Supplementary material to:

J.M. Wagener, M.K. Dithebe, D. Mogano, I. Cukrowski and J.-R. Zeevaart, *S. Afr. J. Chem.,* 2008, **61**, 82–92.

Table S1 Protonation constant of serine determined at 25 °C and ionic strength of 0.15 mol dm^{-3} NaCl.

Equilibrium species	log K (literature values[25])	Hamilton R-factor	Number of data points
H + L = HL	9.09 ± 0.01 (9.05)	0.0200	133
2H + L = H_2L	2.44 ± 0.01 (2.13)		

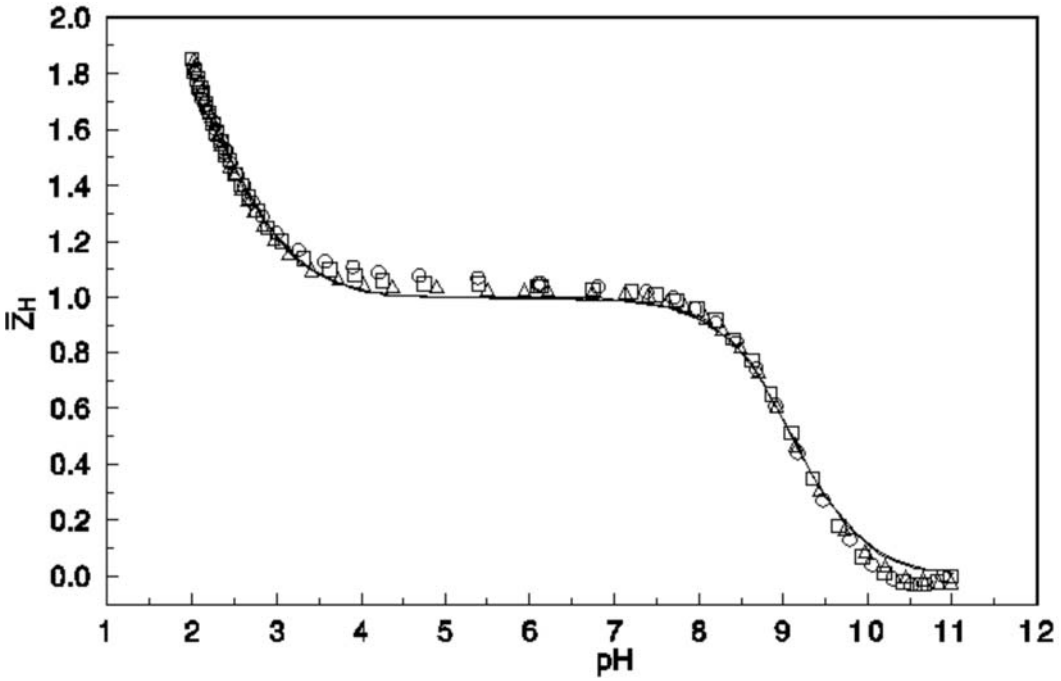

Figure S1 Experimental (points) and modelled (lines) protonation formation curves for serine. The titrations are represented by: (□) 0.001234 mol dm^{-3} serinate, 0.01273 mol dm^{-3} HCl, (△) 0.002469 mol dm^{-3} serinate, 0.012791 mol dm^{-3} HCl and (○) 0.003785 mol dm^{-3} serinate, 0.012709 mol dm^{-3} HCl. All solutions were at 25 °C and 0.15 mol dm^{-3} ionic strength.

