

SUPPLEMENTARY INFORMATION: Measuring Biotic Ligand
Model (BLM) Parameters *In Vitro*: Copper and Silver Binding to
Rainbow Trout Gill Cells as Cultured Epithelia or in Suspension

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Calculation of Inorganic Bound Metal

In order to correct total metal for inorganic complexation National Institute of Standards (NIST) certified $\log K$ values were used^[?]. These corrections were used to determine free ion from total metal at low pH during the one-point internal calibration method, and to determine metal-cell binding isotherms. For silver the hydroxide and carbonate complexation are sufficiently weak that they were ignored. Chloride complexation of silver was also ignored because gluconate rather than chloride salts were used in solution preparations.

Determination of Cu chloride binding at low pH

Similar to Tait et al. (2015)^[?] the complexation of chloride at acidic pH is taken into account to correct total copper for CuCl^+ formation. Other ligand complexation can be neglected because the pH is too low for hydroxide or carbonate binding.

Assuming that free chloride is equal to the total chloride (Cl_T), because $[\text{Cl}^-] \gg \text{Cu}_T$ (total copper), and using the one to one formation constant $K_{\text{CuCl}} = 10^{0.3}$ it is possible to write the following expression to calculate $[\text{CuCl}^+]$:

$$[\text{CuCl}^+] = \frac{K * \text{Cl}_T * \text{Cu}_T}{1 + K_{\text{CuCl}} * \text{Cl}_T};$$

Determination of metal binding to cells at titration pH

For these calculations, measured $[\text{Cu}^{2+}]$ was used to determine each possible inorganic species from the certified $\log K$ values. The values (as association constants) used from NIST are listed below.

CuOH^+ $\log K = 6.48$

$\text{Cu}(\text{OH})_2^0$ $\log K = 11.78$

CuCl^+ $\log K = 0.30$

CuHCO_3^+ $\log K = 1.03$ (reaction $\text{Cu}^{2+} + \text{H}^+ + \text{CO}_3^{2-} \rightleftharpoons \text{CuHCO}_3^+$)

CuCO_3^0 $\log K = 6.55$

$\text{Cu}(\text{CO}_3)_2^{2-}$ $\log K = 9.92$

With the K values representing formation reactions the amount bound is simply the product of K times the measured metal free ion times the free anion concentration. The anion concentrations were determined from pH (i.e. $[\text{OH}]$) or by assuming free anion equals the total (i.e., chloride) or by equilibrium calculation assuming P_{CO_2} of $10^{-3.5}$ atm (i.e, carbonate). An example calculation

for copper carbonate complexation is

$$[\text{CuCO}_3] = 10^{6.55} [\text{Cu}^{2+}] [\text{CO}_3^{2-}]$$

For carbonic acid a pKa1 value of 6.3 was used and 10.3 for pKa2. The Henry's law constant was taken as $10^{-1.47}$ for pressure measured in atmospheres.

References

- [1] Martell AE, Smith RM. NIST Standard Reference Database 46 Version 8.0; 2004. Gaithersburg, MD 20899, USA. Database software developed by R. J. Motekaitis.

- [2] Tait NT, Rabson LM, Diamond RL, Cooper CA, McGeer JC, Smith DS. Determination of cupric ion concentrations in marine waters: an improved procedure and comparison with other speciation methods. *Env Chem.* 2015;13(1):140–148.