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Evaluation of the free ion activity model of metal-organism interaction: extension of the conceptual model

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Abstract

The present study integrates the concepts of the free ion activity model (FIAM) into biological receptor theory (BRT; i.e. pharmacodynamic principles) to obtain a more rigorous conceptual model; one that more precisely quantifies the interaction of chemical species at biological receptor sites. The developed model, which is viewed as an extended FIAM, explains the conditions under which the FIAM will be effective in explaining biological response (BR). It establishes that BR is directly proportional to the activity of the free metal ion in the linear regions of concentration-response curves only. Additionally, it indicates that {X-cell}, the activity of free surface sites on the cell membrane, does not need to be constant in the region of BR, as assumed by the original FIAM. The extended FIAM was tested by re-examining concentration-response data from the literature on aquatic organisms exposed to several ecotoxicologically-relevant trace metals. These data, which would be considered exceptions to the original FIAM, were found to be consistent with the extended FIAM. Due to its more rigorous conceptual basis, the extended FIAM is capable of modelling concentration-response experiments from a wider range of water chemistry conditions (i.e. varying pH, hardness and dissolved organic matter) than the original model and, as such, potentially provides a more useful tool for evaluating metal-organism interactions. This study proposes, for the first time, a quantitative method of uncoupling the biological effects of a metal hydroxide (1:1) complex from that of amelioration of the free metal ion (M^{z+}) by H^+. Since the activities of H^+ and metal-hydroxide cannot be independently varied, it has been previously very difficult to evaluate whether metal-hydroxide species contribute to eliciting a BR. Furthermore, the extended FIAM can directly derive fundamental information from concentration-response curves, such as the binding constants of H^+ or the hardness cations (Ca^{2+} and/or Mg^{2+}) to the cell membrane surface of aquatic organisms.

Keywords: Free ion activity model; Biological receptor theory; Metal speciation; Bioavailability; Concentration-response

1. Introduction

Trace metal interactions with aquatic organisms generally involve the following steps (Campbell, 1995):

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1. advection or diffusion of the metal in the bulk solution to the cell membrane surface;
2. sorption/surface complexation of the metal at binding sites on the cell membrane surface; and
3. uptake (transport) of the metal through the cell membrane into the organism.

Therefore, a metal must first interact with, or traverse, the cell membrane surface to elicit a biological response (BR).

The free ion activity model (FIAM) of metal-organism interaction was initially developed to rationalise experimental observations and to explain what was initially perceived as "the universal importance of free metal ion activities in determining the uptake, nutrition and toxicity of cationic trace metals" (Morel, 1983). It has been effective in explaining the central role of the activity of the free metal ion as a regulator of interactions (i.e. uptake or toxicity) between metals and aquatic organisms. Concepts of the FIAM have been comprehensively reviewed by Morel (1983), Pagenkopf (1983) and Campbell (1995).

The dependence of the free metal ion activity (M^{z+}) has often been misinterpreted to demonstrate that the species of a trace metal that binds to cell surface (transport) sites is the hydrated metal ion ($M^{z+}\cdot nH_2O$). Such a conclusion is not appropriate, since the activity of the free metal ion is simply a measure of its chemical reactivity at the cell surface, and is not equivalent to the concentration of the hydrated metal ion (Morel, 1983). The free metal ion (M) is believed to be in rapid equilibrium with cell surface binding sites (Morel, 1983). Certain metal species, however, are not able to react directly with cell surface binding sites, and thus, are considered biologically inactive. Such species include colloidal metals (Rich and Morel, 1990) and those complexed to strong organic ligands (Anderson and Morel, 1982).

Apparent exceptions to the FIAM will arise if, for example, a metal complex ML, where L is a ligand in the aquatic medium, reacts with the cell membrane surface to form a ternary surface complex. In this particular case, the BR will be proportional to the activity of ML and not to the activity of M (Campbell, 1995). Furthermore, if M also causes a BR, the overall response will be a weighted function of the activities of M and ML. For example, Wilkinson et al. (1990) explained the toxicity of Al, in the presence of increasing fluoride concentrations, to Atlantic salmon (*Salmo salar*) in terms of the species Al^{3+} and AlF^{2+}. Other apparent exceptions to the FIAM, which include inorganic and organic ligands forming lipophilic complexes, have been discussed by Campbell (1995).

Similar to the FIAM, biological-receptor theory (BRT), and its associated models, were developed to facilitate an understanding of the relationship between drug activities at the site of action (receptor) and pharmacological response, including biochemical and physiological effects that influence the interaction of a drug with the receptor (Kenakin, 1997). In essence, therefore, the FIAM and the BRT have been derived for the same purpose since, in this context, a metal can be thought of as a drug reacting at a cell surface. However, the FIAM and BRT differ with respect to their mathematical models and assumptions. Consequently, the primary aim of this study was to examine the conceptual development of the two models to determine how they differ and to determine the significance of the differences in terms of metal-organism interaction.

2. Development of the conceptual model

2.1. The assumptions of the free ion activity model and biological receptor theory

The key assumptions which underpin the FIAM have been given by Campbell (1995). These are:

(i) the cell membrane is the primary site for metal interactions with aquatic organisms;

(ii) the interaction of a free surface site on the cell membrane, $\{-\text{X-cell}\}$, with a metal, M^{z+}, can be described as a surface complexation reaction, given by Eq. (1) (charges here, and subsequently, will be omitted for brevity).

$$\text{M} + \text{X-cell} \overset{K_1}{\leftrightarrow} \text{M} - \text{X-cell} \quad (1)$$

The activity of the surface complex, $\{\text{M} - \text{X-cell}\}$, is therefore, given by

$$\{M-X\text{-cell}\} = K_1\{X\text{-cell}\}[M] \quad (2)$$

where K_1 is the conditional stability constant and the symbols {} and [ ] refer to the activities of surface and dissolved species, respectively;

(iii) rapid equilibrium is established between metal species in the aqueous medium and those at the cell membrane surface;

(iv) biological response, whether it be expressed as uptake/accumulation and/or lethal (i.e. survival) or sub-lethal (e.g. behaviour, growth or reproduction) toxicity, is directly proportional to the activity of the surface complex, M − X-cell. Therefore, BR can be derived from

$$BR = kK_1\{X\text{-cell}\}[M] \quad (3)$$

where k is a constant of proportionality;

(v) in the range of metal activities eliciting a BR, the activity of free surface sites, {X-cell}, remains virtually constant and the activity of the surface complex, M − X-cell, is directly proportional to $[M^{z+}]$ in solution; and

(vi) during exposure to a metal, the nature of the cell membrane surface remains unchanged.

Similarly, the key assumptions underpinning classical BRT have been outlined by Pfitzer and Vouk (1986) and Kenakin (1997). These are:

I. a surface receptor site binds reversibly with a metal to form a surface complex (this is consistent with assumption (ii) of the FIAM);
II. biological response results from the equilibrium or steady-state (pseudo-equilibrium) occupation of surface receptor sites, {X-cell} (this is consistent with assumption (iii) of the FIAM);
III. biological response is directly proportional to the fraction of the total number of surface receptor sites, $\{X\text{-cell}\}_T$, bound by the metal (i.e. {M − X-cell}) (this is similar to assumption (iv) of the FIAM);
IV. the maximum BR (BR_{max}) occurs when the total number of receptor sites on the cell membrane, $\{X\text{-cell}\}_T$, are bound by the metal; and
V. the activity of metal bound to surface receptor sites, {M − X-cell}, is small compared to the activity of the metal in the aqueous solution, [M].

