0 | 1 | 8.604 | 0.003 | | | | |
| | 1 | 0 | 2 | 16.356 | 0.004 | $2.6 \times 10^{-7}$ | 0.003 | 234 | 7 |
| | 1 | 0 | 3 | 18.41 | 0.01 | | | | |
| | 1 | 0 | 4 | 20.03 | 0.02 | | | | |
| Cu(II)–cystinate | 2 | 2 | 0 | 27.803 | 0.007 | | | | |
| | 1 | 1 | 1 | 15.788 | 0.004 | $6.1 \times 10^{-8}$ | 0.001 | 306 | 12 |
| | 1 | 2 | 0 | 14.61 | 0.02 | | | | |
| Ni(II)–cystinate | 2 | 2 | 0 | 17.54 | 0.02 | | | | |
| | 1 | 1 | 1 | 13.51 | 0.02 | $1.6 \times 10^{-7}$ | 0.003 | 265 | 8 |
| | 1 | 2 | 0 | 10.21 | 0.02 | | | | |
| | 2 | 1 | 0 | 11.73 | 0.04 | | | | |
| Zn(II)–cystinate | 1 | 1 | 0 | 6.65 | 0.02 | $2.9 \times 10^{-8}$ | 0.003 | 184 | 6 |
| | 1 | 1 | 1 | 12.89 | 0.05 | | | | |
| Glutaminate | 1 | 0 | 1 | 8.697 | 0.001 | $5.6 \times 10^{-7}$ | 0.003 | 226 | 6 |
| | 1 | 0 | 2 | 10.899 | 0.002 | | | | |
| Cd(II)–glutaminate | 1 | 1 | 0 | 3.168 | 0.005 | | | | |
| | 2 | 1 | 0 | 5.694 | 0.009 | $1.4 \times 10^{-7}$ | 0.003 | 206 | 5 |
| | 1 | 1 | −1 | −6.58 | 0.03 | | | | |
| Cu(II)–glutaminate | 1 | 1 | 0 | 7.474 | 0.004 | | | | |
| | 2 | 1 | 0 | 13.600 | 0.009 | $6.3 \times 10^{-7}$ | 0.004 | 299 | 5 |
| | 1 | 1 | −1 | −0.07 | 0.02 | | | | |
| Ni(II)–glutaminate | 1 | 1 | 0 | 4.979 | 0.004 | | | | |
| | 2 | 1 | 0 | 9.015 | 0.004 | $1.2 \times 10^{-7}$ | 0.002 | 244 | 5 |
| | 3 | 1 | 0 | 11.62 | 0.01 | | | | |
| | 2 | 1 | −1 | −1.91 | 0.03 | | | | |

