SUPPLEMENTAL DATA

AMELIORATION OF METAL-INDUCED TOXICITY IN CAENORHABDITIS ELEGANS: UTILITY OF CHELATING AGENTS IN THE BIOREMEDIATION OF METALS

By

James M. Harrington[1], Windy A. Boyd[2], Marjolein V. Smith,[3] Julie R. Rice[2], Jonathan H. Freedman[2,4*], and Alvin L. Crumbliss[1*]

From [1]Department of Chemistry, Duke University, Durham, NC, USA, [2]Biomolecular Screening Branch, National Toxicology Program, Research Triangle Park, NC, USA, and [3]SRA International, Inc, Durham, NC, USA, [4]Laboratory of Molecular Toxicology, National Institute of Environmental Health Sciences, NIH, Research Triangle Park, NC, USA

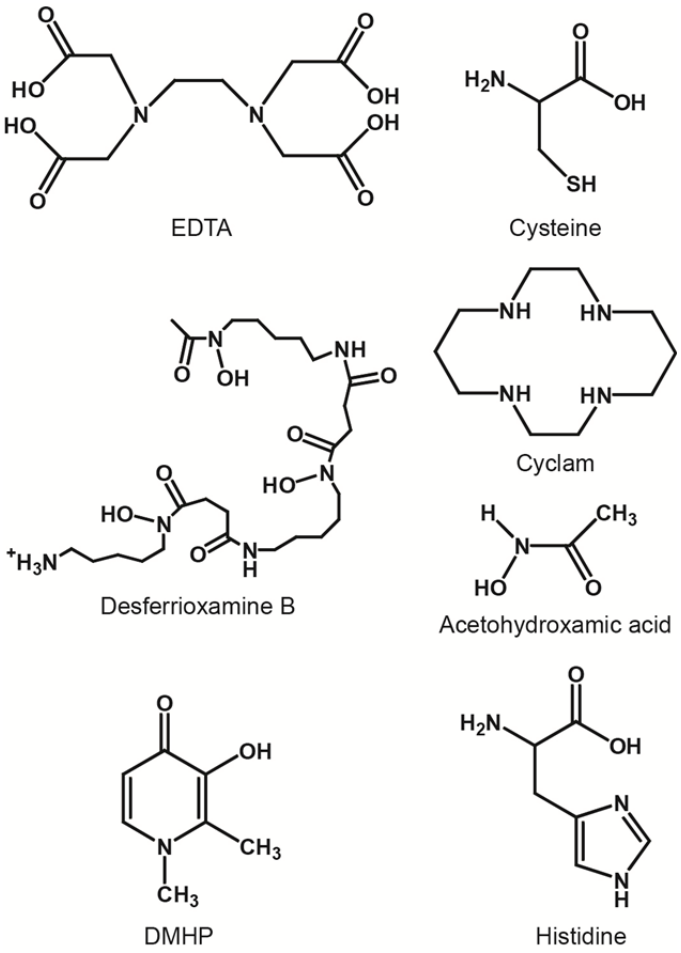


Figure S1. Chemical structures of the chelating agents used in this study.

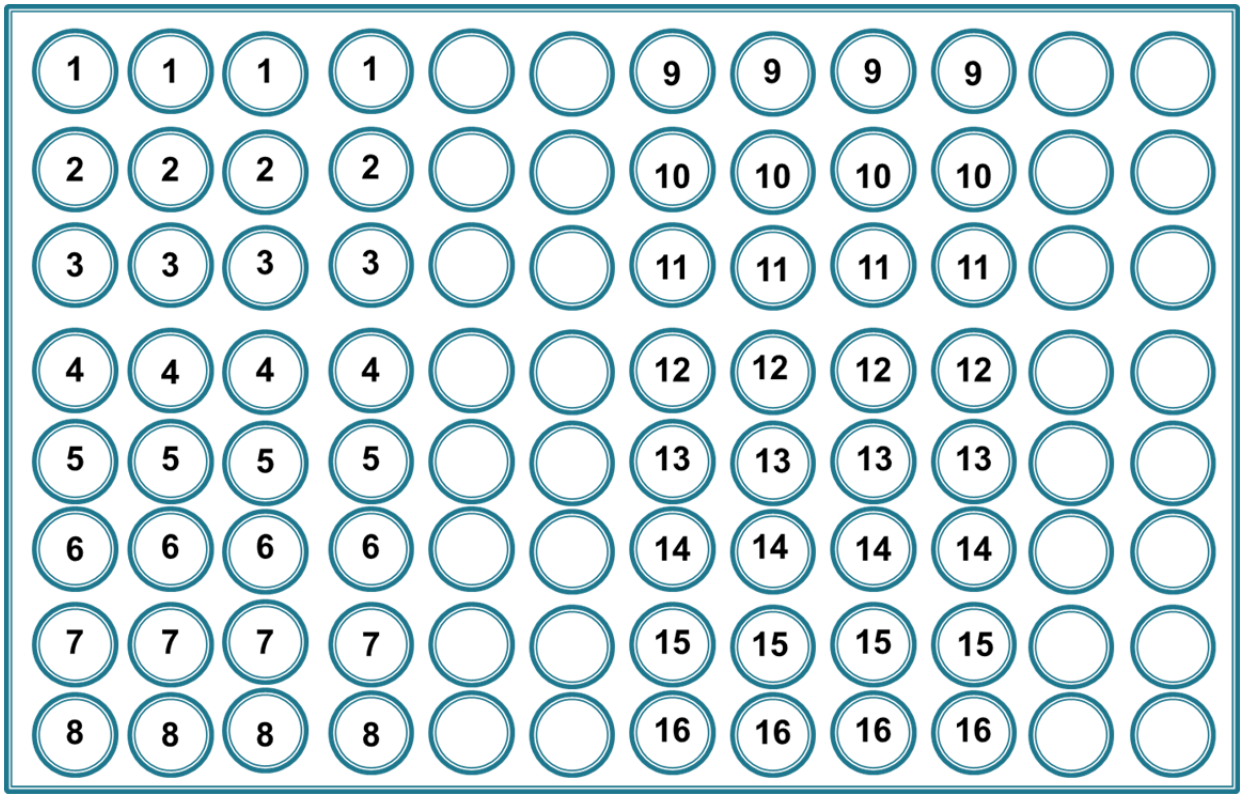


Figure S2. Diagram of metal-chelator assay plate design. Legend: 1 – Control wells; 2 – Treatment by acetohydroxamic acid; 3 – Treatment by cysteine; 4 – Treatment by cyclam; 5 – Treatment by DMHP; 6 – Treatment by desferrioxamine B; 7 – Treatment by EDTA; 8 – Treatment by histidine; 9 – Treatment by metal alone; 10 – Treatment by metal and acetohydroxamic acid; 11 – Treatment by metal and cysteine; 12 – Treatment by metal and cyclam; 13 – Treatment by metal and DMHP; 14 – Treatment by metal and desferrioxamine B; 15 – Treatment by metal and EDTA; 16 – Treatment by metal and histidine; blank circles represent rinse wells filled with deionized water.