In comparing the assumptions of the FIAM and classical BRT, an important contradiction arises. Assumption (v) of the FIAM states that the activity of free surface sites, {X-cell}, remains approximately constant in the region of BR (i.e. {X-cell} is independent of BR), whereas assumptions (iii) and (iv) of the BRT show that {X-cell} must decrease as the BR increases, and further, BR_{max} occurs when all cell surface sites are occupied (i.e. {X-cell} = 0). Despite this contradiction, the FIAM has been successfully used to explain the BR of a wide range of aquatic organisms exposed to a number of different metals (see review by Campbell (1995)). Therefore, it is necessary to examine why the FIAM is apparently successful, given that one of the key assumptions underpinning the model is paradoxical.

2.2. Derivation of the free ion activity model using biological receptor theory

Classical BRT uses the law of mass action, namely

$$\{X\text{-cell}\}_T = \{M-X\text{-cell}\} + \{X\text{-cell}\} \quad (4)$$

to derive an explicit expression for {M − X-cell}, as given by Eq. (5),

$$\{M-X\text{-cell}\} = \frac{K_1\{X\text{-cell}\}_T[M]}{1+K_1[M]} = \frac{\{X\text{-cell}\}_T[M]}{K_{-1}+[M]} \quad (5)$$

where K_{-1} (i.e. $1/K_1$) is the dissociation constant for Eq. (1). Eq. (5) was first used by Michaelis and Menten (1913) to describe the kinetics of enzymatic reactions, and Langmuir (1918) for gas adsorption on solid surfaces. The shape of the curve produced by Eq. (5) is hyperbolic and is often referred to as the occupancy relation or Langmuir curve (Kenakin, 1997). If BR is directly proportional to {M − X-cell} (assumption (iii) of classical BRT), then

$$BR = \frac{BR_{max}[M]}{K_{-1}+[M]} \quad (6)$$

where BR_{max} is the maximum BR induced when all cell receptor sites are occupied. It can be shown from Eq. (6) that at the activity of M which induces half of the maximal response (i.e.

BR_{50}), $[M] = K_{-1}$, and which has often been equated to BR_{50} (Black and Leff, 1983). Thus, Eq. (6) has been given as

$$BR = \frac{BR_{max}[M]}{BR_{50} + [M]} \quad (7)$$

which expresses BR in terms of [M], and is usually referred to as the E_{max} (BR_{max}) model (Wills, 1994). The model is not only justified on empirical grounds, but is an expression of classical BRT (Holford and Sheiner, 1982). However, the form of Eq. (7) is misleading and mathematically incorrect, since BR_{50} is a biological response whereas [M] and K_{-1} are concentration terms.

A number of experiments (see review by Kenakin (1997)) showing hyperbolic response curves, have found that BR_{max} occurs when the receptor sites are not totally occupied. Further interpretation of these experiments has shown that different metals can exert BR_{max} at a different percentage occupancy of receptor sites (see reviews by Black and Leff (1983) and Kenakin (1997)). Collectively, the results of such experiments demonstrate that a direct proportionality between BR and {M − X-cell} is not always appropriate. If BR is not directly proportional to {M − X-cell}, then all concentration-response curves cannot show the same characteristics as the occupancy relation (Eq. (5)). Thus, the BR_{50} will not be equal to K_{-1} for every metal and a 'receptor reserve' should exist (i.e. the presence of receptor sites that are not occupied by M at BR_{max}; Black and Leff, 1983; Kenakin, 1997). Therefore, it is necessary to derive another numerical relationship between BR and {M − X-cell} that is consistent with the concept of 'receptor reserves'.

Non-classical BRT modifies classical theory by relating BR to {M − X-cell} in the same way that {M − X-cell} is related to [M] (i.e. the hyperbolic occupancy relation (Eq. (5)); the first part of classical BRT). This is the only other functional form (apart from the linear form) which will preserve the hyperbolic nature of the BR_{max} model (Black and Leff, 1983). It is well established [see reviews by Black and Leff (1983) and Kenakin (1997)] that two (or more) successive hyperbolic functions, result in a function which also has a hyperbolic form. Thus,

$$BR = \frac{BR_{max}\{M - X\text{-cell}\}}{K_E + \{M - X\text{-cell}\}} \quad (8)$$

where K_E is the concentration of M − X-cell which causes half of the maximal biological response (BR_{max}; Kenakin, 1997). Substitution of Eq. (5) into Eq. (8) gives

$$BR = \frac{BR_{max}\{X\text{-cell}\}_T[M]}{K_E K_{-1} + (\{X\text{-cell}\}_T + K_E)[M]} \quad (9)$$

and it is apparent that Eq. (9) has the same form as Eq. (6) and, as such, preserves the hyperbolic (Langmuir) form of the BR_{max} model.

Often, the concentration-response curve has a more pronounced S-shape (sigmoid) and is not adequately described by the hyperbolic BR_{max} model (Wills, 1994). To improve the fit of sigmoidal concentration-response curves, Wagner (1968) and Holford and Sheiner (1982) utilised the Hill equation (Hill, 1910) to produce the sigmoid BR_{max} model. To account for this behaviour, Eq. (8) is converted into the general logistic form:

$$BR = \frac{BR_{max}\{M - X\text{-cell}\}^n}{K_E^n + \{M - X\text{-cell}\}^n} \quad (10)$$

where the exponent, n, determines the slope of the concentration-response curve (Fig. 1), but is not generally considered to have any physiological meaning (Wills, 1994). Comparing Eqs. (8) and (10) it can be seen that when $n = 1$, Eq. (10) reduces to Eq. (8). Substitution of Eq. (5) into Eq. (10) yields

$$BR = \frac{BR_{max}\{X\text{-cell}\}_T^n[M]^n}{K_E^n(K_{-1} + [M])^n + \{X\text{-cell}\}_T^n[M]^n} \quad (11)$$

thereby expressing BR in terms of [M] for the general case. Typical shapes of theoretical concentration-response curves, for different values of n, are illustrated in Fig. 1.

As shown in Fig. 1, regions of the theoretical concentration-response curves can be approximated by linear equations; that is, BR is directly proportional to the activity of M. Further, the range of the linear region (which includes the BR_{50}) increases with increasing values of n. To determine the significance of these linear regions, Eq. (11) needs to be examined more closely. In the case where $n = 1$, for example, and at small activities of M (Fig. 1(a)), $K_{-1} \gg [M]$, Eq. (11) (or Eq. (9)) reduces to