*(continued overleaf)*

TABLE I. *(continued)*

| Interaction | Species | | | $\log \beta_{pqr}$ | Standard deviation | Sum of squared residuals | MINIQUAD R factor | Number of points | Number of curves |
|---|---|---|---|---|---|---|---|---|---|
| | $p$ | $q$ | $r$ | | | | | | |
| Zn(II)–glutaminate | 1 | 1 | 0 | 4.215 | 0.003 | | | | |
| | 2 | 1 | 0 | 7.808 | 0.004 | $2.4 \times 10^{-7}$ | 0.002 | 273 | 6 |
| | 2 | 1 | −1 | −1.35 | 0.02 | | | | |
| Glutamate protonation | 1 | 0 | 1 | 9.260 | 0.001 | | | | |
| | 1 | 0 | 2 | 13.358 | 0.002 | $2.0 \times 10^{-7}$ | 0.002 | 230 | 5 |
| | 1 | 0 | 3 | 15.541 | 0.002 | | | | |
| Cd(II)–glutamate | 1 | 1 | 0 | 3.60 | 0.01 | | | | |
| | 2 | 1 | 0 | 6.21 | 0.01 | $1.6 \times 10^{-6}$ | 0.005 | 262 | 6 |
| | 1 | 1 | −1 | −6.38 | 0.02 | | | | |
| Cu(II)–glutamate | 1 | 1 | 0 | 8.165 | 0.006 | | | | |
| | 1 | 1 | 1 | 12.297 | 0.004 | $1.6 \times 10^{-7}$ | 0.001 | 239 | 6 |
| | 2 | 1 | 0 | 14.599 | 0.005 | | | | |
| | 2 | 1 | 1 | 19.27 | 0.02 | | | | |
| Ni(II)–glutamate | 1 | 1 | 0 | 5.533 | 0.004 | | | | |
| | 2 | 1 | 0 | 9.754 | 0.006 | $3.6 \times 10^{-7}$ | 0.002 | 307 | 6 |
| | 3 | 1 | 0 | 12.02 | 0.02 | | | | |
| | 1 | 1 | 1 | 10.40 | 0.03 | | | | |
| Zn(II)–glutamate | 1 | 1 | 0 | 4.685 | 0.005 | | | | |
| | 2 | 1 | 0 | 8.470 | 0.008 | $5.7 \times 10^{-7}$ | 0.003 | 305 | 8 |
| | 2 | 1 | −1 | −1.19 | 0.02 | | | | |
| Glycinate protonation | 1 | 0 | 1 | 9.216 | 0.002 | $2.7 \times 10^{-6}$ | 0.005 | 295 | 5 |
| | 1 | 0 | 2 | 11.522 | 0.003 | | | | |
| Cd(II)–glycinate | 1 | 1 | 0 | 3.834 | 0.007 | | | | |
| | 2 | 1 | 0 | 6.88 | 0.01 | $3.3 \times 10^{-6}$ | 0.005 | 345 | 6 |
| | 3 | 1 | 0 | 8.92 | 0.03 | | | | |
| | 2 | 1 | −2 | −13.55 | 0.02 | | | | |
| Cu(II)–glycinate | 1 | 1 | 0 | 7.870 | 0.003 | | | | |
| | 2 | 1 | 0 | 14.451 | 0.007 | $1.6 \times 10^{-6}$ | 0.004 | 405 | 6 |
| | 2 | 1 | −1 | 3.30 | 0.01 | | | | |
| Ni(II)–glycinate | 1 | 1 | 0 | 5.587 | 0.004 | | | | |
| | 2 | 1 | 0 | 10.237 | 0.006 | $1.5 \times 10^{-6}$ | 0.003 | 436 | 6 |
| | 3 | 1 | 0 | 13.72 | 0.02 | | | | |
| Zn(II)–glycinate | 1 | 1 | 0 | 4.868 | 0.005 | | | | |
| | 2 | 1 | 0 | 8.74 | 0.01 | $2.0 \times 10^{-6}$ | 0.003 | 438 | 6 |
| | 3 | 1 | 0 | 11.10 | 0.03 | | | | |
| | 1 | 1 | −1 | −2.98 | 0.02 | | | | |
| Histidinate protonation | 1 | 0 | 1 | 8.77 | 0.001 | | | | |
| | 1 | 0 | 2 | 14.60 | 0.002 | $4.3 \times 10^{-7}$ | 0.002 | 250 | 4 |
| | 1 | 0 | 3 | 16.29 | 0.004 | | | | |
| Cd(II)–histidinate | 1 | 1 | 0 | 5.10 | 0.003 | | | | |
| | 2 | 1 | 0 | 9.02 | 0.009 | | | | |
| | 1 | 1 | 1 | 10.47 | 0.03 | $3.2 \times 10^{-7}$ | 0.003 | 238 | 8 |
| | 1 | 1 | −1 | −5.10 | 0.09 | | | | |
| | 3 | 1 | 0 | 10.7 | 0.1 | | | | |
| Cu(II) histidinate | 1 | 1 | 0 | 9.75 | 0.004 | | | | |
| | 1 | 1 | 1 | 13.70 | 0.01 | | | | |
| | 2 | 1 | 0 | 17.40 | 0.006 | | | | |
| | 2 | 1 | 1 | 22.96 | 0.007 | $5.6 \times 10^{-7}$ | 0.002 | 341 | 7 |
| | 2 | 1 | 2 | 26.16 | 0.05 | | | | |
| | 1 | 1 | −1 | 2.4 | 0.1 | | | | |
| | 2 | 2 | −2 | 7.5 | 0.1 | | | | |
| Ni(II)–histidinate | 1 | 1 | 0 | 8.315 | 0.002 | $2.7 \times 10^{-7}$ | 0.002 | 210 | 6 |
| | 2 | 1 | 0 | 14.86 | 0.006 | | | | |
| Zn(II)–histidinate | 1 | 1 | 0 | 6.26 | 0.004 | | | | |
| | 1 | 1 | 1 | 10.38 | 0.01 | $8.0 \times 10^{-7}$ | 0.003 | 289 | 7 |