TABLE S1

pM values of metal-chelators complexes[a]

Chelator	Metal			
	Cadmium	Copper	Nickel	Zinc
AHA	6.00	7.20	6.03	6.03
Cyclam	11.8	6.41	6.01	6.01
Cysteine	6.03	21.6	13.6	9.06
DMHP	[b]	10.4	6.09	6.17
Desferrioxamine B	6.00	11.5	6.38	6.77
CaEDTA	14.6	15.4	16.7	14.7
Histidine	6.01	9.73	7.63	6.09

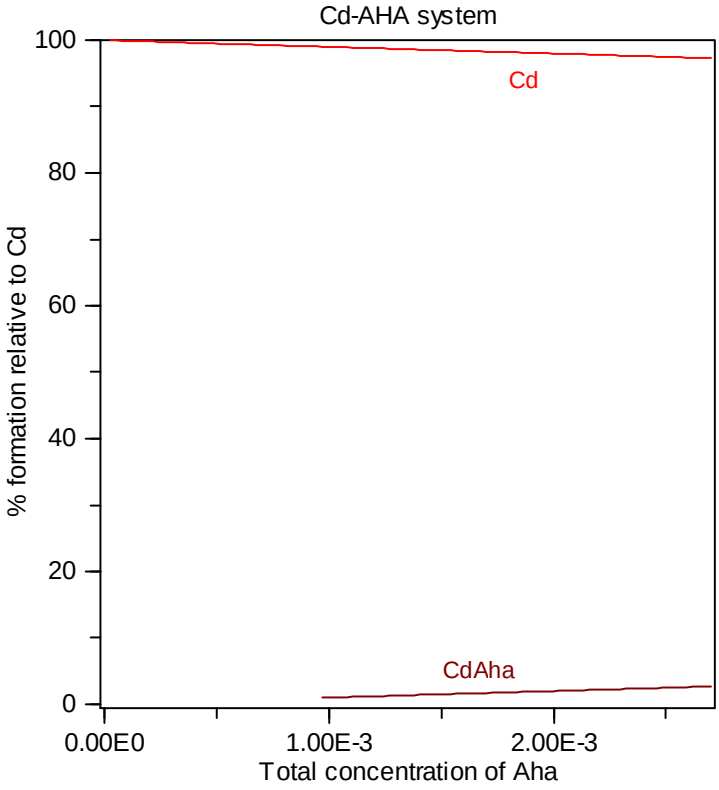
[a] pM values represent the negative log of the free uncomplexed metal concentration at the following conditions: $[M]_{tot} = 10^{-6}$ M, $[L]_{tot} = 10^{-5}$ M, pH = 7.4, T = 25 °C, μ = 0.10 M .(3)

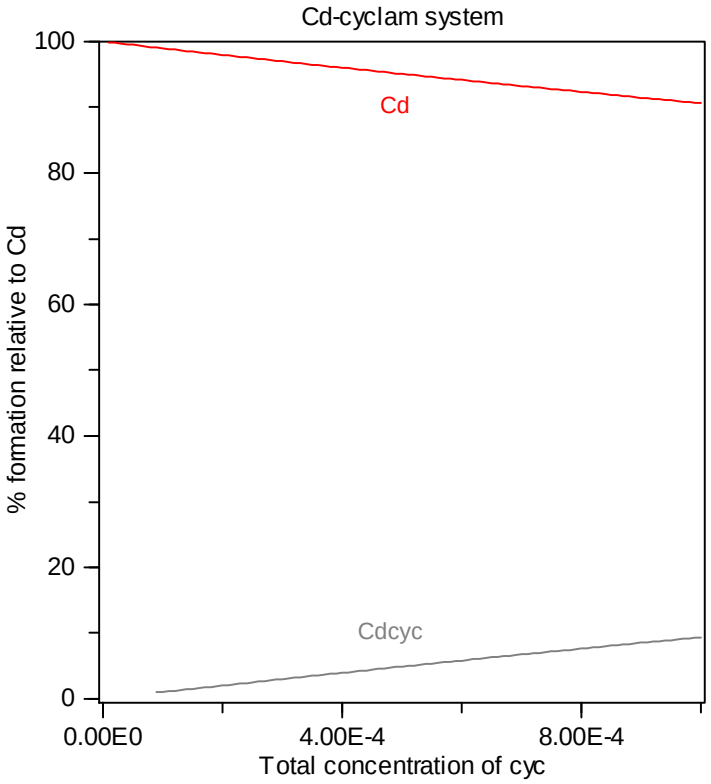
[b]Stability constants for the Cd-DMHP system have yet to be characterized.

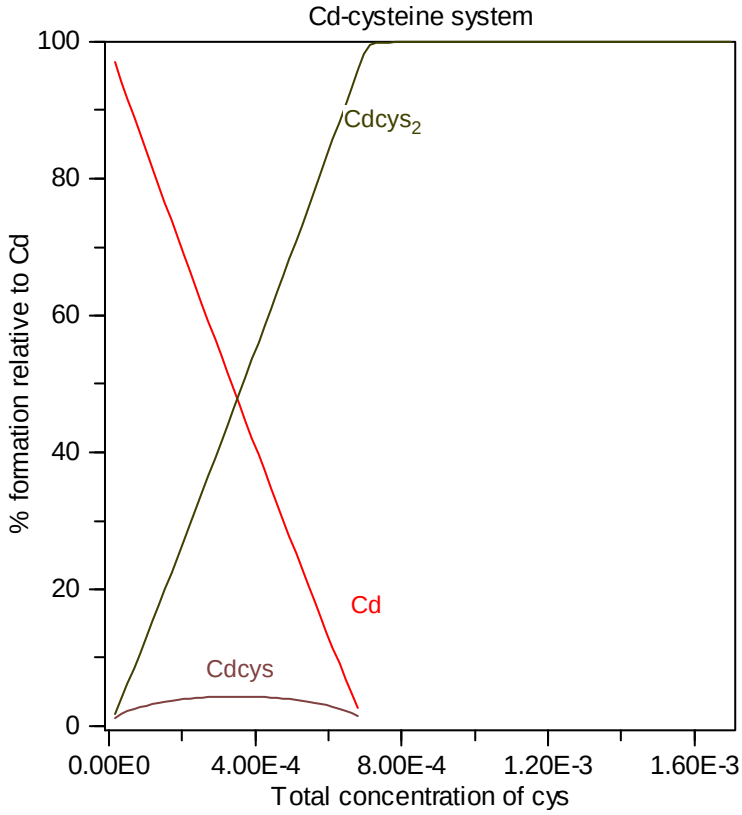
Speciation diagrams for the metal-ligand systems in this study in aqueous environments

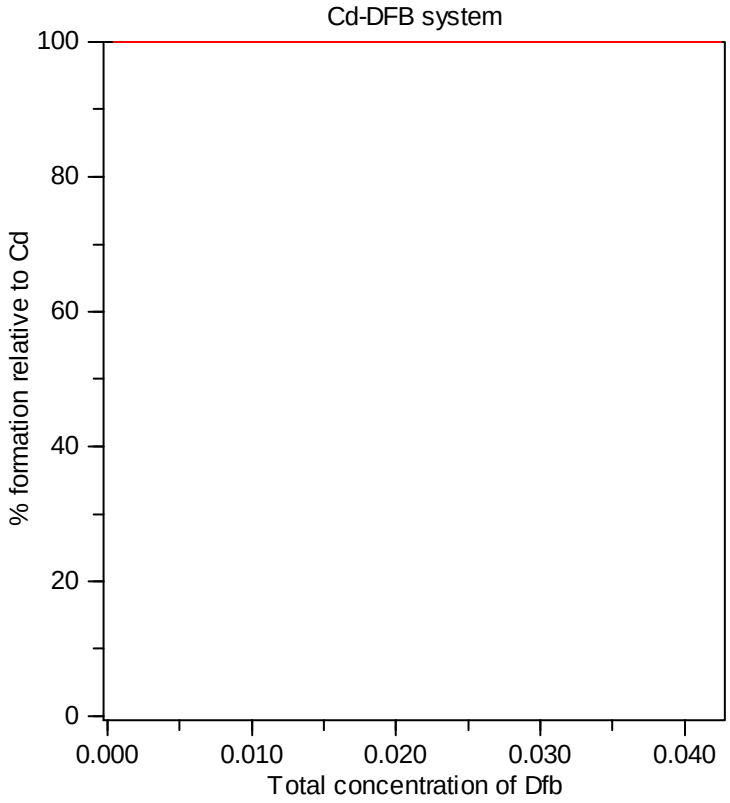
Speciation diagrams were calculated for metal-ligand systems using stability constants obtained from the IUPAC Stability Constant Database using the program, HySS by Protonic Software.[1, 2] Speciation diagrams were calculated at a constant pH and metal ion concentration, which are noted above each section of speciation diagrams, and varying ligand concentration. Speciation diagrams represent the relative amount of each metal that will be present in any form of complex (plotted on the vertical axis as relative percent total metal concentration) while varying the conditions of the system. In these speciation diagrams, the concentration of the metal and the system pH are kept constant, while the concentration (M) of the ligand present in solution is varied (horizontal axis).

