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Critical Review

Dynamic Speciation Analysis and Bioavailability of Metals in Aquatic Systems

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Dynamic metal speciation analysis in aquatic ecosystems is emerging as a powerful basis for development of predictions of bioavailability and reliable risk assessment strategies. A given speciation sensor is characterized by an effective time scale or kinetic window that defines the measurable metal species via their labilities. Here we review the current state of the art for the theory and application of dynamic speciation sensors. We show that a common dynamic interpretation framework, based on rigorous flux expressions incorporating the relevant diffusion and reaction steps, is applicable for a suite of sensors that span a range of time scales. Interpolation from a kinetic spectrum of speciation data is proposed as a practical strategy for addressing questions of bioavailability. Case studies illustrate the practical significance of knowledge on the dynamic features of metal complex species in relation to biouptake, and highlight the limitations of equilibrium-based models.

Introduction

Natural aquatic systems generally are subject to changing conditions and are practically never at chemical equilibrium. To date, the vast majority of experimental and modeling approaches to compound speciation and relationships with bioavailability/toxicity have ignored dynamic aspects. For example, the widely used simplified models for chemical speciation, e.g., WHAM (*1*), and for biouptake and toxicity of pollutants, such as the biotic ligand model, BLM (*2*), and the free ion activity model, FIAM (*3*), for metals, and QSARs for organics (*4*), are all based on equilibrium principles and generally have limited predictive value. The increased recognition of the importance of a quantitative understanding of dynamic compound speciation, taking into account the kinetic features of the interconversion of different species allows such equilibrium approaches to be placed in context: they only approximate simplified limiting cases of the general dynamic situation in which chemical reactivity and transport of the various pollutant species play crucial roles (*5*−*7*). Knowledge of dynamic factors is fundamental for establishing a rigorous quantitative basis for the relationship between metal ion speciation, bioavailability, and biouptake (*8*, *9*) and thus for establishing the foundations for dynamic risk assessment.

A correct interpretation of the fate and environmental impact of metal complexes must consider the importance of the reactivity and fluxes of compounds, their exchange between compartments and biota via interfacial processes, and the relative time scales of processes. Diffusion is a key transport process for both environmental systems and sensor functioning. Figure 1 (*10*) shows the relationship between diffusion time scale and key dimensions of environmental systems and sensors. This diffusion time scale is the one to compare to chemical reaction times, e.g., in discriminating between labile and nonlabile complexes (see below). Note that at least three types of time scales are involved. (*i*) The diffusion time scale relevant for the transport of chemicals over distances typically of the order of 10−100 μm (Figure 1). For example, this is the time scale of transport through diffusion layers in mildly stirred media, or through membrane or gel layers (in permeation liquid membrane (PLM) and diffusive gradients in thin film (DGT) techniques, respectively). (*ii*) The accumulation time scale, as relevant for sensors with accumulation (see below) or in the chronic accumulation of a pollutant by an organism over hours, days, or longer. (*iii*) The equilibrium time scale, i.e., the time required to reach equilibrium, e.g., for an environmental sample after adding a reagent or after changing the conditions

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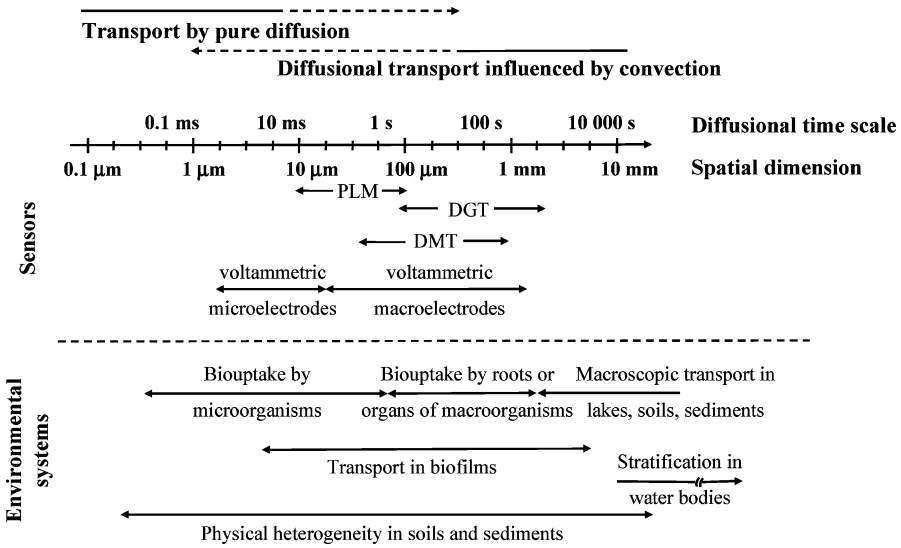