*(continued on facing page)*

TABLE I. *(continued)*

| Interaction | Species | | | $\log \beta_{pqr}$ | Standard deviation | Sum of squared residuals | MINIQUAD R factor | Number of points | Number of curves |
|---|---|---|---|---|---|---|---|---|---|
| | *p* | *q* | *r* | | | | | | |
| | 2 | 1 | 0 | 11.45 | 0.009 | | | | |
| | 2 | 1 | 1 | 16.67 | 0.009 | | | | |
| Lysinate protonation | 1 | 0 | 1 | 10.269 | 0.001 | | | | |
| | 1 | 0 | 2 | 19.103 | 0.002 | $8.5 \times 10^{-7}$ | 0.002 | 300 | 5 |
| | 1 | 0 | 3 | 21.16 | 0.03 | | | | |
| Cd(II)–lysinate | 1 | 1 | 1 | 13.33 | 0.02 | | | | |
| | 2 | 1 | 0 | 7.10 | 0.04 | $1.8 \times 10^{-6}$ | 0.004 | 259 | 5 |
| | 2 | 1 | 1 | 16.88 | 0.04 | | | | |
| | 2 | 1 | 2 | 26.31 | 0.03 | | | | |
| Cu(II)–lysinate | 1 | 1 | 0 | 10.37 | 0.03 | | | | |
| | 1 | 1 | 1 | 17.682 | 0.008 | | | | |
| | 2 | 1 | 0 | 14.18 | 0.04 | $1.4 \times 10^{-6}$ | 0.003 | 368 | 4 |
| | 2 | 1 | 1 | 24.50 | 0.02 | | | | |
| | 2 | 1 | 2 | 34.16 | 0.01 | | | | |
| | 1 | 1 | −1 | 0.78 | 0.03 | | | | |
| Ni(II)–lysinate | 1 | 1 | 0 | 5.6 | 0.1 | | | | |
| | 1 | 1 | 1 | 15.08 | 0.01 | | | | |
| | 2 | 1 | 0 | 9.76 | 0.09 | $1.5 \times 10^{-6}$ | 0.003 | 312 | 5 |
| | 2 | 1 | 1 | 20.12 | 0.02 | | | | |
| | 2 | 1 | 2 | 29.50 | 0.01 | | | | |
| | 1 | 1 | −1 | −3.26 | 0.07 | | | | |
| Zn(II)–lysinate | 1 | 1 | 1 | 14.307 | 0.02 | | | | |
| | 2 | 1 | 2 | 28.34 | 0.02 | $1.35 \times 10^{-6}$ | 0.003 | 237 | 5 |
| | 1 | 1 | −1 | −2.06 | 0.04 | | | | |

[a] $I$ = 150 mmol dm$^{-3}$ NaCl. $\beta_{pqr} = [M_qL_pH_r]/[M]^q[L]^p[H]^r$.

program [25]. Values for the stepwise formation constants were estimated using Bjerrum's half z bar method [26] and the constants subsequently refined using MINIQUAD [27]. Superimposability of the experimental and simulated formation curves [28] was used as a criteria to distinguish between statistically equivalent models.

