# The Selectivity of Meso-dimercaptosuccinic Acid for Lead(II) in Blood Plasma

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In our search for drugs which will effectively remove polluting metal ions from humans, we have previously listed several criteria which ought to be satisfied [1, 2]. First, the agent ought to be selective for the metal ion concerned and not remove too much of the biologically essential metal ions. Secondly, it ought to be of low toxicity and since there is insufficient incidence of lead overload in humans, this is tantamount to stating that we must restrict our search to drugs which are already acceptable in the clinic. Thirdly, we have recently researched the concept of synergistic chelation therapy whereby one drug is used to remove the metal ions from within cells into blood plasma and then a second agent is introduced in order to promote the excretion of lead complexes from blood plasma through the renal or biliary routes [3]. References 1 and 2 describe our investigations into EDTA, D-Penicillamine, and Razoxane based drugs in respect of lead(II) removal.

One of the agents not fully exploited in our studies was British Anti Lewisite (BAL), *i.e.*, 2,3-dimercaptopropanol. Although there is clinical evidence that it is a useful chelating agent for heavy metals, the drug possesses a nauseating odour and is readily hydrolysed. In addition, it cannot be taken through the oral route and injections are often extremely painful. Mesodimercaptosuccinic acid (DMSA) has been investigated as a less toxic, more stable, derivative of BAL, in particular, by researchers in the USSR [4]. Indeed, this agent has been shown to be effective in the treatment of lead poisoning [5, 6], and it has a toxicity which is thirty times lower than that of BAL [7]. It is water soluble and can be administered orally to patients [8] although intraperitoneal administration is claimed to be more effective [9].

Surprisingly, there is a lack of formation constant data for lead(II) reacting with DMSA, there being just one other report available in the literature and this arose from spectrophotometric determinations at low concentrations [10]. Thus, this letter reports the protonation constants for DMSA and also the formation constants for lead–DMSA interactions which were determined by using EDTA as a competing ligand in order to overcome some serious insolubility problems with the simple lead–DMSA system.

## Experimental

Analytical grade reagents were used throughout. All solutions were prepared using distilled and degassed double deionised water. Stock solutions of lead chloride were prepared and analysed by EDTA complexometric titration [11] and hydrogen ion concentration By Gran Plots [12, 13]. DMSA was supplied by the Aldrich Chemical Co. Ltd. (CH Analysis found: C, 35.1; H, 3.23; calcd. for $C_4H_6O_4S_2$: C, 35.2; H, 3.19%). Ligand solutions were freshly prepared each day to minimize hydrolysis.

### *Computing*

The MAGEC [14] and MINIQUAD [15] computer programs were employed in a cycling refinement procedure to evaluate protonation constants. ESTA [16] generated speciation plots of the metal ligand systems and ECCLES [17] was employed to compute plasma distribution models.

### *Measurement of Formation Constants*

Potentiometric titrations using a micro-titration apparatus were carried out at 37 °C and $I$ = 150 mmol $dm^{-3}$ [NaCl]. Ranges of ligand concentration (13–18 and 5–10 mmol $dm^{-3}$ for DMSA and EDTA, respectively) were used in the protonation studies whilst for the ternary systems absolute concentrations of ligands and metal were restricted by the limits of solubility (2–10 and 3–4 mmol $dm^{-3}$, respectively).

## Results and Discussion

The protonation and lead complex formation constants are shown in Table I. DMSA has four possible protonation sites, there being two sulphydryl groups and two carboxylate groups. Other workers have reported values for all four constants [10, 18, 19], however, the log $K$ for the second sulphydryl group protonation is the order of 11. This is beyond the limits of glass electrode potentiometry and so the constant cannot be determined with any great precision in this work. The values arising from the MAGEC-MINIQUAD cycling approach are given in Table I and are in fairly good agreement with those reported in references 20 and 21.

The poor dissolving power of DMSA was overcome in the protonation studies by commencing titrations in the alkaline region. When metal ligand titrations were attempted, precipitation rapidly occurred in the acid region of the pH scale. We surmised that this was insoluble lead–DMSA complex, probably $PbDMSAH_2$ which is neutral and occurs in that part of the titration. These difficulties were overcome by using EDTA as a competing ligand in order to keep the free

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TABLE I. Formation Constants for Proton and Lead(II)–DMSA Interactions at 37 °C and $I$ = 150 mmol dm$^{-3}$ [NaCl].

| p | q | r | lg $\beta_{pqr}$[a] | Stnd. devn. | Sum of Squares | $R$ Factor | n[b] | N[b] |
|---|---|---|---|---|---|---|---|---|
| 1 | 1 | 0 | 12.00 | – | | | | |
| 1 | 2 | 0 | 21.33 | 0.0025 | $2.03 \times 10^{-6}$ | 0.0031 | 349 | 7 |
| 1 | 3 | 0 | 24.91 | 0.0051 | | | | |
| 1 | 4 | 0 | 27.30 | 0.0091 | | | | |
| 1 | 1 | 1 | 24.82 | 0.063 | $3.3 \times 10^{-6}$ | 0.006 | 312 | 5 |
| 1 | 1 | –1 | 19.98 | 0.049 | | | | |

[a] $\beta_{pqr} = [L_pM_qH_r]/[L]^p[M]^q[H]^r$. [b] n = no. of points and N = no. of titrations.

lead ion concentration low. The formation constants for EDTA had been previously determined [2] and were used as input into the MAGEC-MINIQUAD cycling. We titrated an acidic mixture of lead(II) and EDTA in the vessel with an alkaline solution of DMSA from the burette. In this manner, a wide pH range was covered and precipitation was avoided. Computational analysis suggested that two mono lead(II)–DMSA complexes are formed, one being protonated (MLH) and one being a hydroxy (MLH-1) species. In addition, simple ML, dimeric and polymeric species were tested for but were not found.

These constants can then be used as input for the ECCLES computer program in order to calculate Plasma Mobilising Index figures [22]. Figure 1 shows

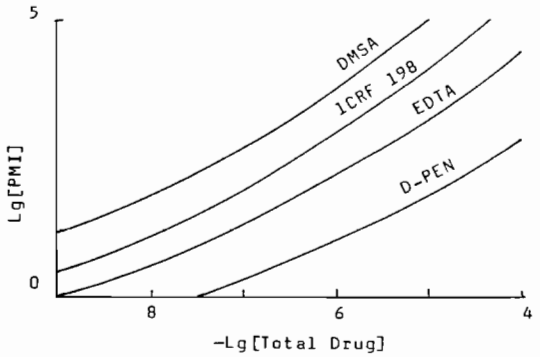


Fig. 1. Plasma Mobilizing Index curves for 4 ligands mobilizing lead(II) from proteins into low molecular mass species in plasma as computed by ECCLES.

the PMI curves for DMSA, ICRF 198 (the hydrolysis product of Razoxane), EDTA, and D-Penicillamine. Clearly, DMSA is by far the best lead mobilising agent in blood plasma and would merit further investigations, especially if EDTA or D-Penicillamine became unacceptable. The complexes formed are negatively charged and thus amenable to excretion through the kidneys. Thus, there are now several agents available for removing lead from the extracellular fluid of plasma but the search for a really effective intracellular chelator of lead(II) ions continues.

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