FIGURE 1. Schematic representation of diffusional time scales and spatial dimensions for a range of environmental processes and analytical sensors: PLM = permeation liquid membrane, DGT = diffusive gradients in thin film, DMT = Donnan membrane technique.

of the medium (or for an equilibrium-based speciation sensor).

Equilibrium schemes such as the FIAM and the BLM only apply if full equilibrium exists in the environmental surroundings of the organism. This implies that the rate of diffusion outside the organism should be much faster than the rate of transfer through the membrane (*11*). This exemplifies the different roles of various time scales and shows that an appropriate understanding of dynamic speciation processes is required not only to interpret nonequilibrium processes, but also to establish a rational basis for the range of applicability of equilibrium approaches.

Here we present a critical overview of the state of the art in dynamic metal speciation analysis. The main features of available sensors are described, and the magnitude of the analytical signal is explained in terms of rigorous flux expressions that consider the dynamic features of the technique and the geometry of the sensor. Practical examples are given to illustrate the principles involved. The focus herein is on trace metal species, although the concepts are applicable to any analyte.

Examples of Dynamic Sensors and Comparison with Microorganism Uptake

A given analytical technique will detect species with physicochemical properties within a certain range, e.g., of size, mobility, stability/lability, etc. Analytical thermodynamic and kinetic windows for several methods are well-known (*12*−*14*). The upper limit of the thermodynamic window is reached when equilibrium can no longer be attained within the measurement time scale, at which point the signal becomes of a kinetically controlled nature. Below we focus on the concept of a *kinetic* window, as determined by the effective time scale of the technique.

Among the available sensors for metal speciation analysis in aquatic ecosystems, some have an analytical signal based on dynamic processes (e.g., DGT or stripping voltammetry (SV)), while for others an equilibrium characteristic is involved (e.g., Donnan membrane technique (DMT), or diffusive equilibration in thin film (DET)). Depending on their mode of employment, some sensors may function on either a dynamic or an equilibrium basis (e.g., DMT, PLM). These techniques are briefly described below. All the techniques presented are deployable in situ which is a major advantage over sampling-based, sequential step "kinetic speciation schemes" (*15*). Figure 2 gives schematic drawings of a few sensors (GIME, DGT, PLM, Figure 2A−C) and generalized representations of chemical and diffusion processes at sensors (Figure 2D) and at microorganisms (Figure 2E) (see also refs *8*, *16*).

Specific techniques are briefly described below. Dynamic techniques (Figure 2A−D) are based on detection of either metal fluxes, or metal accumulated over a given period, or both. In dynamic analytical sensors, the *diffusion time* (see Introduction) is related to the diffusion of metal species in solution (for all techniques) and through a gel (DGT) and/or across a membrane (PLM, DMT). The *accumulation time* is the length of time over which the metal ion is accumulated in the electrode (GIME), the resin (DGT), or the receiving/strip solutions (DMT, PLM). The signal resulting from the accumulation step represents an integration of all fluctuations in the test medium and thus provides an average value for this time period.

Diffusive Gradients in Thin Film, DGT (*17*−*19*); Figure 2B. A DGT sensor consists of a layer of hydrogel (0.4−2 mm thick) overlying a layer of Chelex resin beads. Concentration gradients are established in the gel layer as species diffuse through it (planar diffusion) and accumulate in the resin. The lability and diffusion coefficients of penetrating complexes determine the amount of metal collected in the resin. The thickness of the gel, δ_g, strongly impacts on the metal flux and thus on the required deployment time, as well as the operational lability of the measured species (see below). For conventional applications, the time required to attain steady-state diffusion in the gel (typically of the order of 100 s) should be negligible relative to the deployment (accumulation) time (typically hours to days). The measured accumulated metal, divided by the deployment time, gives the flux, *J*, from which the concentration of DGT dynamic species is readily attained (Table 1). Note that when δ_g is not significantly greater than δ_s, the labile complexes can contribute to the accumulated metal even when they do not penetrate the gel. Detailed dynamic features of DGT are discussed in refs *5* and *17*. Limitations at low ionic strength (*20*, *21*), due to Donnan effects arising from residual charge in the gel layer are discussed in refs *22*, *23*. DET involves *equilibration* of the gel alone, in the absence of a resin layer, with a test solution, followed by analysis of the entrapped species (*19*, *24*, *25*).