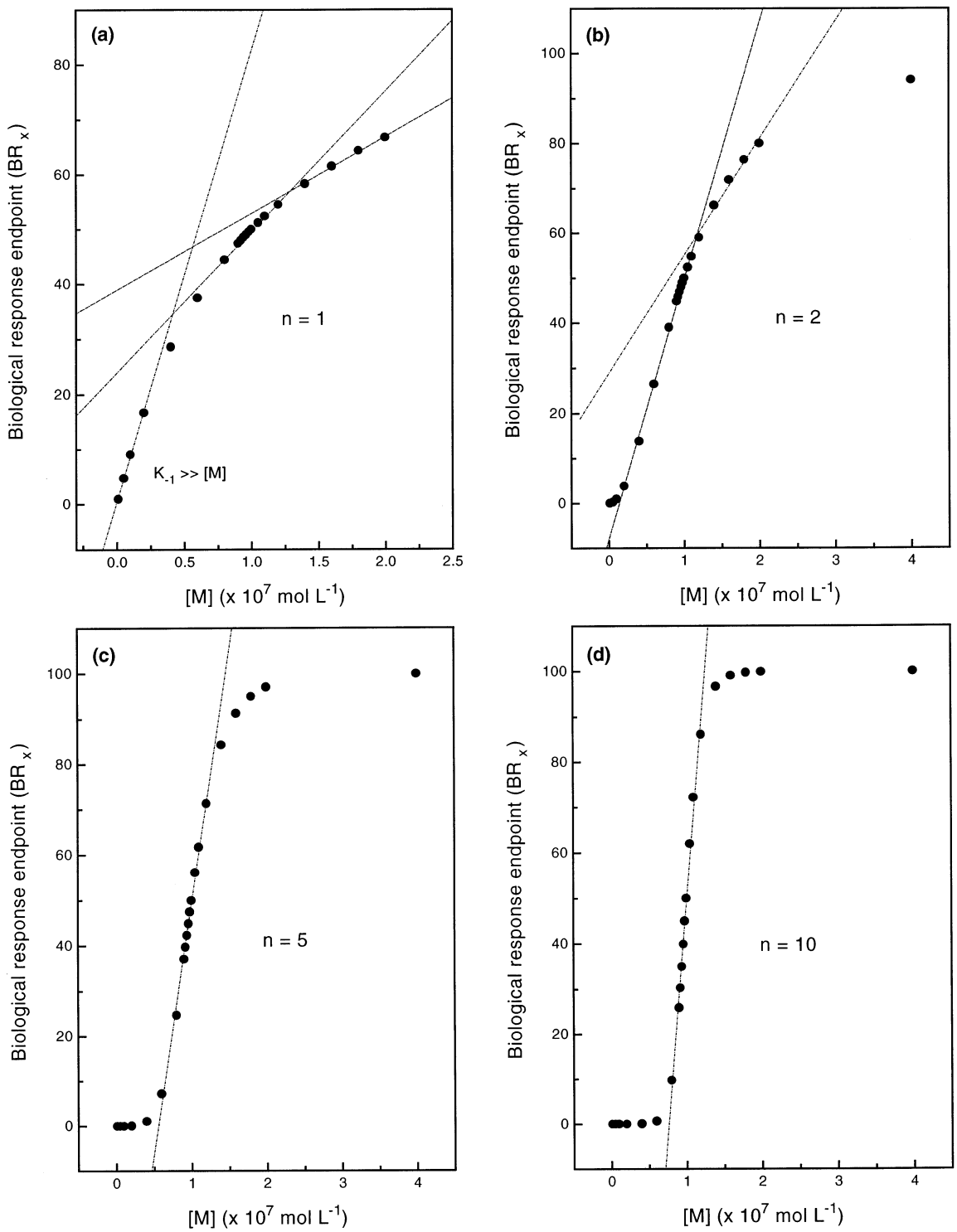

Fig. 1. Theoretical hyperbolic or sigmoidal concentration-response curves for different slope values ($n = 1$ (a); $n = 2$ (b); $n = 5$ (c); and $n = 10$ (d)) indicating various linear regions (dotted lines).

$$BR = (BR_{max}/K_E)K_1\{X\text{-cell}\}_T[M] \quad (12)$$

which is analogous to the FIAM, given by Eq. (3). For other linear regions of the concentration-response curve where $n = 1$ (Fig. 1(a)), the two equations comprising Eq. (11), namely Eqs. (5) and (8), will be examined. It follows from Eq. (8), that for any BR endpoint (e.g. BR_{25}) the activity of $M - X$-cell is related to K_E, as shown in Eq. (13)

$$\{M - X\text{-cell}\} = \frac{\chi_1}{1-\chi_1}K_E \quad (13)$$

where χ_1 is the fraction of the maximum response (BR_{max}) induced by $\{M - X\text{-cell}\}$ (i.e. $BR_{100\chi_1}$). Similarly, from Eq. (5), [M] is related to the value of the constant K_{-1}, as given in Eq. (14)

$$[M] = \frac{\chi_2}{1-\chi_2}K_{-1} \quad (14)$$

where χ_2 is the fraction of cell surface sites required to be occupied (complexed) to elicit $BR_{100\chi_1}$. Substitution of Eqs. (13) and (14) into Eqs. (8) and (5), respectively, yields

$$BR_{100\chi_1} = (BR_{max}/K_E)(1-\chi_1)\{M - X\text{-cell}\} \quad (15)$$

and

$$\{M - X\text{-cell}\} = (1-\chi_2)K_1\{X\text{-cell}\}_T[M] \quad (16)$$

It follows, therefore, that for any linear region of the concentration-response curve where $n = 1$ (Fig. 1(a)), the BR will be given by

$$BR = a + bK_1\{X\text{-cell}\}_T[M] \quad (17)$$

where a and $bK_1\{X\text{-cell}\}_T$ are the intercept and slope, respectively, of the line of best fit for the region. The magnitude of a and b depends on the location of the linear approximation of the hyperbolic curve (Fig. 1(a)), and therefore, incorporates the parameter χ_1 and the multiplication factor (BR_{max}/K_E) given in Eq. (15) and χ_2 given in Eq. (16).

For concentration-response curves where $n > 1$ (Fig. 1(b–d)), Eq. (17) is also applicable for any linear region, irrespective of the value of n. In such cases, as was shown for the case where $n = 1$, the activity of $M - X$-cell at any BR endpoint is related to K_E. The general equation for $n > 1$ is given by Eq. (18).

$$\{M - X\text{-cell}\} = \left(\frac{\chi_1}{1-\chi_1}\right)^{1/n}K_E \quad (18)$$

Substitution of Eq. (18) into Eq. (10) yields

$$BR_{100\chi_1} = \left(\frac{BR_{max}}{K_E}\right)\chi_1^{(n-1)/n}[(1-\chi_1)^{1/n}]\{M - X - \text{cell}\} \quad (19)$$

which reduces to Eq. (15) when $n = 1$. Thus, in the general case, the values of a and b in Eq. (17) will also be a function of n. Furthermore, for concentration-response curves where n is large ($n \geq 5$), the value of the slope ($bK_1\{X\text{-cell}\}_T$) given in Eq. (17) is related to the value of n, since n determines the steepness of the sigmoidal concentration-response curve. This implies a potential relationship between the value of n and the magnitude of the metal binding constant (K_1) at the cell membrane surface. Eq. (17), like Eq. (12), is also analogous to the mathematical description of the FIAM (Eq. (3)). However, {X-cell} in Eq. (3) is replaced by $\{X\text{-cell}\}_T$ in Eq. (17). This infers, therefore, that rather than the necessity of {X-cell} being constant in the region of BR (assumption (v) of the FIAM, but contrary to assumption (iii) of the BRT), Eq. (17) identifies no such dependence on {X-cell}. Instead, the equation introduces $\{X\text{-cell}\}_T$ which is, by definition, a constant.

Eq. (17) also identifies that for the linear regions of concentration-response curves, BR is directly proportional to the activity of M. Although this proportionality is implicit in the development of the FIAM, the formulation of Eq. (17) establishes that the proportionality only holds for linear regions of concentration-response curves, and not necessarily the whole of the curve, as might be implied by the FIAM. Thus, the operational success of the FIAM in predicting BR will increase as the slope (n) of the concentration-response curve also increases. Additionally, at large values of n ($n \geq 5$), the FIAM will be more effective in predicting BR in regions of lower response (i.e. BR_{10}), than it will at higher response (i.e. BR_{90}; Fig. 1(c, d)). Therefore, Eq. (17) can be thought of as a modified form of the FIAM, derived from BRT, and which, explains the conditions under which the FIAM will be effective in explaining BR. Since the most common form of concentration-response curves in aquatic toxicology is sig-

moidal, with large slopes ($n \geq 5$) (Rand et al., 1995), it is not surprising, therefore, that the FIAM has largely been successful in interpreting the BR of a wide range of aquatic organisms.