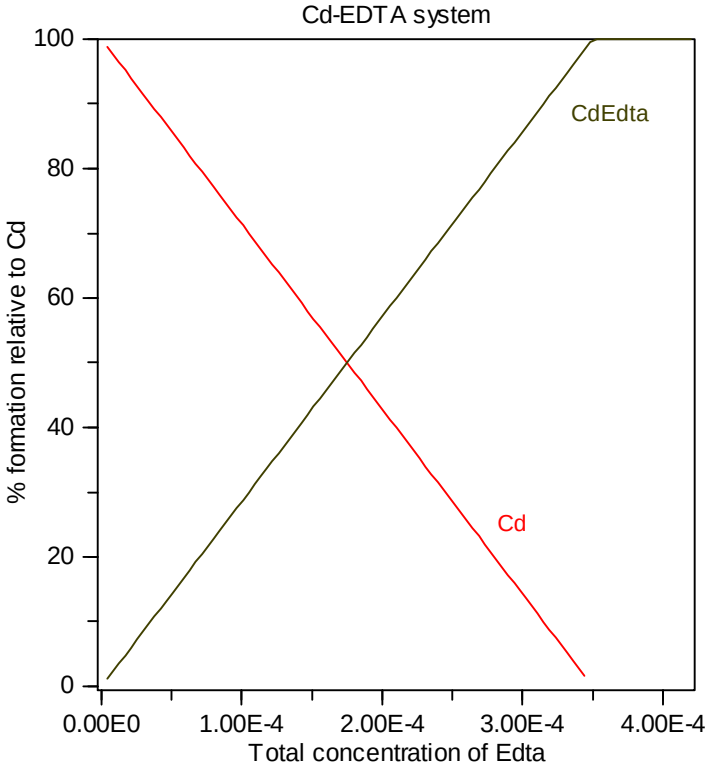
Conditions: pH 6.5, 350 µM Cd^{2+}

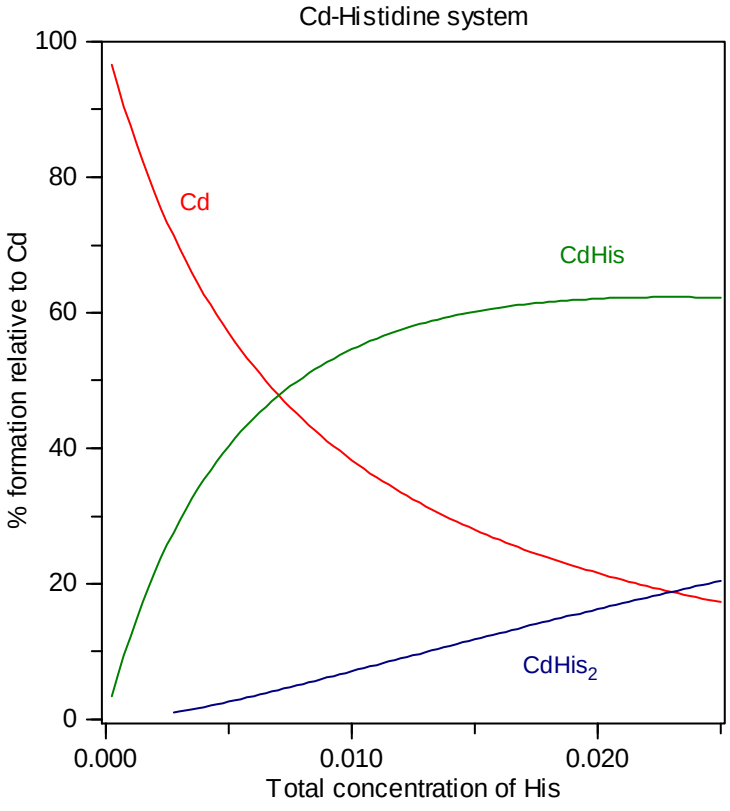




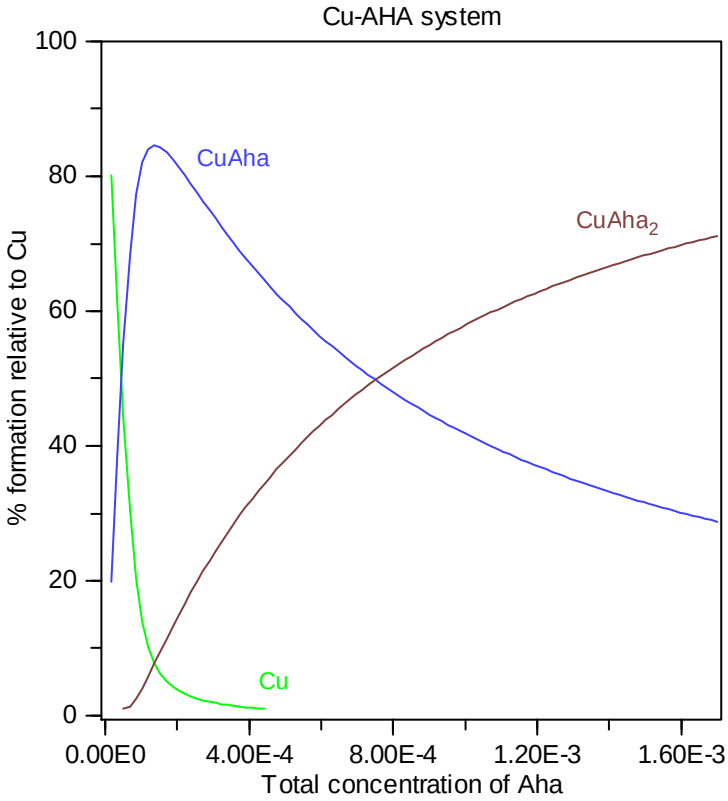

Cd-cysteine system
100
80
60
40
20
0
% formation relative to Cd
Cdcys2
Cd
Cdcys
0.00E0
4.00E-4
8.00E-4
1.20E-3
1.60E-3
Total concentration of cys

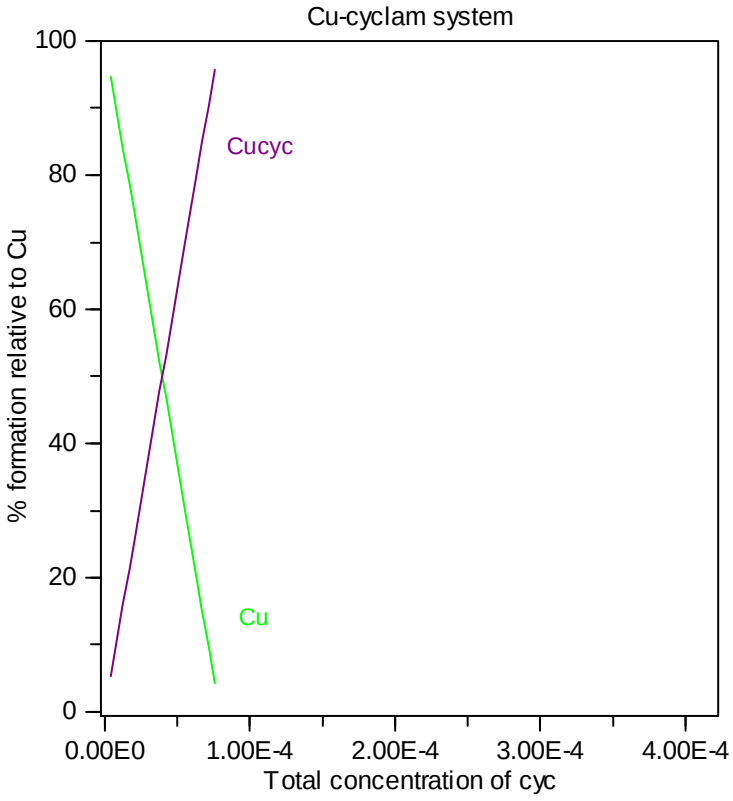

Cd-DFB system
100
80
60
40
20
0
% formation relative to Cd
0.000
0.010
0.020
0.030
0.040
Total concentration of Dfb