Voltammetries. Various modes of voltammetry have been applied to metal speciation analysis. Selectivity is ensured by an electrochemical reaction, $M_{aq} + e^- \rightarrow M^0$ and the

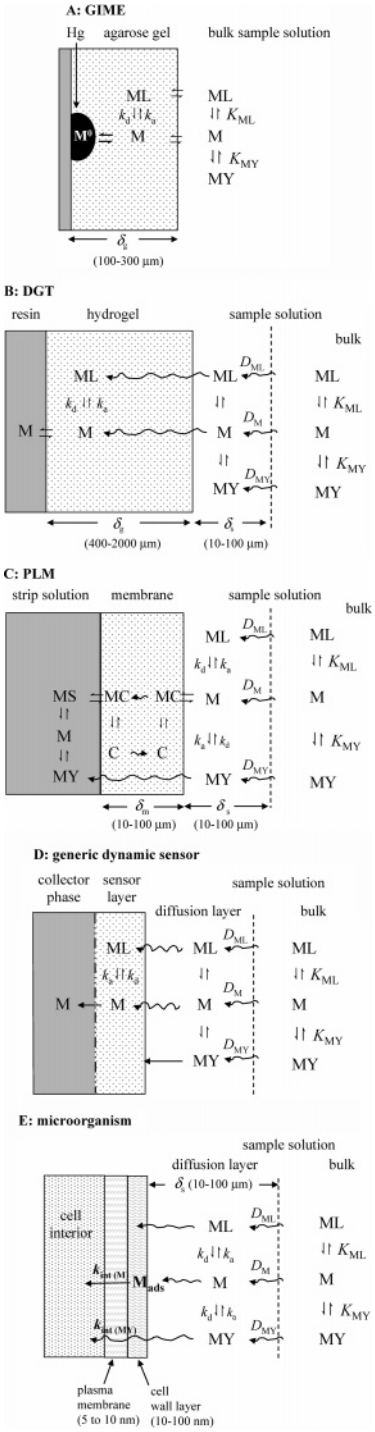

FIGURE 2. Schematic diagram of (A) gel-integrated microelectrode, GIME, (B) diffusive gradients in thin film, DGT, (C) permeation liquid membrane, PLM, and (D) a generic dynamic sensor, as compared to (E) uptake of M at microorganism/solution interface (not drawn to scale). In (D) the sensor layer may be an uncharged gel layer (DGT), a membrane with a carrier (PLM), or a membrane with negative electric charge (DMT); the collector may be an electrode amalgam (GIME), a resin (DGT), or a receiving solution (PLM, DMT). MY represents a lipophilic complex which may be transported intact through the PLM and the microorganism membranes. ML represents complexes that can only contribute to accumulation in a sensor or biouptake via prior dissociation into M. Symbols: k_a, complex association rate constant ($dm^3\ mol^{-1}\ s^{-1}$); k_d, complex dissociation rate constant (s^{-1}); K, equilibrium constant ($dm^3\ mol^{-1}$); δ_g, gel layer thickness (m); δ_s, solution diffusion layer thickness (m); δ_m, membrane thickness (m); D, diffusion coefficient ($m^2\ s^{-1}$); k_{int}, internalization rate constant (s^{-1}).

potential chosen to reduce the target metal ion and accumulate it inside the electrode. The reduction current derives from the fluxes of the free metal ion and the dynamic complexes (see below). For adequate sensitivity in environmental samples, accumulation is performed for a significant time (typically 10−20 min), and a so-called stripping mode is used to quantify the metal by a reoxidation potential scan with various types of modulations (stripping voltammetry, SV; if the stripping step is involved with reoxidation of metals it is often denoted as ASV (*26*, *27*)) or if the stripping mode is by constant current as scanned deposition potential stripping chronopotentiometry, SSCP (*28*−*30*).