2.3. Amelioration of biological response by protons or hardness cations

To examine whether the binding of M^{z+} at the cell membrane surface is ameliorated by H^+ or hardness cations (Ca^{2+} and/or Mg^{2+}), equations describing the interaction of these three cations with the cell membrane surface need to be considered (Pagenkopf, 1983). These equations will have the same form as Eqs. (1) and (2) for the metal ion. The interaction of H^+ with the cell membrane surface is described in Eqs. (20) and (21). Identical equations (not given) can also be derived for the hardness cations (Pagenkopf, 1983).

$$H + \text{X-cell} \overset{K_2}{\leftrightarrow} H - \text{X-cell} \tag{20}$$

$$\{H - \text{X-cell}\} = K_2\{\text{X-cell}\}[H] \tag{21}$$

From the law of mass action, the total activity of surface sites, $\{\text{X-cell}\}_T$, is given by

$$\{\text{X-cell}\}_T = \{\text{X-cell}\} + \{H - \text{X-cell}\} + \{M - \text{X-cell}\} \tag{22}$$

and by substitution of Eqs. (2) and (21) into Eq. (22), the activity of the surface complex, $M - X$-cell, is given by

$$\{M - \text{X-cell}\} = \frac{K_1\{\text{X-cell}\}_T[M]}{1 + K_1[M] + K_2[H]} \tag{23}$$

By analogy with the theoretical development of $\{M - X\text{-cell}\}$ in the absence of $\{H - X\text{-cell}\}$ (i.e. Eq. (3)), it follows here that the BR will be given by

$$BR = a + \frac{bK_1\{\text{X-cell}\}_T[M]}{1 + K_2[H]} \tag{24}$$

which has the same form as the equation developed by Pagenkopf (1983). For any particular BR ($BR_{100\chi_1}$: a constant), Eq. (24) can be rewritten as

$$[M] = \frac{BR_{100\chi_1} - a}{bK_1\{\text{X-cell}\}_T} + \frac{(BR_{100\chi_1} - a)K_2}{bK_1\{\text{X-cell}\}_T}[H] \tag{25}$$

Thus, if [M] is ameliorated by [H] then, for a given BR (e.g. EC_{50}), Eq. (25) shows that a linear relationship should exist between [M] and [H], with a slope of $(BR_{100\chi_1} - a)K_2/bK_1\{\text{X-cell}\}_T$ and an intercept of $(BR_{100\chi_1} - a)/bK_1\{\text{X-cell}\}_T$. This also applies for the hardness cations, [Ca] and/or [Mg].

2.4. Biological response induced by metal species additional to M^{z+}

To examine whether metal species other than the free metal ion, M, elicit a BR, it is necessary to derive equations that describe the reaction of a metal complex, ML, with the cell membrane surface. These equations are similar to those developed for both the metal ion (Eq. (3)) and the proton (Eq. (21)), namely

$$ML + \text{X-cell} \overset{K_3}{\leftrightarrow} LM - \text{X-cell} \tag{26}$$

As can be seen from Eq. (26), the reaction, in this case, does not involve ligand exchange; that is, the ligand, L, remains bound to the metal ion after the latter has reacted with the cell membrane surface. From Eq. (26), an expression for the activity of the surface complex can be derived as follows

$$\{LM - \text{X-cell}\} = K_3\{\text{X-cell}\}[ML] \tag{27}$$

However, the reaction of M with L, to give ML, can be written as

$$M + L \overset{K_4}{\leftrightarrow} ML \tag{28}$$

from which the following expression can be obtained

$$[ML] = K_4[M][L] \tag{29}$$

Substitution of Eq. (29) into Eq. (27), leads to the following expression for $\{LM - X\text{-cell}\}$.

$$\{LM - \text{X-cell}\} = K_3K_4\{\text{X-cell}\}[M][L] \tag{30}$$

Again, from the law of mass action, the total activity of surface sites, $\{\text{X-cell}\}_T$, is given by

$$\{\text{X-cell}\}_T = \{\text{X-cell}\} + \{M - \text{X-cell}\} + \{LM - \text{X-cell}\} \tag{31}$$

By analogy with the theoretical development of Eqs. (5) and (23), expressions for $\{M - X\text{-cell}\}$

and {LM − X-cell} are given by Eqs. (32) and (33), respectively.

$$\{\mathrm{M-X\text{-}cell}\} = \frac{K_1\{\mathrm{X\text{-}cell}\}_T[\mathrm{M}]}{1 + K_1[\mathrm{M}] + K_3K_4[\mathrm{M}][\mathrm{L}]} \quad (32)$$

$$\{\mathrm{LM-X\text{-}cell}\} = \frac{K_3K_4\{\mathrm{X\text{-}cell}\}_T[\mathrm{M}][\mathrm{L}]}{1 + K_1[\mathrm{M}] + K_3K_4[\mathrm{M}][\mathrm{L}]} \quad (33)$$

The total activity of M bound to cell membrane surface sites will be the sum of Eqs. (32) and (33), as shown in Eq. (34).

$$\{\mathrm{M-X\text{-}cell}\} + \{\mathrm{LM-X\text{-}cell}\} = \frac{\{\mathrm{X\text{-}cell}\}_T(K_1 + K_3K_4[\mathrm{L}])[\mathrm{M}]}{1 + K_1[\mathrm{M}] + K_3K_4[\mathrm{M}][\mathrm{L}]} \quad (34)$$

By analogy with the theoretical development of Eq. (17), an expression for BR is derived in Eq. (35).

$$\mathrm{BR} = a + b(K_1 + K_3K_4[\mathrm{L}])\{\mathrm{X\text{-}cell}\}_T[\mathrm{M}] \quad (35)$$

For any particular BR ($BR_{100_{\chi_1}}$: a constant), Eq. (35) can also be rewritten as

$$\frac{1}{[\mathrm{M}]} = \frac{bK_1\{\mathrm{X\text{-}cell}\}_T}{BR_{100_{\chi_1}} - a} + \frac{bK_3K_4\{\mathrm{X\text{-}cell}\}_T}{BR_{100_{\chi_1}} - a}[\mathrm{L}] \quad (36)$$

Thus, Eq. (36) shows that if both [M] and [ML] elicit a given BR (e.g. EC_{50}), a linear relationship should exist between 1/[M] and [L], with a slope of $bK_3K_4\{\mathrm{X\text{-}cell}\}_T/(BR_{100_{\chi_1}} - a)$ and an intercept of $bK_1\{\mathrm{X\text{-}cell}\}_T/(BR_{100_{\chi_1}} - a)$.

An equivalent way of expressing Eq. (35), which warrants some attention, is given in Eq. (37).

$$\mathrm{BR} = a + bK_1\{\mathrm{X\text{-}cell}\}_T[\mathrm{M}] + bK_3\{\mathrm{X\text{-}cell}\}_T[\mathrm{ML}] \quad (37)$$

This equation indicates that, for all BR values over a linear range, multiple linear regression analysis can be used to demonstrate that M and ML jointly elicit a BR. Although Eq. (37) has been developed specifically for ML (i.e. metal complexes with a 1:1 stoichiometry, e.g. UO_2OH^+, $A1F^{2+}$, and similar complexes with both inorganic and organic ligands), it can be generalised to include other metal complexes, such as M_pL_q (e.g. $Cu_2(OH)_2^{2+}$ and $Al_{13}(OH)_{32}^{7+}$ etc.) or ML_1L_2 (e.g. $UO_2(OH)Cit^{2-}$ or $Cu(OH)Mal^-$; where Cit and Mal are citrate and malonate, respectively). The form of the model given in Eq. (37) assumes that M^{z+} and one other metal species jointly predict BR. This represents the simplest model (i.e. conforms with Ockam's razor) and is consistent with the results of previous studies (Section 3). More importantly, Eq. (37) extends the modified FIAM developed in Section 2.2, to account for a BR elicited by the free metal ion, M^{z+}, together with one other metal species. A more generalised form of this equation may also be derived to account for a BR elicited by the free metal ion together with two or more metal species (see Markich (1998) for further discussion).