Ternary metal–ligand data were analysed using MINIQUAD holding formation constants for the corresponding binary systems at fixed values and refining for the ternary species.

## Results and Discussion

The results of the MINIQUAD analysis of the titration data for the proton–ligand and metal–ligand binary systems studied are shown in Table I. The majority of metal ion–amino acid anion interactions considered are characterised by the formation of ML and $ML_2$ complexes. In addition, many of these systems exhibit the formation of hydroxy species which are characterised by curl-backs in the formation curves. Of the ligands studied, only lysinate displays a pronounced formation of protonated species.

The sulphur-containing amino acids cystinate and cysteinate exhibit a strong tendency to form polynuclear complexes, 220 and 320 type species being formed by copper, nickel and zinc. Such species are commonly formed by this type of ligand [29], undoubtedly as a result of sulphur-bridging. Formation constants for the interaction of Cu(II) with cysteine could not be determined due to the reduction of Cu(II) to Cu(I) by thiol ligands. Cadmium forms an insoluble complex with cysteinate and this has hitherto precluded the determination of the Cd(II)–cysteinate formation constants [30, 31]. However, precipitation only occurs in the pH range 5.0–7.5 and by titrating either side of this region, a large proportion of the formation curve could be obtained. The resulting data were analysed using MINIQUAD. In all, over seventy possible models were considered. The set of formation constants considered to be most representative of the experimental data are given in Table I. The high value for the standard deviation of the ML complex is due to the absence of data in the pH region 5.0–7.5 where the greatest formation of this complex occurs.

Complexation of Cd(II) ions by cystinate also results in the formation of an insoluble complex.

TABLE II. Formation Constants for Zn(II)–Glutaminate–Cysteinate Ternary Interaction at 37 °C.[a]

| Species | | | | log $\beta_{pp'qr}$ | Standard Deviation | Sum of squared residuals | MINIQUAD R factor | Number of points | Number of curves |
|---|---|---|---|---|---|---|---|---|---|
| $p$ | $p$ | $q$ | $r$ | | | | | | |
| 1 | 1 | 1 | 1 | 19.66 | 0.05 | $3.2 \times 10^{-7}$ | 0.003 | 240 | 4 |

[a]$I$ = 150 mmol dm$^{-3}$ NaCl. $\beta_{pp'qr} = [M_qL_pL'_{p'}H_r]/[M]^q[L]^p[L']^{p'}[H]^r$. L = Glutaminate. L′ = Cysteinate.

TABLE III. Formation Constants for Cd(II)– and Ni(II)–Alaninate–Histidinate Ternary Interaction at 37 °C.[a]

| Interaction | Species | | | | log $\beta_{pp'qr}$ | Standard deviation | Sum of squared residuals | MINIQUAD R factor | Number of points | Number of curves |
|---|---|---|---|---|---|---|---|---|---|---|
| | $p$ | $p'$ | $q$ | $r$ | | | | | | |
| Cd(II)–alaninate–histidinate | 1 | 1 | 1 | 0 | 8.165 | 0.009 | $5.2 \times 10^{-7}$ | 0.003 | 355 | 6 |
| | 1 | 1 | 1 | −1 | −2.35 | 0.01 | | | | |
| Ni(II)–alaninate–histidinate | 1 | 1 | 1 | 0 | 12.600 | 0.008 | $3.7 \times 10^{-7}$ | 0.003 | 381 | 6 |
| | 1 | 2 | 1 | 0 | 14.51 | 0.06 | | | | |

[a]$I$ = 150 mmol dm$^{-3}$ NaCl. $\beta_{pp'qr} = [M_qL_pL'_{p'}H_r]/[M]^q[L]^p[L']^{p'}[H]^r$. L = Alaninate. L′ = Histidinate.