The metal reduction flux, and thus the lability, depends on the diffusion time scale which in turn may depend on the size of the working electrode: at a conventional macroelectrode (typically millimeter dimensions) the hydrodynamic conditions govern the size of the diffusion layer, δ, while for a microelectrode (typically micrometer dimensions: see (*26*) for exact definition) its radius, r_0, is the predominant parameter on practical time scales (see eq 5 below). Lability is manifest in a shift of the characteristic potential, and, for unequal diffusion coefficients, also in the magnitude of the analytical signal.

Gel-Integrated Microelectrode, GIME (*26*, *31*); Figure 2A. GIME is a sensor comprising a single microelectrode (*32*) or an array of microelectrodes (*33*), separated from the test solution by a layer (typically 0.1−0.3 mm thick) of hydrogel, e.g., agarose. In practice, the GIME is equilibrated with the sample, then metal reduction and SV is performed as described above, inside the gel. As in DGT, the gel serves to exclude the larger (voltammetrically interfering) colloids (radius >30 nm for agarose (*34*)) and to provide pure molecular diffusion conditions, thus enabling reliable interpretation of the signals in terms of rigorous physicochemical metal flux expressions. This is of key importance for in situ measurements in natural water bodies with inherent uncontrolled hydrodynamic conditions. For agarose it has been shown that the diffusion of species with radius <2−3 nm is not affected by steric effects (*35*), and while such effects are not fully negligible for species with radius in the range 10−30 nm (*34*), such species anyway make an inherently small contribution to the steady-state metal reduction flux at microelectrodes. In GIME, electrostatic and complexation effects in the gel should also be considered (*35*), but they are well controlled and taken into account in calibration curves.

GIME has been modified to design sensors with improved sensitivity/selectivity for the target species, e.g., by incorporation of a thin layer of complexing resin at the interface between the voltammetric electrode and the gel layer (CGIME) for specific measurement of the free metal ion (*36*), or by embedding hydrophobic microparticles in the gel to minimize interfering adsorption of hydrophobic compounds (*37*).

Permeation Liquid Membrane, PLM (*37*−*41*); Figure 2C. A water-immiscible organic solvent, containing a carrier ligand, C, selective for the target metal, M, is imbedded in a porous hydrophobic membrane sandwiched between two aqueous phases: the sample source solution on one side, and the receiving (strip) solution on the other. Species separation is based on liquid/liquid partition, $A^{n-} + M_{aq}^{n+} + C_{org} \rightleftharpoons AMC_{org}$ (where A^{n-} is a counteranion). A metal flux occurs when the complexation strength increases from the test solution to the hydrophobic membrane and to the strip solution; it depends on diffusive transport in the hydrophobic membrane, the aqueous source, and the strip solutions, and it is measured by determining the metal concentration in the strip solution vs time. The flux, and thus the nature of the test species measured, can be varied by manipulation of these diffusion-controlling steps: if the diffusion in the source

TABLE 1. Expressions for the Lability Criterion, L, the Limiting Diffusive Flux, J^*_{Dif}, for Labile Systems, and the Limiting Kinetic Flux, J^*_{Kin}, for Nonlabile Systems[a]

	Transient	Steady-state macroscopic [b]	Steady-state microscopic sphere with radius r_0 [b]
L	$\frac{k_d^{1/2}(1+\varepsilon K')t^{1/2}}{\varepsilon^{3/2}K'(1+K')^{1/2}}$	$\frac{k_d^{1/2}(1+\varepsilon K')^{1/2}\delta}{D_{ML}^{1/2}\varepsilon K'}$	$\frac{k_d^{1/2}(1+\varepsilon K')^{1/2}r_0}{D_{ML}^{1/2}\varepsilon K'}$
L for $\varepsilon K' >> 1$	$(k_d t/\varepsilon K')^{1/2}$	$(k_d/D_{ML}\varepsilon K')^{1/2}\delta$	$(k_d/D_{ML}\varepsilon K')^{1/2}r_0$
J^*_{dif} for $L >> 1$	$\left[\frac{1}{(\pi\bar{D}t)^{1/2}}+\frac{1}{r_0}\right]\bar{D}c^*_{M_T}$	$\bar{D}c^*_{M_T}/\delta$	$\bar{D}c^*_{M_T}/r_0$
J^*_{kin} for $L << 1$	$k_d c^*_{M_T}(D_M/k'_a)^{1/2}$	$k_d c^*_{M_T}(D_M/k'_a)^{1/2}$	$k_d c^*_{M_T}(D_M/k'_a)^{1/2}$

[a] $\bar{D} = D_M(1 + \varepsilon K')/(1 + K')$, $\varepsilon = D_{ML}/D_M$ [b] "Macroscopic" means that the diffusion layer thickness, δ, is much smaller than the dimensions or radius of curvature, r_0, of the sensor or microorganism surface; "microscopic" means $r_0 \ll \delta$.

solution is made to be rate limiting, then both free metal and labile complexes are determined; if diffusion across the membrane is governing the flux, then only the free metal ion is measured. The rate controlling step can be tuned by varying δ_m and the carrier ligand concentration over a wide range of values (*37, 41*).