2.5. Uncoupling the biological effects of a metal hydroxide (MOH) complex from amelioration of the free metal ion (M) by protons

In discussing the biological effects of a metal hydroxide (MOH) complex, it is important to state that mechanistically the MOH complex can only elicit a BR if M also elicits a response. The free metal, M, forms a stronger complex at the cell membrane surface than MOH (Markich et al., 2000). Thus, the biological effects of MOH complexes can only be considered in conjunction with those elicited by M. Nevertheless, it is perhaps counter-intuitive that MOH binding at the cell membrane surface can be uncoupled from the amelioration of M by H, since the formation of MOH itself is dependent on H. Let us suppose that metal binding to a cell surface can be represented by the following four equations (charges omitted for brevity):

$$\mathrm{Cell}\begin{bmatrix} \mathrm{Z} \\ \\ \mathrm{YH} \end{bmatrix} + \mathrm{M} \Leftrightarrow \mathrm{Cell}\begin{bmatrix} \mathrm{Z} \\ \\ \mathrm{YH} \end{bmatrix} \quad (38)$$

(I)

$$\mathrm{Cell}\begin{bmatrix} \mathrm{Z} \\ \\ \mathrm{YH} \end{bmatrix}\mathrm{M} + \mathrm{OH} \Leftrightarrow \mathrm{Cell}\begin{bmatrix} \mathrm{Z} \\ \mathrm{YH} \end{bmatrix}\mathrm{MOH} \quad (39)$$

(II)

$$\text{Cell}\begin{bmatrix} Z \\ \\ YH \end{bmatrix}M + OH \Leftrightarrow \text{Cell}\begin{bmatrix} Z \\ \\ YH \end{bmatrix}M + H_2O \quad (40)$$

(III)

$$\text{Cell}\begin{bmatrix} Z \\ \\ YH \end{bmatrix}M + H \Leftrightarrow \text{Cell}\begin{bmatrix} Z \\ \\ YH \end{bmatrix}H + M \quad (41)$$

(IV)

Eqs. (38)–(41) assume that the binding of metal, M, involves at least two distinct functional groups on the cell surface, Z and Y, and that at least one of these (Y) is subject to protonation in the pH range of interest. Eq. (38) corresponds to a simple reaction involving X-cell and M (i.e. Eq. (1)), Eq. (39) corresponds to a modified simple reaction between X-cell and MOH, and is thermodynamically equivalent to Eq. (40), and Eq. (41) corresponds to proton competition with M for binding to X-cell. It is assumed that surface complex (I) contributes to the BR, as do surface complexes (II) or (III).

Acidification of the system will drive Eqs. (39) and (40) to the left, and Eq. (41) to the right, leading to decreased BR. Therefore, Eqs. (39) and (40) will give rise to the same relationship between BR and [H]. However, mechanistically, this relationship will differ from that given by Eq. (41). It is evident that Eqs. (39) and (40) show a dependence on the concentration of OH^-, whereas Eq. (41) shows a dependence on the concentration of H^+. Indeed, Eqs. (39) and (40) imply that MOH is ameliorated by H at the cell surface, whereas Eq. (41) identifies that M is ameliorated by H at the cell surface. Since these equations have a different functional dependence (i.e. OH^- and H^+), it allows their individual effects to be uncoupled, as indicated by the differences between Eqs. (25) and (36). Furthermore, even though OH^- and H^+ are inversely related to one another, it can be shown mathematically that a direct dependence of M on H is not equivalent to a direct dependence of 1/M on OH.

3. Literature examples that support the extended FIAM

The original FIAM postulates that BR, whether it be uptake or toxicity, is proportional to the activity of the free metal ion, M^{z+} (Morel, 1983; Campbell, 1995). Data that do not fit this postulate are typically regarded as 'apparent exceptions' to the FIAM (Campbell, 1995). Although a number of apparent exceptions to the original FIAM have been reported in the literature, these are few in comparison to studies that support the original FIAM. Apparent exceptions may be classified as:

1. Hydrophilic metal complexes, including inorganic (e.g. AlF^{2+}: Wilkinson et al. (1990); $CuOH^+$: Blust et al. (1991); UO_2OH^+: Markich et al. (2000)) and organic (e.g. $CdCit^-$: Pärt and Wikmark (1984); $CuGly^+$: Daly et al. (1990); $ZnHis^+$: Vercauteren and Blust (1996); where Gly is glycine and His is histidine) species; and
2. Lipophilic, neutral, metal complexes (e.g. Cu-oxine: Ahsanullah and Florence (1984) and Florence and Stauber (1986); Cd-xanthate: Block and Wicklund-Glynn (1992); Pb-carbamate: Phinney and Bruland (1994); AgCl, Engel et al. (1981) and $HgCl_2$; Mason et al. (1996)).

Assumption (ii) of both the FIAM and BRT (Section 2.1) state that metals are presumed to exist in the exposure solution as hydrophilic species. Therefore, lipophilic metal species, that might traverse the cell membrane without first forming a surface complex, are not considered. Consequently, only the apparent exceptions that relate to hydrophilic metal complexes will be discussed.

Two potentially different mechanisms may be operating at the cell membrane surface with respect to hydrophilic metal complexes, but with the same end result. Work by Wilkinson et al. (1990) and Markich et al. (2000) indicates that inorganic ligands, such as F^- or OH^-, may form a ternary metal complex at the cell membrane surface, L – M – X-cell, but not traverse the cell membrane. Alternatively, work by Vercauteren and Blust

(1996) and Errécalde et al. (1998) indicates that small molecular weight organic ligands, such as histidine or citrate, accidentally traverse the cell membrane with the metal (ML). In such cases, it is proposed that the ligands are also metabolites for which exists a separate binding site and a separate membrane transport system (e.g. permease). Further work is required to elucidate these two potential mechanisms.

Five case examples of apparent exceptions to the original FIAM, in addition to one case example, which is consistent with the original FIAM, are outlined below. All examples, however, are consistent with the extended FIAM developed in this study. In the first case example, only the free metal ion elicits a BR. In the next three cases examples, the free metal ion and a metal complex elicit a BR by forming complexes at the cell membrane surface; thus, BR is explained jointly by the activities of the free metal ion and an additional metal complex. In the last two case examples, BR is explained by either protons (H^+) or the hardness ion, Ca^{2+}, ameliorating the complexation of the free metal ion at the cell membrane surface.

3.1. Case example 1 — free metal ion

Markich et al. (2000) demonstrated that the valve movement response of the freshwater bivalve, *Velesunio angasi*, to Mn, under conditions of varying pH and/or fulvic acid concentration, was directly proportional to the activity of Mn^{2+}. However, their analysis of the concentration-response data showed that direct proportionality was valid only between the EC_{10} and EC_{75}. As such, the valve movement response of *V. angasi* to Mn exposures is consistent with the original FIAM only between these biological endpoints, and not the whole range of biological response (i.e. entire concentration-response curve), as might be implied by the original FIAM. As indicated in Section 2.2, the formulation of Eq. (17) establishes that the proportionality between M^{z+} and BR only holds for linear regions of concentration-response curves. Therefore, the BR of *V. angasi* to Mn exposures is completely consistent with the extended FIAM.

3.2. Case example 2 — free metal ion + an inorganic metal complex

Markich et al. (2000) demonstrated that the valve movement response of *V. angasi* to U, under conditions of varying pH and/or fulvic acid concentration, was clearly an exception to the original FIAM, since both UO_2^{2+} and UO_2OH^+ caused a BR. However, the authors did not demonstrate whether or not H^+ was ameliorating the binding of UO_2^{2+} at the cell membrane surface. If UO_2^{2+} was ameliorated by H^+, then a linear relationship should exist between the activity of UO_2^{2+} and H^+, as shown by Eq. (25). A plot of UO_2^{2+} versus H^+, for the EC_{20}, EC_{50} and EC_{80} values given in Table 6 of Markich et al. (2000), is shown in Fig. 2. Clearly, from the non-linearity of the plot, in the pH range examined in the study, there is indeed no convincing evidence to suggest that H^+ reduces the binding of UO_2^{2+} at the cell membrane surface.