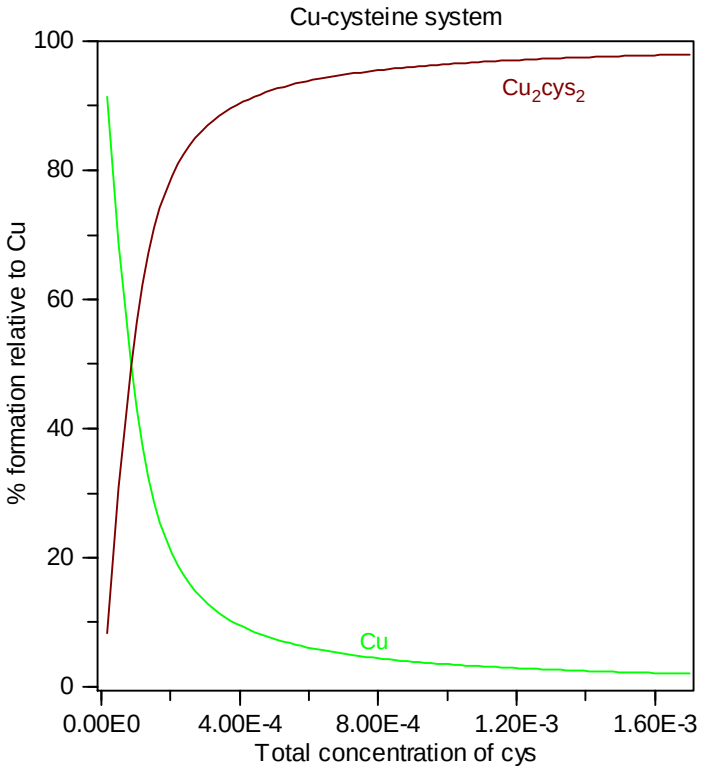

Cd-EDTA system
100
80
60
40
20
0
% formation relative to Cd
CdEdta
Cd
0.00E0
1.00E-4
2.00E-4
3.00E-4
4.00E-4
Total concentration of Edta

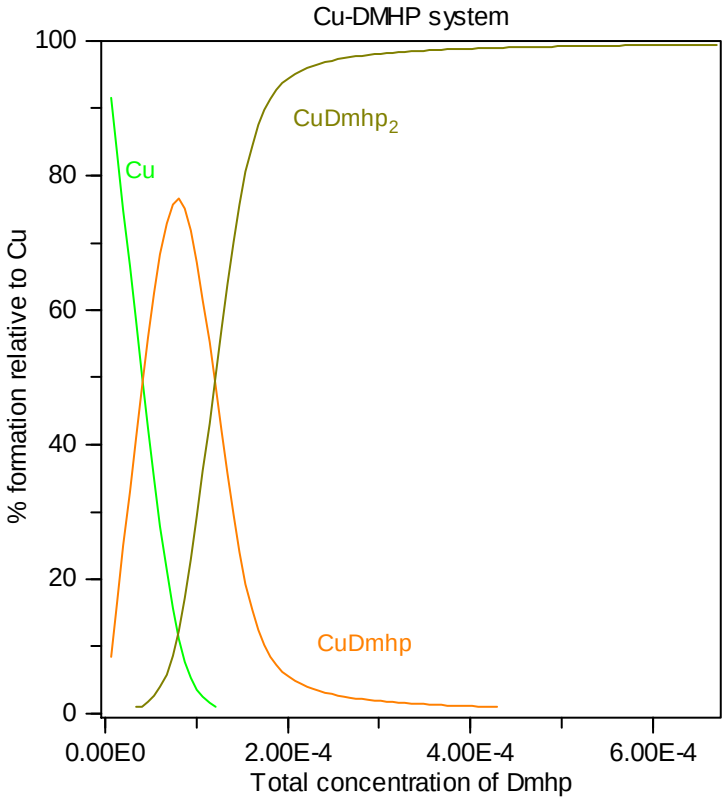

Cd-Histidine system
100
80
60
40
20
0
% formation relative to Cd
Cd
CdHis
CdHis2
0.000
0.010
0.020
Total concentration of His