In addition to its dynamic flux mode, PLM can also be used to determine the free metal ion concentration in the test solution after equilibrium is reached in the whole system (compare DET and DMT).

The following three techniques are generally based on attainment of equilibrium, yet their dynamic features must be considered to verify whether equilibrium has been established within the measurement time (*5*).

Donnan Membrane Technique, DMT (*42*). A charged porous membrane is placed between the sample solution (donor) and a receiving solution (acceptor). Discrimination between species is primarily based on their charge. The measurement is typically made after equilibrium has been attained (currently of the order of days), but recent developments also consider a faster steady-state flux-based mode (*43*).

Ion selective electrodes, ISE (*44, 45*). ISEs are based on equilibrium partition of the test ion between an ionic hydrophobic liquid, or solid, membrane and the test solution. The resulting equilibrium or steady-state membrane potential is measured and related to the free metal ion concentration. Recently, dynamic principles have been applied leading to improved detection limits by control of ion fluxes (*46, 47*).

Competing Ligand Exchange−Adsorptive Stripping Voltammetry, CLE−AdSV (*48, 49*). The CLE−AdSV technique involves an exchange reaction in the bulk test solution with an added ligand, L_{ad}: $ML + L_{ad} \rightleftharpoons ML_{ad} + L$, followed by detection of the resulting ML_{ad} complexes by adsorptive accumulation and subsequent reduction of the metal. Assuming that equilibrium has been attained between M, ML, and ML_{ad} in the bulk sample, the free metal plus sample complexes weaker than ML_{ad} are contained in the signal. Analysis of the kinetic features of CLE−AdSV when applied to environmental samples has shown that in a number of cases an equilibrium-based interpretation is questionable (*14, 50*). When equilibrium with L_{ad} is not attained, the ensuing stability constants overestimate the real values. This situation is of particular concern for Fe(III), Ni(II), Co(II), and Cu(II) (*50, 51*).

Theory

It is evident that lability is an important concept for dynamic metal speciation sensors. Here we explain the meaning of this concept and its importance for understanding and applying the information obtained by a given technique.

Significance of Lability. The concept of *lability* describes the ability of complexes to maintain equilibrium with the free metal ion, M, within the context of an ongoing interfacial process in which a particular species, usually M, is consumed (*52*).

Equilibration Time in the Bulk Solution. In terms of a volume reaction only, maintenance of equilibrium is derived from the pertaining reaction rate constants and the relevant time scale. Consider the simplest case of a metal ion M forming complexes with ligand L:

$$M + L \underset{k_d}{\overset{k_a}{\rightleftharpoons}} ML \quad (1)$$

where k_a and k_d are the rate constants for complex association and dissociation, respectively, and the thermodynamic stability constant, K, equals k_a/k_d. Under conditions of sufficient excess ligand over metal, the association reaction is quasi-monomolecular with rate constant $k'_a = k_a c_L$. On time scales, t, much larger than the characteristic lifetimes of M ($1/k'_a$) and ML ($1/k_d$), a given metal ion undergoes frequent interchange between M and ML. The complex system is then sufficiently dynamic to maintain bulk equilibrium and obeys the double condition:

$$k'_a\, t,\ k_d t \gg 1 \quad (2)$$

In the usual situation of practical interest, K' ($= Kc_L = k'_a/k_d$) > 1, so eq 2 reduces to $k_d t \gg 1$. At the other extreme, the system is static, that is ML is inert, if the lifetimes of M and ML are much larger than the operational time scale, t:

$$k'_a\, t,\ k_d t \ll 1 \quad (3)$$

As above, with $K' > 1$, eq 3 reduces to $k'_a\, t \ll 1$.