Conversely, if a BR was caused by both UO_2^{2+} and UO_2OH^+, then Eq. (36) requires that a plot of $1/[UO_2^{2+}]$ versus $[OH^-]$, for a particular BR (e.g. EC_{50}), should be linear. Fig. 3 confirms such a relationship for the EC_{20}, EC_{50} and EC_{80}; the highly linear fits ($r^2 > 0.997$; $P \leq 0.001$) demonstrate that the valve movement responses of *V. angasi* can be explained by the combined activities of $(1.86)UO_2^{2+}$ and UO_2OH^+ (Markich et al., 2000). Thus, the valve movement responses of *V. angasi* to U, although an exception to the original FIAM, are clearly consistent with the extended FIAM. Valve movement response was found to be directly proportional to the weighted function of the sum of the activities of UO_2^{2+} and UO_2OH^+ between the EC_{10} (BR_{10}) and EC_{80} (BR_{80}). More importantly, however, the results of Markich et al. (2000) demonstrate that the biological effects of a metal hydroxide species $[MOH^{(z-1)+}]$ (e.g. UO_2OH^+) can be uncoupled from the amelioration of the free metal ion (e.g. UO_2^{2+}) by H^+.

3.3. Case example 3 — free metal ion + an inorganic metal complex

Wilkinson et al. (1990) studied the effect of

fluoride complexation of Al on the mortality of juvenile Atlantic salmon (*Salmo salar*). From 168 h (7 days) static-renewal toxicity tests, they showed that the toxicity of Al to *S. salar* was enhanced in the presence of fluoride. Based on the results of multiple linear regression analysis, the authors postulated that both Al^{3+} and AlF^{2+} were jointly responsible for explaining the toxicity

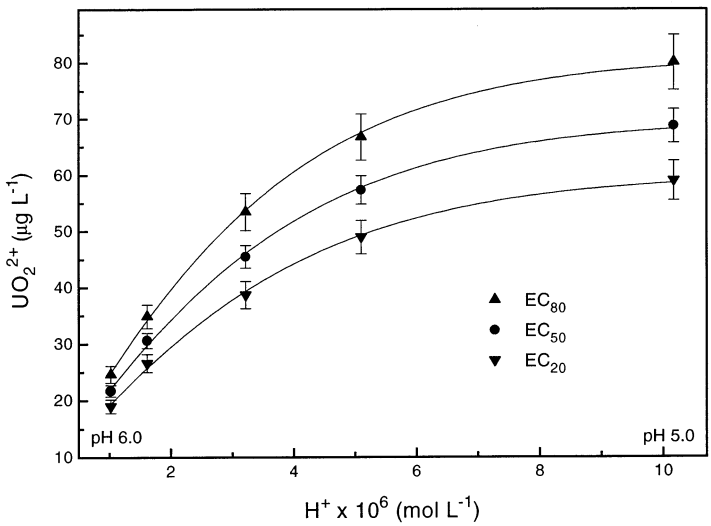

Fig. 2. Non-linear relationships between UO_2^{2+} and H^+ for *V. angasi* at different biological response endpoints (EC_{20}, EC_{50} or EC_{80}). Each plotted point represents the mean (and 95% confidence limit) response of six (pH 5.3 and 5.8) or 18 (pH 5.0, 5.5 and 6.0) individuals.

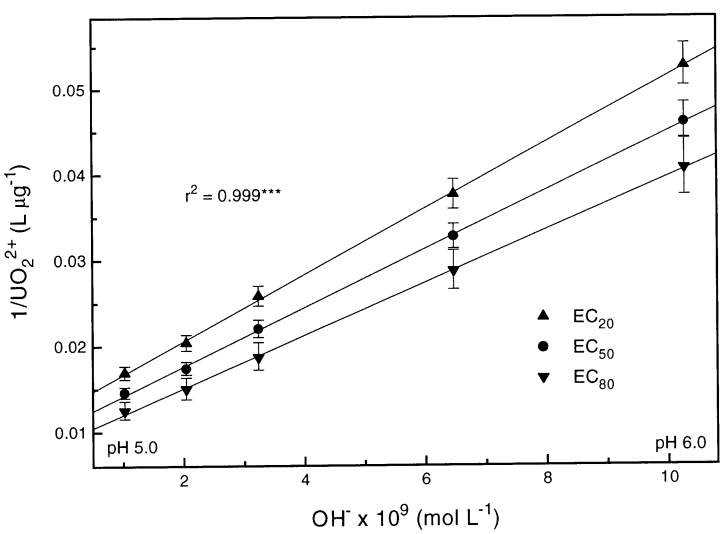

Fig. 3. Linear regressions between $1/[UO_2^{2+}]$ and $[OH^-]$ for *V. angasi* at different biological response endpoints (EC_{20}, EC_{50} or EC_{80}). Each plotted point represents the mean (and 95% confidence limit) response of six (pH 5.3 and 5.8) or 18 (pH 5.0, 55 and 6.0) individuals. ***$P \leq 0.001$.

of inorganic Al to *S. salar* ($r^2 = 0.93$, $P \leq 0.01$), namely:

$$\text{Predicted mortality (\%)} = 16.3[Al^{3+}] + 10.3[AlF^{2+}] \quad (42)$$

A second equation, however, was required to relate the predicted mortality with observed mortality, namely

$$\text{Observed mortality (\%)} = 1.5[\text{Predicted mortality (\%)}] - 33 \quad (43)$$

The combination of Eqs. (42) and (43) gives

$$\text{Mortality (\%)} = 24.5[Al^{3+}] + 15.5[AlF^{2+}] - 33 \quad (44)$$

which has exactly the same form as Eq. (37) developed above; in this case, BR is expressed in terms of mortality, rather than valve movement response (Markich et al., 2000). The y-intercept of the multiple linear regression equation given in Eq. (44) indicates that a concentration threshold exists, below which no mortality is observed. The ratio of the regression coefficients (i.e. binding constants at the cell membrane surface) for Al^{3+} and AlF^{2+} is 1.6; that is, Al^{3+} binds more strongly than AlF^{2+} at the cell membrane surface.

It follows from Eq. (44) that the mortality of *S. salar* is primarily dependent on the activity of Al^{3+} and secondarily dependent on the activity of fluoride. The secondary dependence on $[F^-]$, however, does not imply that fluoride alone will be toxic to *S. salar*, since an examination of Eq. (37) shows that if [M] (i.e. $[Al^{3+}]$) equals zero then no adverse BR (i.e. mortality) would occur. Further, in accord with Eq. (36), a plot of (% mortality)/$[Al^{3+}]$ versus $[F^-]$, at a fixed mortality (%), will be linear. However, based on the data of Wilkinson et al. (1990), BR appears to diverge from linearity at high % mortalities (80–100%), a finding consistent with that of bivalves exposed to Mn (Markich et al., 2000). Overall, the study of Wilkinson et al. (1990) is consistent with the extended form of the FIAM proposed in this study. Wilkinson et al. (1993) subsequently provided physicochemical evidence (NMRS and electrophoresis) for the formation of F – Al – X-cell surface complexes.

3.4. Case example 4 — free metal ion + an organic metal complex

Vercauteren and Blust (1996) determined the effect of complexation of organic ligands (ethylenediamine tetraacetic acid (EDTA), nitrilotriacetic acid (NTA), histidine, citrate and glycine) on the uptake rate of Zn by the marine bivalve, *Mytilus edulis*, in synthetic seawater (salinity 35‰, pH 8.0, 15°C) over 24 h. The authors showed that in the presence of either EDTA, NTA, citrate or glycine, the rate of Zn uptake by *M. edulis* was directly proportional to the activity of Zn^{2+}. However, in the presence of histidine, the activity of Zn^{2+} alone did not explain the majority of variability in the Zn uptake rate. Vercauteren and Blust (1996) found that correlations between the activity of $ZnHis^+$ and Zn uptake rate were significantly ($P \leq 0.05$) positive and concluded, therefore, that $ZnHis^+$ promotes Zn uptake. Based on the results of multiple linear regression analysis, the authors postulated that the activities of Zn^{2+} and $ZnHis^+$ were jointly responsible for explaining the majority of variability ($r^2 = 0.84$–0.87; $P \leq 0.001$) in the rate of Zn uptake by *M. edulis*, namely

$$\text{Zn uptake} = C_1[Zn^{2+}] + C_2[ZnHis^+] \quad (45)$$

where C_1 and C_2 are constants, which depend on the site of uptake (i.e. gills, digestive system or hemolymph). Eq. (45) is clearly in accord with Eq. (37). The y-intercept value is zero in Eq. (45), since uptake will occur even at very low concentrations of aqueous Zn; a marked contrast with metal toxicity studies (Wilkinson et al., 1990), including valve movement response (Markich et al., 2000). Further, in accord with Eq. (36), a plot of (uptake rate)/$[Zn^{2+}]$ versus $[His^-]$ will be linear at a fixed uptake rate. Again, the study of Vercauteren and Blust (1996) is consistent with the extended form of the FIAM proposed in this study.