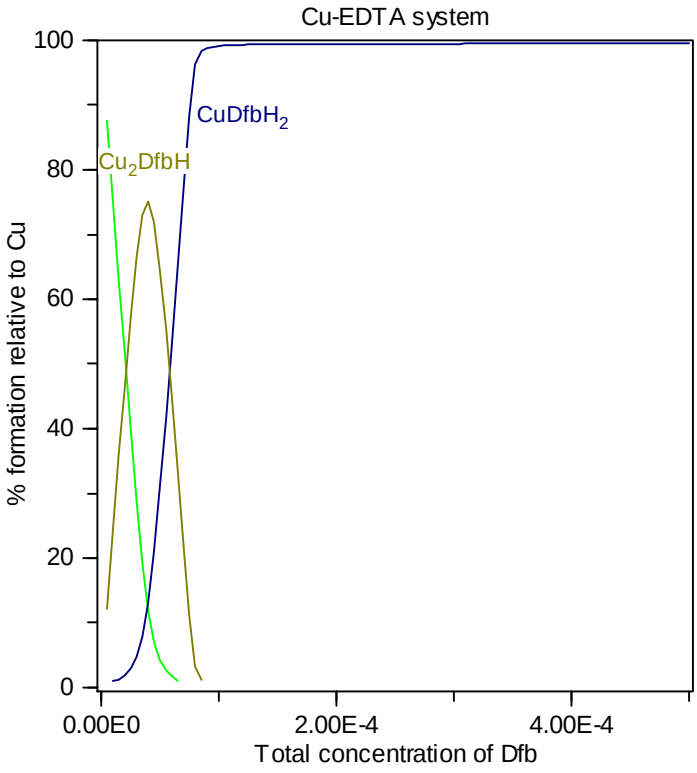
Conditions: *pH 6.5, 79 μM* Cu^{2+}

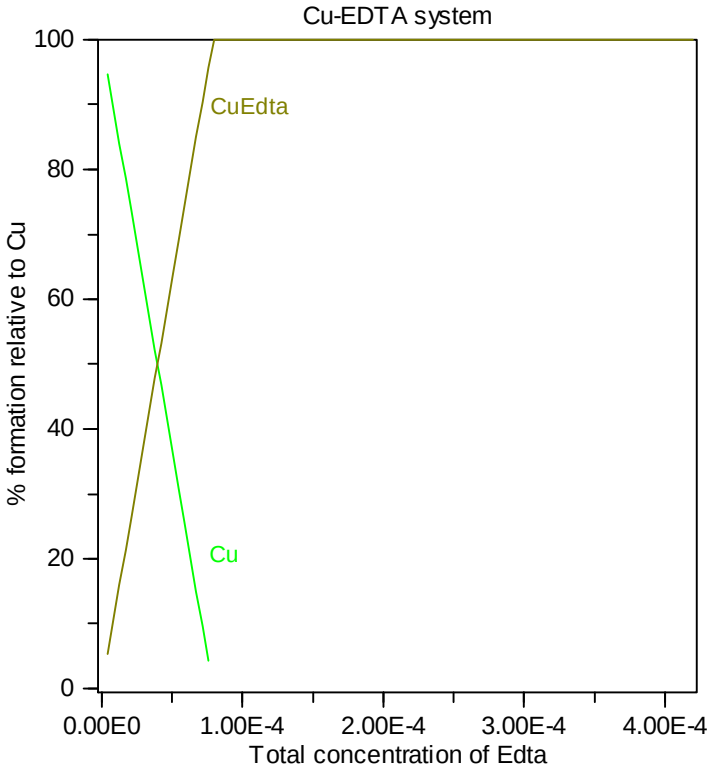


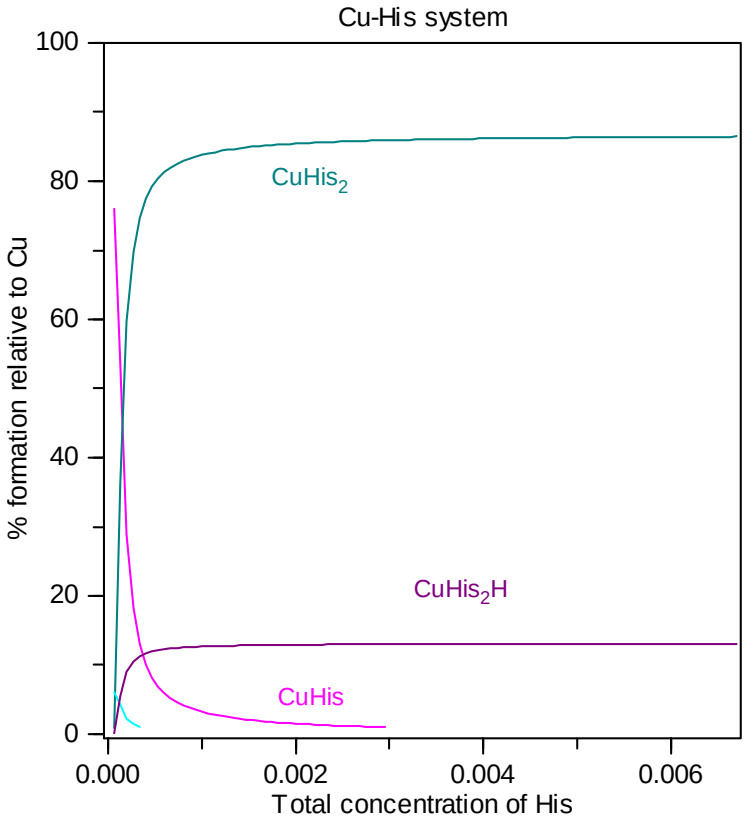

Cu-cyclam system
100
80
60
40
20
0
% formation relative to Cu
Cucyc
Cu
0.00E0
1.00E-4
2.00E-4
3.00E-4
4.00E-4
Total concentration of cyc


Cu-cysteine system
100
80
60
40
20
0
% formation relative to Cu
Cu2cys2
Cu
0.00E0
4.00E-4
8.00E-4
1.20E-3
1.60E-3
Total concentration of cys

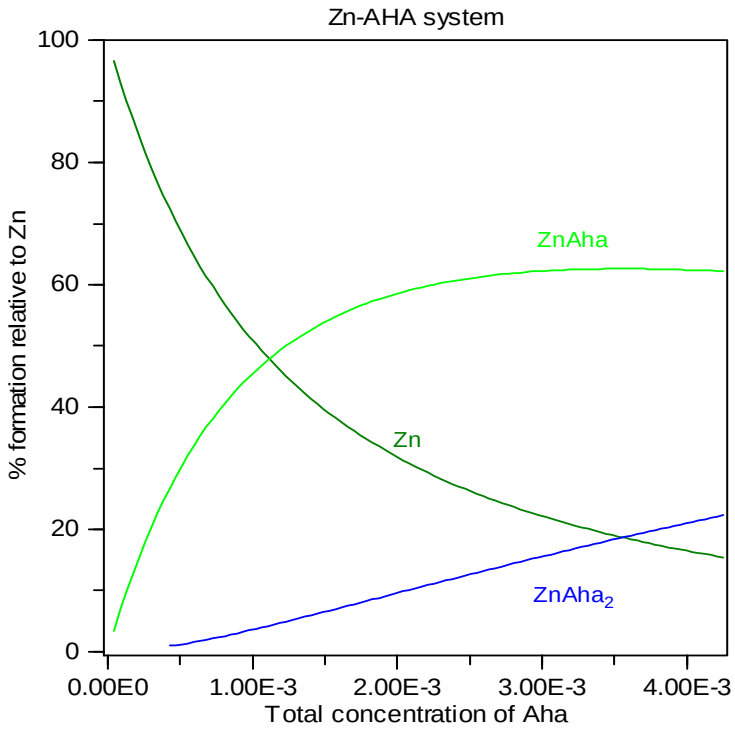

Cu-DMHP system
100
80
60
40
20
0
% formation relative to Cu
Cu
CuDmhp2
CuDmhp
0.00E0
2.00E-4
4.00E-4
6.00E-4
Total concentration of Dmhp

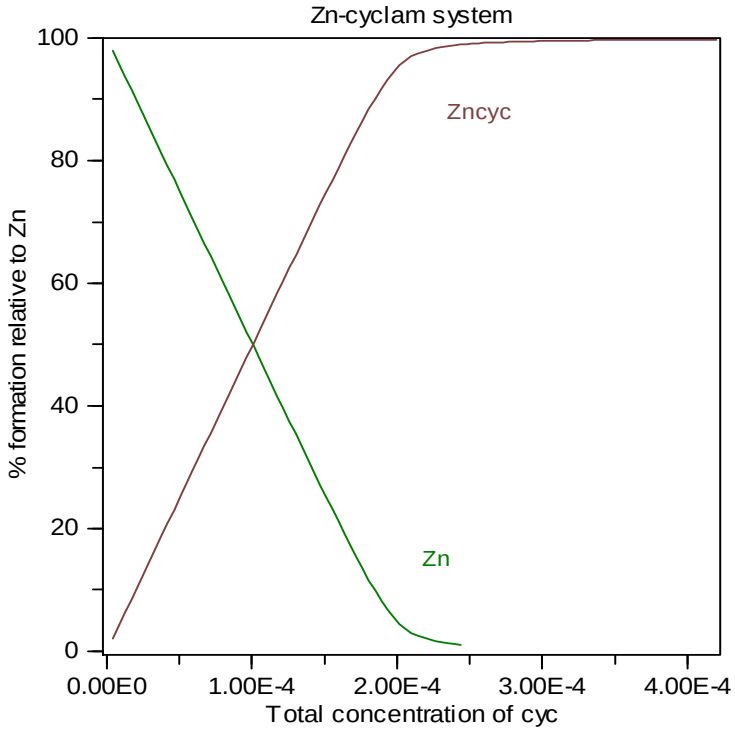

Cu-EDTA system
100
80
60
40
20
0
% formation relative to Cu
CuDfbH2
Cu2DfbH
0.00E0
2.00E-4
4.00E-4
Total concentration of Dfb

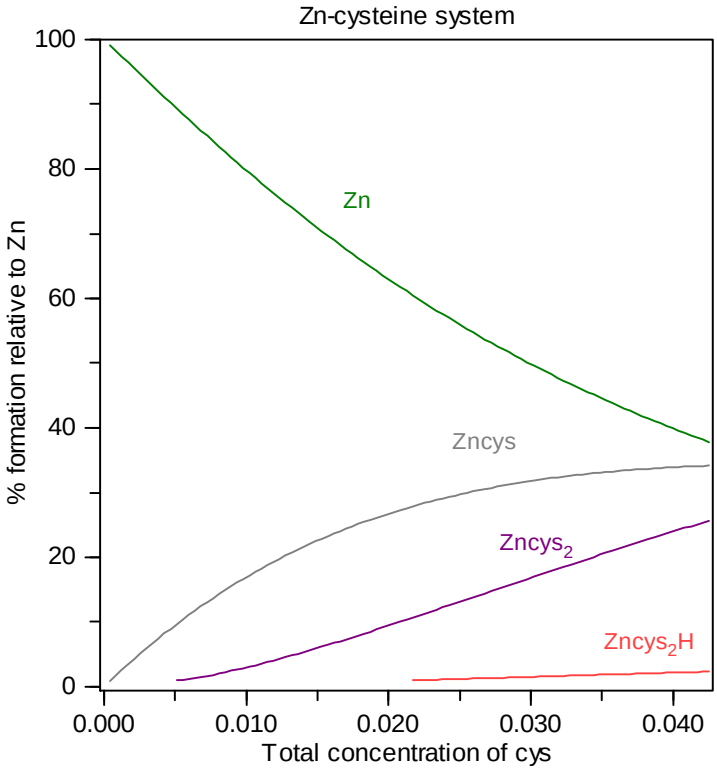

Cu-EDTA system
100
80
60
40
20
0
% formation relative to Cu
CuEdta
Cu
0.00E0
1.00E-4
2.00E-4
3.00E-4
4.00E-4
Total concentration of Edta