Unlike the Cd(II)–cysteinate system, precipitation occurs over the whole pH range precluding any analysis of the binary system. However, formation constants for the Cd(II)–cystinate system have previously been determined using NTA as a competing ligand [32] and these values were used in the present simulations.

Formation constants for the ternary systems investigated are given in Tables II and III. Glutamine and cysteine form a protonated complex with Zn(II) ions. In the alanine–histidine systems, cadmium forms an MLL′ complex together with a hydroxy species, whereas in the case of nickel, a species is present in which the metal is complexed to an alanine and two histidine molecules.

*Low-molecular-weight Distribution of Cd(II) and Ni(II) in Blood Plasma*

The LMW distributions of Cd(II) and Ni(II) ions in blood plasma were computed using the ECCLES program [1]. The total ligand concentrations were as used in previous simulations of the speciation of essential metals in plasma [1]. Since both nickel and cadmium are pollutants, their plasma concentrations are likely to vary considerably depending on the extent of exposure. Blood cadmium levels of $1.3–1.7 \times 10^{-7}$ mol dm$^{-3}$ have been reported in industrially exposed workers [6]. However, as this value includes protein-bound metal, the concentration of cadmium associated with the LMW fraction is certain to be much lower than this and the free metal ion concentration very small indeed. In view of the uncertainties in the free Cd(II) and Ni(II) concentrations, these values were scanned over a wide range. The computed distributions were found to be independent of the free metal ion concentration below a value of $10^{-9}$ mol dm$^{-3}$ to a precision of 1%.

TABLE IV. Predominating Low-molecular-weight Cd(II) and Ni(II) Complexes in Blood Plasma as Computed by the ECCLES Program.[a]

| Complex formation | Low-molecular-weight Metal % |
|---|---|
| $Cd(CYS)^o$ | 46.4 |
| $Cd(CIS)^o$ | 27.8 |
| $Cd(CYS)(OH)^-$ | 14.3 |
| $Cd(CYS)_2H^-$ | 8.4 |
| $Cd(CYS)_2^{2-}$ | 1.9 |
| $Cd(CYS)_2H_2^o$ | 0.3 |
| $Cd(CYS)(HIS)^-$ | 0.2 |
| $Cd(HIS)^+$ | 0.1 |
| $Cd(CYS)(HIS)H^o$ | 0.1 |
| $Ni(HIS)_2^o$ | 50.6 |
| $Ni(CYS)(HIS)^-$ | 23.9 |
| $Ni(CYS)_2^{2-}$ | 11.3 |
| $Ni(HIS)^+$ | 4.4 |

[a]Abbreviations: CYS = cysteinate, CIS = cystinate, HIS =histidinate.

The predominating LMW Cd(II) and Ni(II) complexes in blood plasma as computed by ECCLES are given in Table IV.

LMW cadmium is mainly present as a neutral 1:1 complex with cysteinate which accounts for almost 50% of the LMW metal. The neutrality of the com-

plex suggests it may be able to penetrate cellular membranes. This species may, thus, play a role in the transport of cadmium *in vivo*. Other important Cd(II) cysteinate complexes are the MLOH, $ML_2H$, $ML_2$ and $ML_2H_2$ species. The 1:1 complex formed between cadmium and cystinate in plasma is also significant, accounting for some 28% of the LMW metal.

The LMW distribution of Ni(II) ions in blood plasma shows the metal to be largely bound to histidinate and cysteinate. More than half of the LMW Ni(II) is bound to histidinate, which is in agreement with other workers [21]. The other species of significance are a ternary cysteinate–histidinate species and a bis complex with cysteinate.

## Conclusion

These ongoing computer simulation models for the LMW complexation of Ni(II) and Cd(II) ions in blood plasma provide a useful tool for the understanding of the biochemical behaviour of these toxic metals *in vivo*. More importantly, they provide an essential basis for the subsequent assessments of the efficacy of potential chelating drugs in removing such metals from the body.

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