Conflicting results have been reported in the literature on the uptake and toxicity of hydrophilic metal-organic complexes in aquatic or-

ganisms (Campbell, 1995). In the majority of studies, the presence of organic complexes reduces the uptake/toxicity of the relevant metals to the organisms. However, some studies have shown that some organic complexing agents, such as citrate or histidine, enhance the uptake and/or toxicity of metals in aquatic organisms. These latter studies are considered exceptions to the original FIAM (Campbell, 1995).

Two potentially different mechanisms may be operating at the cell membrane surface with respect to hydrophilic metal complexes, but with the same end result. Work by Wilkinson et al. (1990) and Markich et al. (2000) indicates that some ligands can form ternary metal complexes at the cell membrane surface, but may not traverse the cell membrane. Alternatively, work by Errécalde et al. (1998) indicates the metal might accidentally traverse the cell membrane with small molecular weight organic ligands, such as citrate. In such cases, it is proposed that the ligands are metabolites for which there are separate binding sites and separate membrane transport systems (e.g. permease). Further work is required to elucidate these two potential mechanisms. Nevertheless, the current extended FIAM could be further developed to include different binding sites at cell membrane surfaces.

3.5. Case example 5 — amelioration by protons

Cusimano et al. (1986) determined the effects of pH (4.7, 5.7 and 7.0) on the survival (LC_{50}) of juvenile steelhead trout (*Oncorhynchus mykiss*, formerly *Salmo gairdneri*) exposed to Cd, Cu and Zn in soft water over 168 h (7 days). The LC_{50} values of Cd, Cu and Zn increased by a factor of 23, 23 and 8, respectively, as H^+ increased (pH decreased) 200-fold. However, based on results of geochemical speciation modelling of the test waters, the activities of Cd^{2+} and Zn^{2+} remained constant (ca. 97% of total Cd and Zn) across the range of LC_{50} values from pH 4.7 to 7.0. Therefore, according to Eq. (25), a linear relationship should exist between the activities of Cd^{2+} or Zn^{2+} and H^+, if both metal ions are ameliorated by H^+ alone. Fig. 4(a, b) show plots of Cd^{2+} and Zn^{2+} versus H^+, respectively, confirming significant ($P \leq 0.05$), positive, linear relationships for both metals.

In contrast to Cd^{2+} and Zn^{2+}, the activity of Cu^{2+} decreased from 97% of total Cu for the LC_{50} value (2.3 μg l^{-1}) at pH 4.7, to 71% for the LC_{50} value (36.7 μg l^{-1}) at pH 7.0 (Cusimano et al., 1986). However, if the LC_{50} values at each pH are normalised for the activity of Cu^{2+}, then a plot of the normalised LC_{50} values, expressed as Cu^{2+} versus H^+, should also show a linear relationship, if H^+ alone ameliorates Co^{2+}. A normalised, positive, linear relationship ($P \leq 0.05$) of Cu^{2+} versus H^+, is shown in Fig. 4(c). Overall, these results provide convincing evidence that H^+ alone ameliorates the binding of Cd^{2+}, Zn^{2+} and Cu^{2+} at the cell membrane surface of *O. mykiss*, in accord with the extended FIAM proposed in this study.

An important outcome of the development of the extended form of the FIAM is the ability to determine the binding constants of ameliorating cations, such as H^+. From Eq. (25), it was established that the slope and intercept values of the linear region of a concentration-response relationship are equal to $[(BR_{100\chi_1} - a)K_2/bK_1\{\text{X-cell}\}_T]$ and $[(BR_{100\chi_1} - a)/bK_1\{\text{X-cell}\}_T]$, respectively, for amelioration by protons. The ratio of these expressions gives a value of K_2 (i.e. K_2 = slope/intercept); that is, the binding constant for H^+ to the cell membrane surface. The values of log K_2 calculated from linear regressions given in Fig. 4 are 5.58, 6.25 and 6.52 for Cd^{2+}, Zn^{2+} and Cu^{2+}, respectively. Therefore, the mean binding constant ($\pm$95% confidence limit) for H^+ at the cell membrane surface of rainbow trout (*O. mykiss)* is 6.1 ± 0.9. This value is in excellent agreement with that experimentally measured (log $K_2 = 5.9$) for *O. mykiss* by Janes and Playle (1995).

3.6. Case example 6 — amelioration by hardness cations (Ca)

Galvez and Wood (1997) determined the effects of Ca (4.0, 20, 40 and 200 mg l^{-1}) on the median lethal time (LT_{50}) of juvenile rainbow trout (*O. mykiss)* exposed to Ag (100 μg l^{-1}) in a synthetic freshwater. The LT_{50} values decreased four-fold as the Ca concentration increased 50-fold. Since a

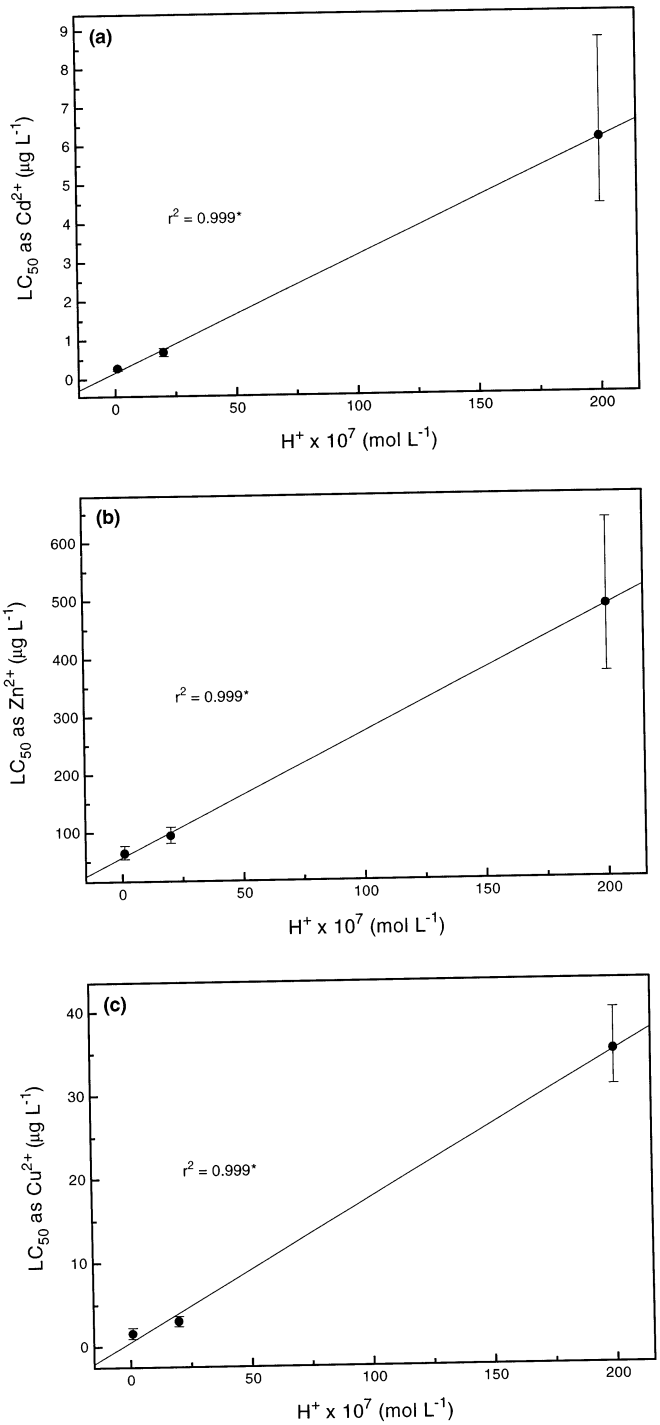

Fig. 4. Linear regressions of 168 h (7 days) LC_{50} values for steelhead trout (*O. mykiss*) expressed as (a) Zn^{2+}, (b) Cd^{2+} and (c) Cu^{2+}, versus H^+. Each plotted point represents the mean (and 95% confidence limit) response of 20 individuals, recalculated from Cusimano et al. (1986). $*P \leq 0.05$.