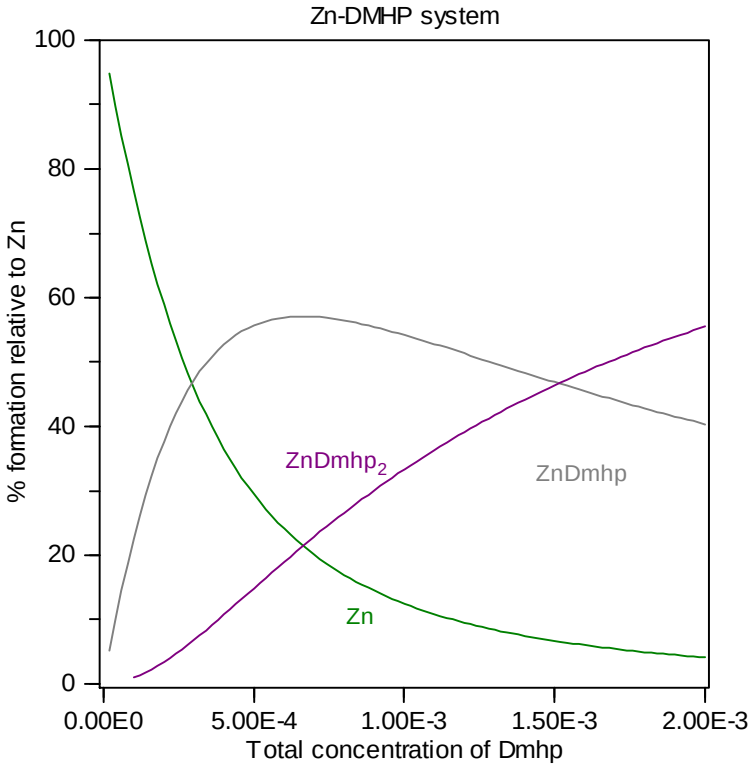

Cu-His system
100
80
60
40
20
0
% formation relative to Cu
CuHis2
CuHis2H
CuHis
0.000
0.002
0.004
0.006
Total concentration of His

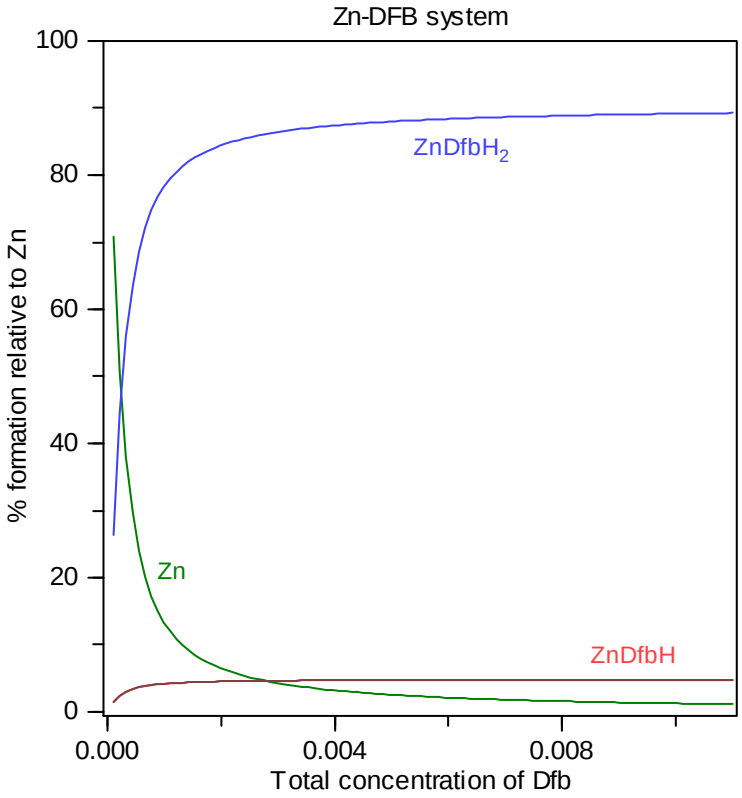
Conditions: pH 6.5, 200 μM Zn^{2+}

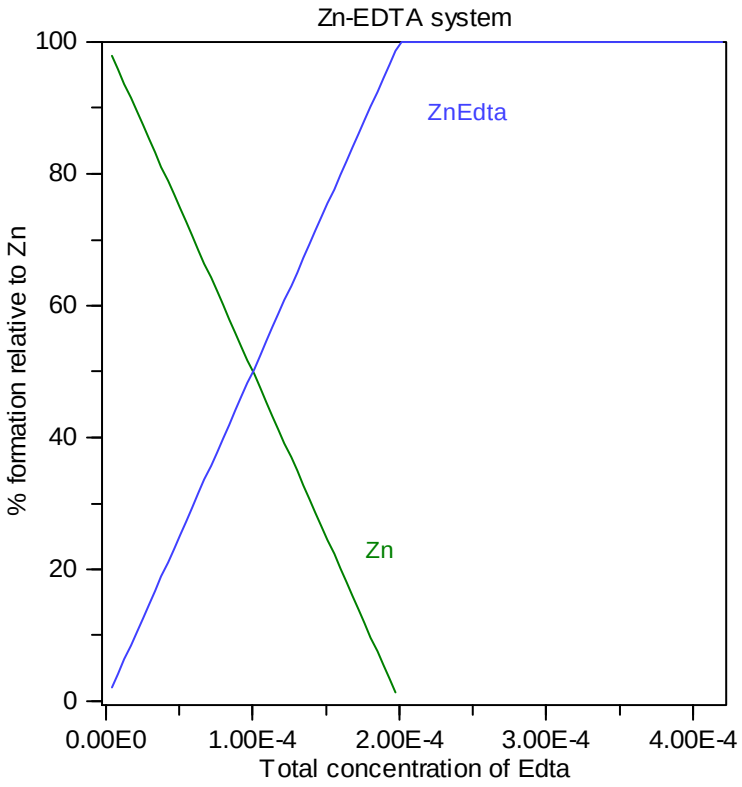


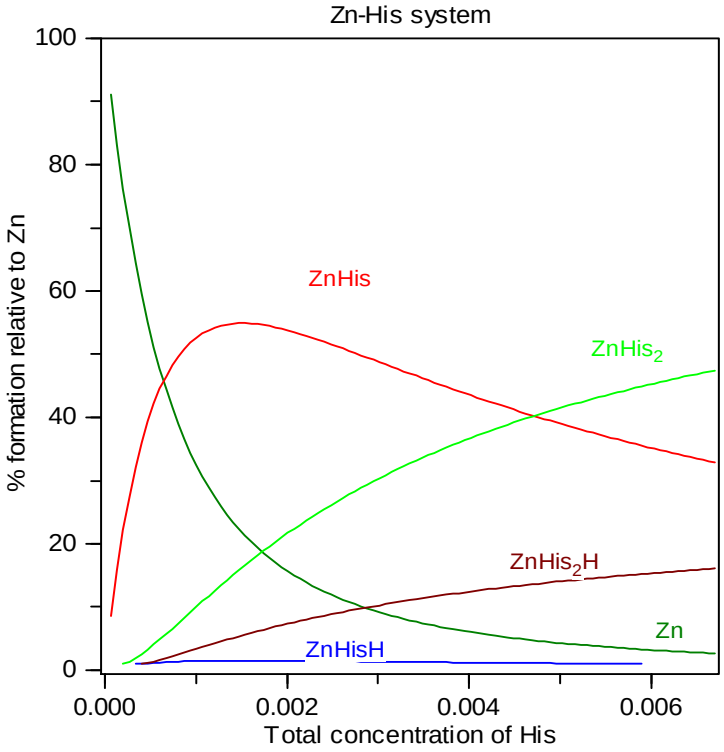



Zn-cysteine system
% formation relative to Zn
100
80
60
40
20
0
Zn
Zncys
Zncys2
Zncys2H
0.000
0.010
0.020
0.030
0.040
Total concentration of cys