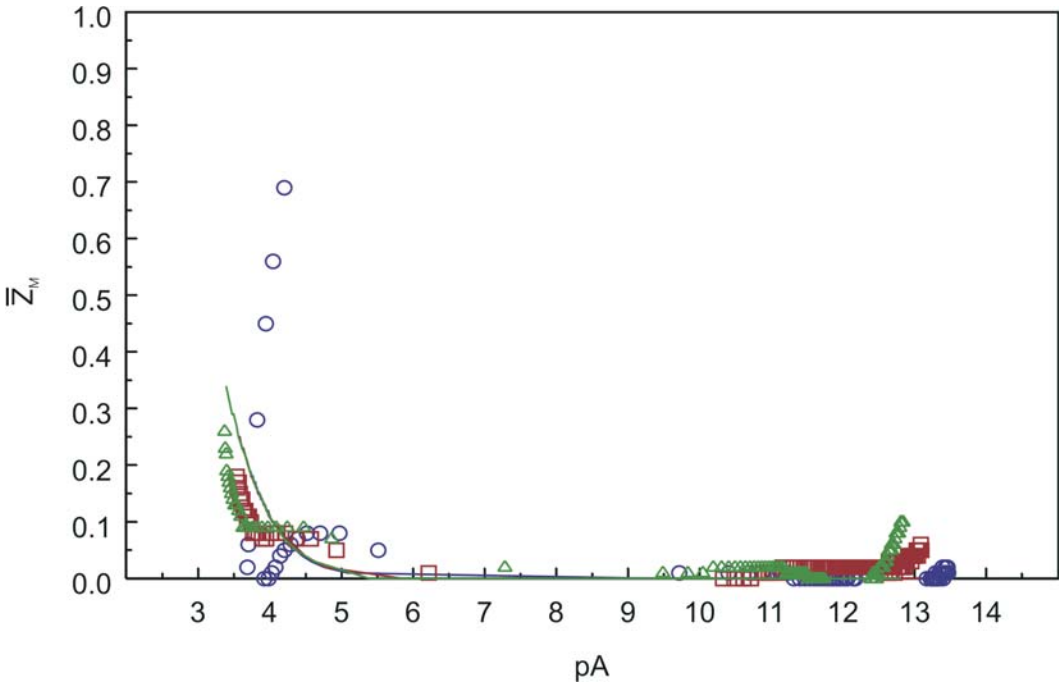

Figure S2 Experimental (symbols) and modelled (lines) formation curves for complexation of DMBG by Mg(II). The titrations are represented by: (○) 0.001001 mol dm^{-3} DMBG, 0.0010050 mol dm^{-3} Mg(II) and 0.010990 mol dm^{-3} HCl; (□) 0.002002 mol dm^{-3} DMBG, 0.0010050 mol dm^{-3} Mg(II) and 0.011991 mol dm^{-3} HCl; (△) 0.003003 mol dm^{-3} DMBG, 0.0010050 mol dm^{-3} Mg(II) and 0.013036 mol dm^{-3} HCl; *versus* 0.0500 mol dm^{-3} NaOH in 0.10 mol dm^{-3} NaCl at 25 °C and 0.15 mol dm^{-3} total ionic strength.

As indicated in the text for a few complexes described, one could argue that the potentiometry results would not be able to describe the complex MLH that is formed by the reaction M + LH → MLH, as potentiometry will not be able to record them, because no protons are released in this reaction. This would also happen from *ca.* pH 4 to pH 9, which is the area of interest for blood plasma modelling. It was assumed in the text that these complexes do not exist and that the deduction above, that there is no competition of the blood plasma metal ions for these ligands, could therefore be an underestimation. To check this assumption, other ligand analogues, for which ML and MLH complexes are reported, were selected from the literature. This information was then reinserted into the blood plasma model to see whether these ligands experience complexation by the blood plasma metal ions.

1. Biguanide (BIG)

The protonation and formation constants found in the NIST database[15] are listed in Table 2. No constants for Ca(II), Mg(II) or Zn(II) were found. In a conservative approach, the constants for Cu(II) were inserted as Zn(II), meaning that the concentration of Zn(II) was used for this complex. This is conservative, because the constants for Zn(II) are by definition lower than those for Cu(II) (first hydrolysis constant for Zn(II) is one log unit lower than that of Cu(II)) and the free concentration of Cu(II) in blood plasma is five orders of magnitude lower than for Zn(II). The same approach was used for Ni(II), where the same constants were taken as for Mg(II). The results of the modelling were that 93.1 % of BIG stays unbound in blood plasma in the form of LH. 1.9 % formed a ternary complex of Mg(II), BIG and lactate. 4.4 % formed other Mg complexes. This is probably due to the overestimation for the Mg(II) complex by using the constants recorded for Ni(II). Therefore, substantial complexation by blood plasma occurs even when an MLH complex is included. The deductions above with regard to DMBG (structurally related to BIG) therefore seem to be correct.

2. Carbamic acid (CBA)

The protonation and formation constants found in the NIST database[15] are listed in Table 2. CBA (Fig. 1) could be viewed as a part of the BIU molecule. Constants for Zn(II) and Cd(II) were found. The same conservative approach was followed, taking Mg(II) instead of Cd(II). The results of the modelling were that 99 % of CBA stays unbound in blood plasma in the form of L. If Ca(II) is assigned the Cd(II) constants, 2.2 % Ca-CBA forms. Therefore, no substantial complexation by blood plasma occurs even when an ML complex is included. The deductions above with regard to BIU (structurally related to CBA) therefore seem to be correct.

3. N-Acetylthiourea (ATU)

The formation constants found in the NIST database[15] are listed in Table 2. ATU (Fig. 1) could be viewed as an analogue of ITB. Constants for Cu(II) were found, while the protonation constant for ITB was used, as the protonation constants for ATU in the NIST database are listed as non-critical. The same conservative approach was followed, taking Zn(II) instead of Cu(II). The results of the modelling were that 90 % of ATU stays unbound in blood plasma in the form of L, 1.9 % as HL, 3.4 % as ZnL_2 and the rest as various Zn(II) ternary complexes. This compares favourably with the calculated speciation for ITB (Fig. 7). Therefore, no substantial complexation by blood plasma occurs, even when an ML_x complex is included. The deductions above with regard to ITB (structurally related to ATU) therefore seem to be correct.

4. β-Alanine (β-ALA)

The protonation and formation constants found in the NIST database[15] are listed in Table 2. β-ALA (Fig. 1) could be viewed as a part of the CBIG molecule. Constants for Ni(II), Zn(II) and Cu(II) were found. The same conservative approach was followed, taking Ni(II) instead of Mg(II). The results of the modelling were that 95.8 % of β-ALA stays unbound in blood plasma in the form of HL, 3.4 % as MgL and the rest as various Mg(II) ternary complexes. This is probably due to the overestimation for the Mg(II) complex by using the constants recorded for Ni(II). This compares favourably with the calculated speciation for CBIG (Fig. 7). Therefore no substantial complexation by blood plasma occurs, even when an ML_x complex is included. The deductions above with regard to CBIG (structurally related to β-ALA) therefore seem to be correct.