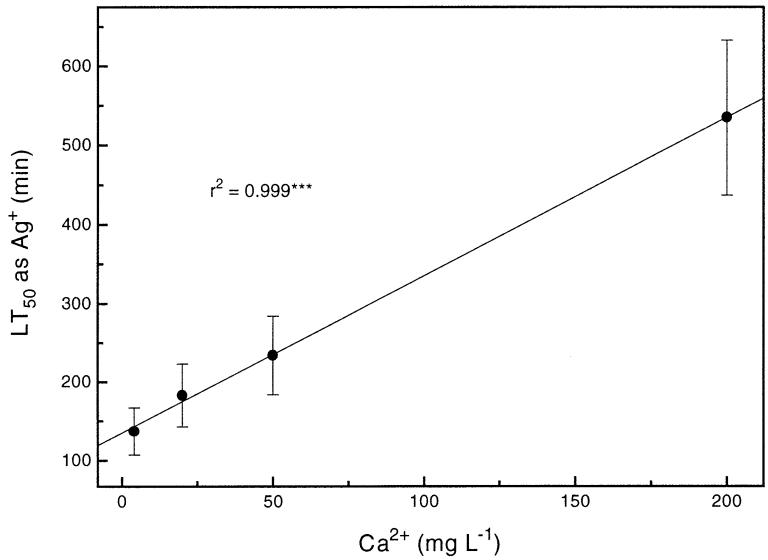

Fig. 5. Linear regression of LT_{50} values for juvenile rainbow trout (*O. mykiss*) versus $[Ca^{2+}]$. Each plotted point represents the mean (and 95% confidence limit) response of ten individuals, recalculated from Galvez and Wood (1997). $***P \leq 0.001$.

fixed Ag concentration was used, $[Ag^+]$ remained constant (97% of total Ag as determined by geochemical modelling). An important feature of this study is that BR is expressed as a function of time (LT_{50}), rather than concentration (EC_{50} or LC_{50}; as described in the above case examples). This required that an expression of time (t) be incorporated into the interaction of Ca^{2+} with the cell membrane surface (Eq. (24); except that $[Ca^{2+}]$ replaces $[H^+]$ and K_5, the Ca binding constant, replaces K_2), as given in Eq. (46).

$$BR = a + \frac{bK_1\{\text{X-cell}\}_T[M]t}{1 + K_5[Ca]} \quad (46)$$

By analogy with Eq. (25), Eq. (46) can be re-expressed in terms of t, as follows

$$t = \frac{BR_{100\chi_1} - a}{bK_1\{\text{X-cell}\}_T[M]} + \frac{(BR_{100\chi_1} - a)K_5}{bK_1\{\text{X-cell}\}_T[M]}[Ca] \quad (47)$$

According to Eq. (47), when [M] is fixed (as is the case in this example for Ag^+), a linear relationship should exist between time (LT_{50}) and Ca^{2+}, if Ag^+ is ameliorated by Ca^{2+} alone. Fig. 5 shows a plot of time (LT_{50}) versus Ca^{2+}, confirming a highly significant ($P \leq 0.001$), positive, linear relationship ($r^2 = 0.999$). These results provide evidence that Ca^{2+} ameliorates the binding of Ag^+ at the cell membrane surface of *O. mykiss*, in accord with the extended FIAM proposed in this study. Prima facie, this result is somewhat surprising, since Ca^{2+} (divalent and 'hard') is inherently unlikely to compete with Ag^+ (monovalent and 'soft') for the same binding sites on the cell membrane surface. Indeed, it has been established that Ag^+ binds to Na-K-ATPase and is inhibited by Na^+ (Wood et al., 1999). Nevertheless, it has been suggested that Ca^{2+} ameliorates Ag toxicity by reducing the diffusive permeability of Ag^+ at the cell membrane surface (McDonald, 1983).

Analogous to the calculation of K_2 in case example 5, K_5, the binding constant for Ca^{2+} at the cell membrane surface, can be calculated from the slope and intercept values of the fitted linear regression given in Fig. 5, using Eq. (47). The value of log K_5 calculated from the data of Galvez and Wood (1997) (Fig. 5) is 2.8 ± 0.2 (95% confidence limit) for *O. mykiss* using Ag^+. This value is

consistent with those measured experimentally by Reid and McDonald (1991) (log $K_5 = 3.0$) and Janes and Playle (1995) (log $K_5 = 3.3$) for the same species, and Playle et al. (1993) (log $K_5 = 3.4$) for the fathead minnow (*Pimephales promelas*).

4. Conclusions

The original FIAM postulates that the biological effects of metals are best predicted by the activity of the free metal ion (M^{z+}), rather than the concentration of total metal. It was developed using chemical thermodynamics to explain experimental observations, and has been effective in explaining the central role of the activity of the free metal ion as a regulator of interactions between metals and aquatic organisms.

The present work has integrated the concepts of the FIAM into BRT (i.e. pharmacodynamic principles) to obtain a more rigorous conceptual model; one that more precisely quantifies the interaction of chemical species at biological receptor sites. This study proposes, for the first time, a quantitative method of uncoupling the biological effects of a metal hydroxide (1:1) complex from that of amelioration of the free metal ion (M^{z+}) by H^+. This is a major outcome that stems directly from the development of the conceptual model. Since the activities of H^+ and metal-hydroxide cannot be independently varied, it has not been a simple process to evaluate whether metal-hydroxide species contribute to eliciting a BR.

Another extension of the FIAM is the fish gill surface interaction model (see review by Playle (1998)). This model attempts to predict metal bioavailability by incorporating measured (conditional) metal-gill surface binding constants into a geochemical speciation model. The approach explicitly considers competitive (e.g. Ca^{2+} or H^+) and complexation effects (e.g. by DOM) on metal binding to freshwater fish gills thereby making it more predictive and mechanistically-based than descriptive empirical equations. The models developed to date, however, have only considered the acute biological effects of several metals (e.g. Ag, Cd, Cu and Co in addition to protons, Ca and Mg) at the gills of rainbow trout or fathead minnows (Playle, 1998).

Implicit in the extended FIAM developed in this study, is the ability to determine metal binding constants for Ca, Mg or protons at cell membrane surfaces directly from concentration-response experiments (conducted over a range of pH or hardness). As such, a simpler and more direct method of determining proton and hardness binding constants is provided than using experimental techniques (i.e. gill isolation/exposure; Playle, 1998). Future development of the extended FIAM should focus on the possibility of deriving cell membrane binding constants for metals of ecotoxicological concern, directly from concentration-response experiments, for a variety of aquatic organisms. The development of the extended FIAM recognises that the value of the slope factor (n) of concentration-response curves, which is widely believed to have no physiological relevance, may indeed be positively related to the magnitude of the metal binding constant at the cell membrane surface.

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