Equations 2 and 3 indicate whether a system will attain equilibrium in the bulk solution within a certain time. The concepts are therefore useful in bulk solution techniques such as various modes of titration, CLE-AdSV, or DMT, as a means to confirm whether equilibrium is attained (*5*).

Lability at Consuming Interfaces. Let us now consider the situation of a surface (sensor or organism; Figure 2), in contact with a homogeneous solution containing M and ML, at which the free metal species M is consumed. The pertaining transport equations and boundary conditions are given in the Supporting Information. The overall flux of metal M to the analytical or biological interface results from the coupled

diffusion and kinetics of interconversion between M and its various species in the complex system. Inert complexes represent a simple case in this context: ML dissociates so slowly that it does not contribute significantly to the flux; the analytical signal will correspond to the free metal ion.

For operationally dynamic systems, in which there is an interfacial flux of free metal species (Figure 2), metal complexes are labile if there is frequent interconversion between M and ML during their transport through the diffusion layer. Thus, lability is conveniently expressed by J_{kin}/J_{dif}, where J_{dif} is the diffusion-limited flux of M and ML, i.e., the flux in the limit of infinitely fast dissociation of ML, and J_{kin} is the kinetic flux, as ensuing from the rate of dissociation of ML into M on the basis of the reaction layer concept (see below). Systems range from fully labile (for $J_{kin} \gg J_{dif}$) to nonlabile (for $J_{kin} \ll J_{dif}$). The ratio J_{kin}/J_{dif} is denoted as the lability criterion, L (*5*,*53*):

$$L \equiv J_{kin} / J_{dif} \tag{4}$$

Several expressions are available for L, depending on the pertaining conditions, and these are collected in Table 1. We note that the conventional formulation of lability criteria, which essentially are given as inequalities, uses the maximum kinetic flux, based on the bulk concentration of ML. A more quantitative approach to kinetic responses would describe J_{kin} in terms of the relevant species concentration in the reaction layer; for more details see ref *54*.

There are two important limiting cases, corresponding to extreme values of L.

For $L \gg 1$ (labile complexes), the flux reduces to eq 5:

$$J^*(t) = \left[\frac{1}{(\pi \bar{D} t)^{1/2}} + \frac{1}{r_0}\right] \bar{D} c^*_{M_T} \tag{5}$$

where $\bar{D}$ is the mean diffusion coefficient of M and ML:

$$\bar{D} = \frac{D_M c_M + D_{ML} c_{ML}}{c_{M_T}} = D_M \left(\frac{1 + \varepsilon K'}{1 + K'}\right) \tag{6}$$

where $\varepsilon = D_{ML}/D_M$. The flux corresponds to a purely diffusion-controlled coupled transport of M and ML, i.e., ML $\rightleftharpoons$ M equilibrium is maintained on the relevant spatial scales (*55*).

For the other extreme, $L \ll 1$ (nonlabile complexes), the flux is reduced to its kinetically controlled limit, which for sufficiently large K' ($\varepsilon K' \gg 1$, hence $c^*_{ML} \approx c^*_{M_T}$) comes to:

$$J^*(t) = k_d c^*_{M_T} (D_M / k'_a)^{1/2} \tag{7}$$

The reader is referred to ref *5* for full details on the various kinetic categories that emerge from rigorous theory. Thus, labile systems are characterized by diffusion-controlled responses, governed by an effective diffusion coefficient that averages the various species by their number and mobilities.

The Reaction Layer Concept. Eq 7 corresponds to the well-known electrochemical concept of a kinetic current originating *only* from the dissociation of ML. In practice, truly nonlabile systems, for which the contribution from the complex is purely kinetic, are probably rare (*27, 56*), and rigorous interpretation would require simultaneous consideration of kinetics of dissociation of ML and diffusional flux of M (Figure 3). Equation 7 is derived from reaction layer theory (*57*). $(D_M/k'_a)^{1/2}$ corresponds to the thickness of the reaction layer, μ (Figure 3), defined by the mobility and mean lifetime of free M, i.e., D_M and $1/k'_a$, respectively. In the simplifying Koutecký−Koryta approach for planar semi-infinite diffusion (*58, 59*), this μ is the basis for the spatial division of the concentration profiles for M and ML into a nonlabile and a labile region, separated by the boundary of the reaction layer, with thickness μ. Figure 3 illustrates that within the region $\mu < x < \delta$, M and ML are in equilibrium all along their concentration gradients, i.e., their diffusion is coupled with no net chemical reaction so that the concentration ratio is always given by K'. The kinetic flux due to dissociation of ML in the reaction layer ($0 < x < \mu$) equals the diffusive flux of ML, $D_{ML}(dc_{ML}/dx)$, toward the reaction layer boundary ($\mu < x < \delta$). This approximation has recently been found to be quite helpful in the complete kinetic range from nonlabile to labile complexes for any metal-to-ligand ratio (*59*). In case of mixtures of different complexes of the same metal with different ligands forming complexes with different degrees of lability, and hence different reaction layer thicknesses for each of the various species, the situation becomes more intricate (*60*).