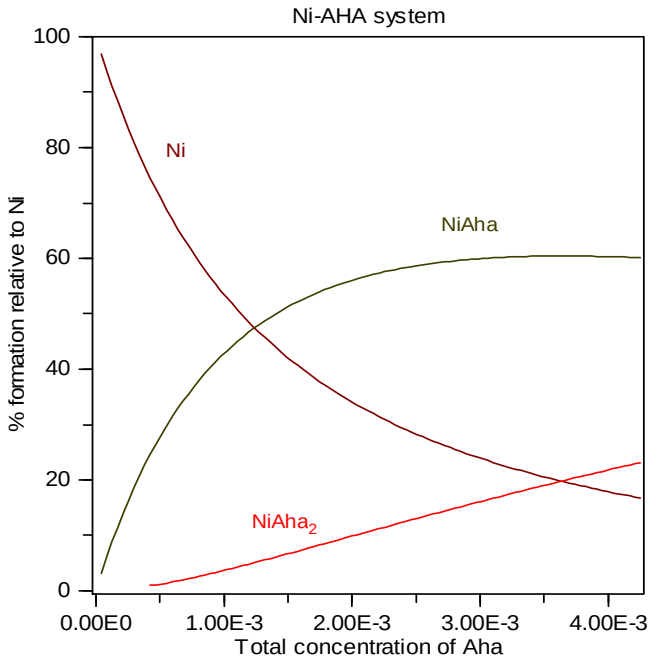

Zn-DMHP system
% formation relative to Zn
100
80
60
40
20
0
ZnDmhp2
ZnDmhp
Zn
0.00E0
5.00E-4
1.00E-3
1.50E-3
2.00E-3
Total concentration of Dmhp

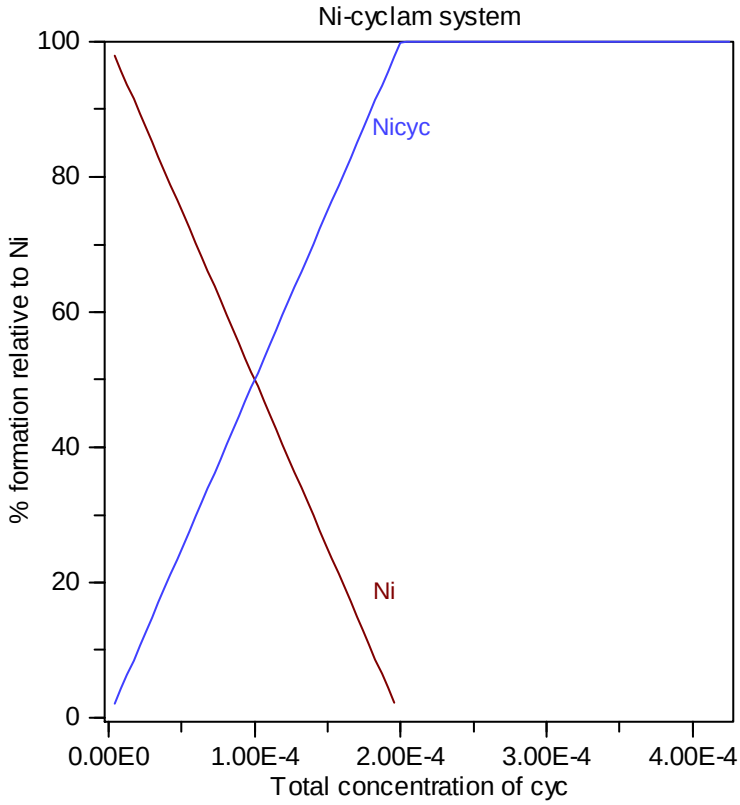

Zn-DFB system
100
80
60
40
20
0
% formation relative to Zn
ZnDfbH2
Zn
ZnDfbH
0.000
0.004
0.008
Total concentration of Dfb

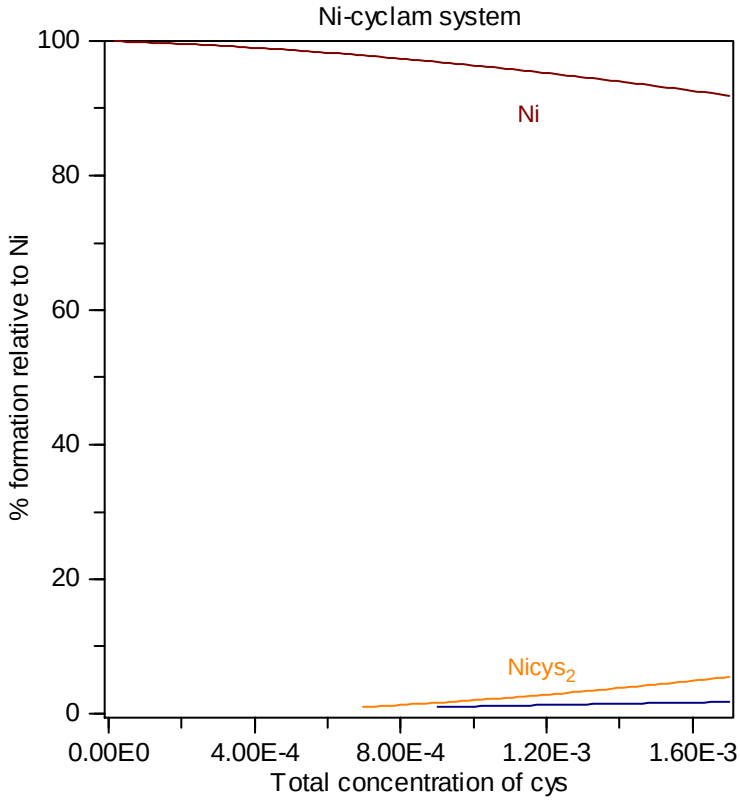

Zn-EDTA system
100
80
60
40
20
0
% formation relative to Zn
ZnEdta
Zn
0.00E0
1.00E-4
2.00E-4
3.00E-4
4.00E-4
Total concentration of Edta

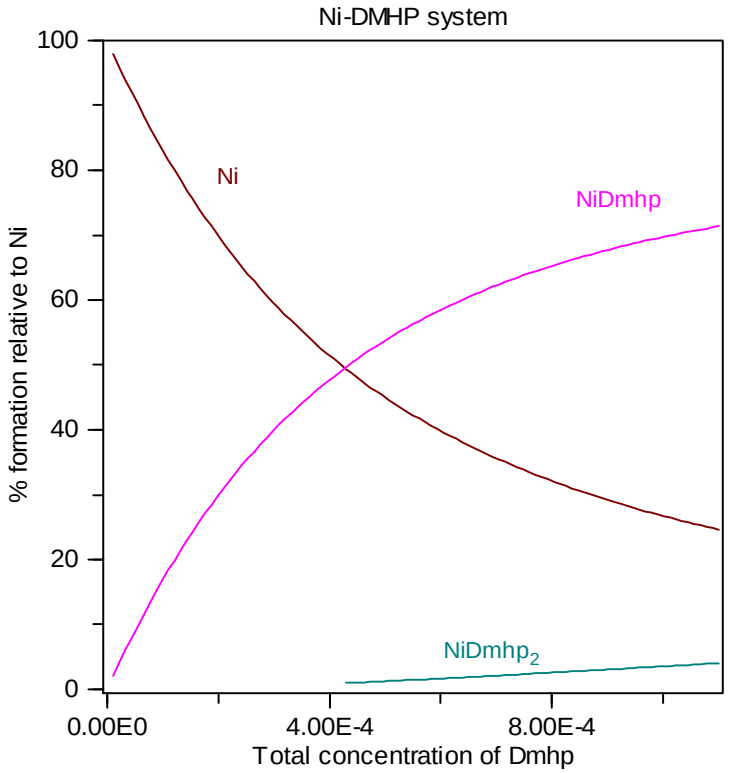

Zn-His system
100
80
60
40
20
0
% formation relative to Zn
ZnHis
ZnHis2
ZnHis2H
Zn
ZnHisH
0.000
0.002
0.004
0.006
Total concentration of His