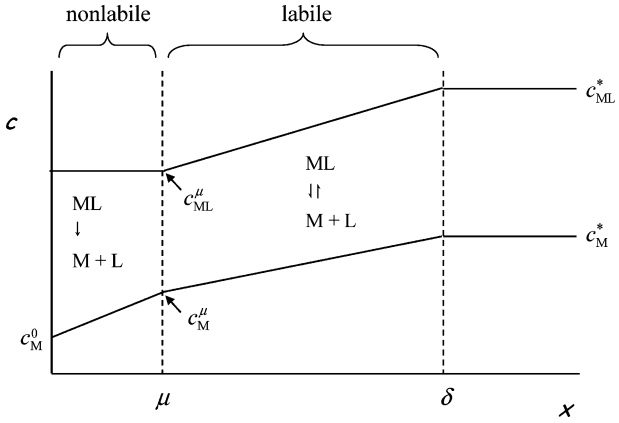

FIGURE 3. Illustration of the reaction layer and its role in the Koutecký−Koryta approximation, showing schematic concentration profiles for M and ML as a function of distance, *x*, from the electrode surface.

Steady-State Conditions. For the situation in which a steady-state flux is established toward a consuming interface, the diffusion layer thickness is constant (e.g., DGT, Figure 2B), and hence the concentration gradient is also constant and the term $(\pi \bar{D} t)^{1/2}$ in the denominator of eq 5 is replaced by the diffusion layer thickness, δ. The ensuing steady-state lability criteria, and the limiting diffusive and kinetic fluxes for steady-state conditions are collated in Table 1, together with the corresponding ones for the transient case. As considered in the present work, analytical signals from the dynamic sensors are obtained under steady-state conditions; the operationally measured dynamic fraction thus incorporates all labile species in proportion to their mobilities. For each technique, the analytical signal is taken as quantitatively proportional to the integrated flux over the accumulation time. As explained above, the magnitude of the flux is determined by the effective lability and mobility. In the context of this work, L is used in terms of an inequality, and is not related to absolute concentrations; for a quantitative expression in terms of the degree of lability see ref *61*.

Microsensors. An important situation arises when the dimensions of the consuming interface become comparable to or smaller than $(\pi \bar{D} t)^{1/2}$, as, e.g., may occur at a microelectrode or a microorganism. In such case the diffusional flux is of a nonlinear, convergent nature and various geometries have been considered (*5, 61*). The diffusive flux at a spherical microscopic consuming interface is given by eq 5 where for small r_0 the planar term, $1/(\pi \bar{D} t)^{1/2}$, becomes negligibly small with respect to the spherical term, $1/r_0$. The relevant expressions for the lability criterion are given in Table 1. It is evident from eq 5 that as the radius of a microsensor, r_0, decreases, J_{dif} increases, thus requiring a greater J_{kin} for the system to remain labile. This enhanced diffusion means that the lability of a given metal species will

TABLE 2. Features of Dynamic Metal Speciation Techniques, and Comparison with equilibrium-based sensors