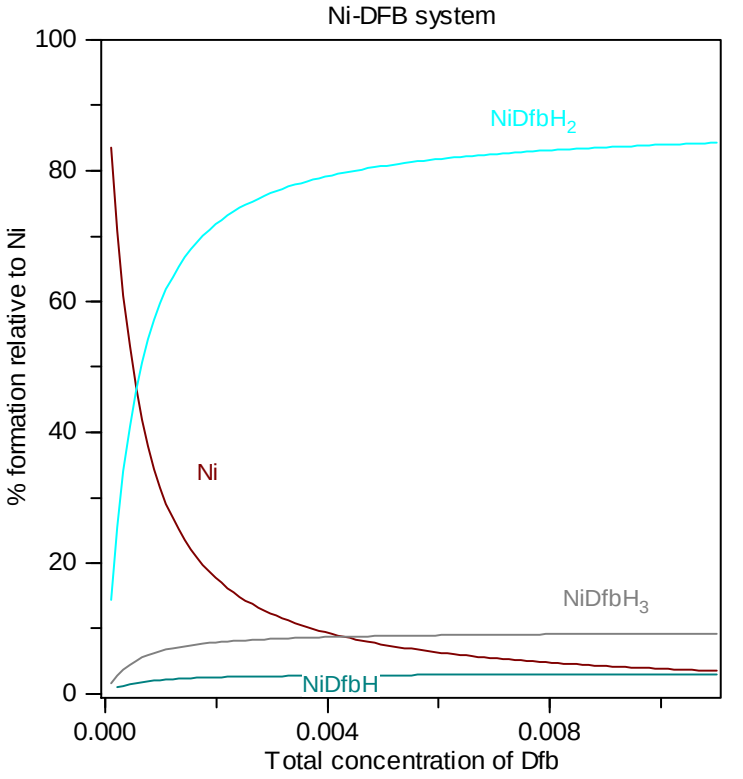
Conditions: pH 6.5, 200 µM Ni^{2+}

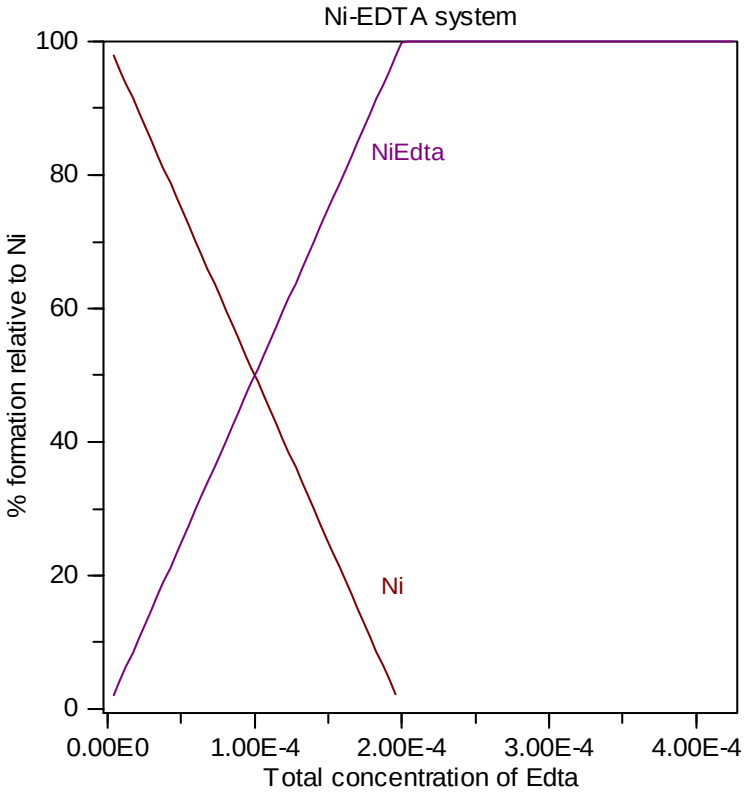


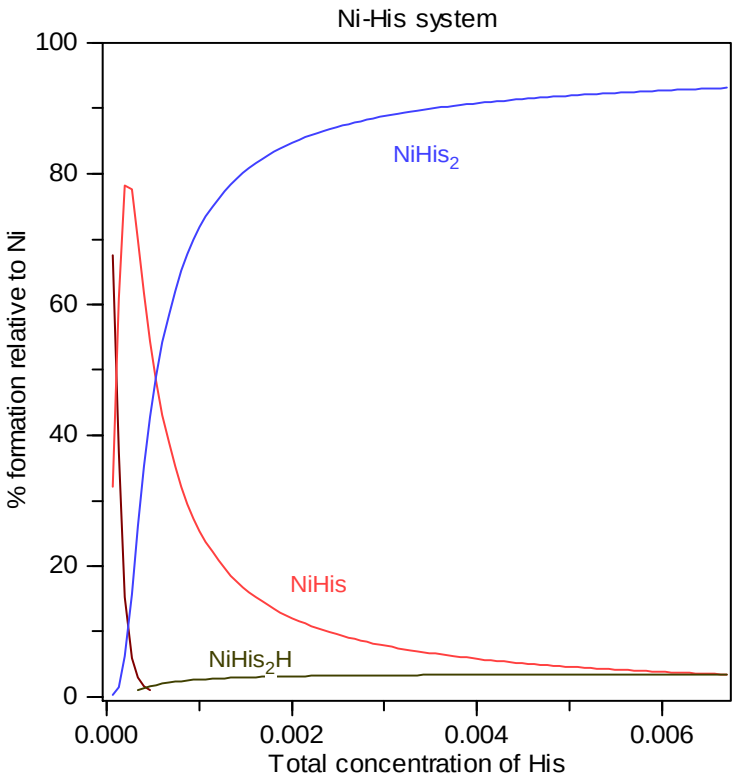



Ni-cyclam system
100
80
60
40
20
0
% formation relative to Ni
Ni
Nicys2
0.00E0
4.00E-4
8.00E-4
1.20E-3
1.60E-3
Total concentration of cys


Ni-DMHP system
100
80
60
40
20
0
% formation relative to Ni
Ni
NiDmhp
NiDmhp2
0.00E0
4.00E-4
8.00E-4
Total concentration of Dmhp


Ni-DFB system
100
80
60
40
20
0
% formation relative to Ni
NiDfbH2
Ni
NiDfbH3
NiDfbH
0.000
0.004
0.008
Total concentration of Dfb


Ni-EDTA system
100
80
60
40
20
0
% formation relative to Ni
NiEdta
Ni
0.00E0
1.00E-4
2.00E-4
3.00E-4
4.00E-4
Total concentration of Edta


Ni-His system
100
80
60
40
20
0
% formation relative to Ni
NiHis2
NiHis
NiHis2H
0.000
0.002
0.004
0.006
Total concentration of His

References

(1) Alderighi, L.; Gans, P.; Ienco, A.; Peters, D.; Sabatini, A.; Vacca, A. *Coord. Chem. Rev.* **1999**, *184*, 311-318.

(2) Powell, K. J.; Academic Software: Timble, Otley, Yorks, UK.

(3) Harris WR, Carrano CJ, Raymond KN. 1979. Coordination chemistry of microbial iron transport compounds. 16. Isolation, characterization, and formation constants of ferric aerobactin. J Am Chem Soc 101: 2722-2727.