method	physicochemical basis	characteristic diffusion length	species measured	lability criterion, L[a]	typical analysis time/s
		Dynamic Techniques			
voltammetries [b]	diffusion in test medium	macroelectrode, δ_s/ microelectrode, r_0	free metal plus dynamic complexes	$\Lambda\delta_s$/ Λr_0	10^2-10^3
GIME	radial diffusion in gel	microelectrode radius, r_0	free metal plus dynamic penetrating complexes	Λr_0	10^2-10^3 [c]
DGT	planar diffusion in gel	gel layer thickness, δ_g	free metal plus dynamic penetrating complexes [d]	$\Lambda\delta_g$	10^3-10^5
PLM	planar diffusion in sample and membrane	diffusion layer thickness, δ_s (solution diffusion control)	free metal plus dynamic complexes	$\Lambda\delta_s$	10^2-10^3
		membrane thickness, δ_m (membrane diffusion control)	free metal ion	na [e]	
		Equilibrium Techniques			
ISE	equilibrium or steady-state membrane potential	na	free metal ion	na	1−10
DET	equilibrium gel/sample	na	all penetrating species	na	10^5
PLM	equilibrium source/ acceptor solutions	na	free metal ion	na	10^4
DMT	equilibrium sample/ acceptor	na	free metal plus part of cationic penetrating complexes	na	10^5
CLE- AdSV	equilibrium with ML_{ad} in sample	na	free metal plus complexes weaker than ML_{ad}	na	10^2-10^3

[a] $L \gg 1$ for the labile case, $\Lambda = k_d D_M^{1/2}/k_a^{1/2} D_{ML}$ ($D_{ML}K'/D_M \gg 1$). [b] Traditional techniques, including stripping ones. [c] Equilibration in gel. [d] For $\delta_g \gg \delta_s$. [e] na = not applicable.

decrease as the radius of the consuming interface decreases (*61*), as verified experimentally (*62*, *63*).

The Lability Window for Some Analytical Techniques. It is evident from the above discussion that lability changes drastically with time scale and spatial scales (Table 1). Thus, various types of nonequilibrium techniques for speciation analysis will each have their own characteristic kinetic window, and accordingly measure a given operationally dynamic proportion of metal species. For example, the lability of complex species will be greater at a DGT device (δ_g of the order of 10^{-3} m) than at a GIME (δ_s in the range 10^{-5} to 10^{-4} m for the deposition step).The kinetic features and lability criteria for the dynamic sensors described in previous sections are summarized in Table 2; the characteristics of some equilibrium-based sensors are included for comparative purposes.

Experimental Aspects of Dynamic Measurements of Metal Speciation

Chemical Aspects of Lability. In aqueous systems, k_a, and hence μ, is generally controlled by (*i*) the rate constant for water substitution in the inner coordination sphere of the metal ion, k_{-w}, (*64*) multiplied by (*ii*) the stability constant for the intermediate outer-sphere complex, K_{os}, that is typically estimated on the basis of metal−ligand ion pair electrostatics (*65*). This relationship holds for the so-called Eigen mechanism which is applicable to many types of complexes. Any dynamic characteristic of a given metal complex system, e.g., its lability on a given time scale t, can thus be estimated from tabulated values of its formation rate constant, k_a, and the thermodynamic stability constants, K. Such estimates are subject to significant uncertainties which may become as high as 0.5 units on a logarithmic scale. For a given K value, the lower is the k_a value (e.g., as may occur on changing the metal) the lower the corresponding k_d value, and thus the lower is the lability as determined by a given technique. Thus, from k_{-w} values (*65*) we can explain why metal ions such as Pb^{2+} ($k_{-w} = 7 \times 10^9$ s^{-1}), Cu^{2+} ($k_{-w} = 10^9$ s^{-1}), and Cd^{2+} ($k_{-w} = 3 \times 10^8$ s^{-1}) form complexes that are generally more labile than those of Co^{2+} ($k_{-w} = 2 \times 10^6$ s^{-1}) or Ni^{2+} ($k_{-w} = 3 \times 10^4$ s^{-1}).

For complexes of a given ligand, lability differences are observed between metal ions due to the different rates of water exchange, e.g., Cu(II)NTA is fully labile by DGT whereas Ni(II)NTA is not (*66*). For systems that have been studied by more than one dynamic speciation technique, the results are consistent with the relative time scale of diffusion, e.g., Cd(II) complexes with NTA are electrochemically semilabile at a voltammetric microelectrode (*63*), and fully labile at DGT, due to its much longer diffusion time scale (*67*).

For complexes of natural ligands, e.g., isolated humic substances, their lability follows the same trends as for more well-defined systems, in particular its dependence on the nature of the metal involved. Quantitative interpretation is more involved due to the heterogeneity of these ligands which results in a multiplicity of complexes, i.e., they span a range (distribution) of stabilities and labilities (*27*). Consequently, the measured lability depends on the metal-to-ligand ratio (*27*, *68*−*73*), on pH (*70*, *72*−*76*), and on the degree of heterogeneity itself (*68*, *69*, *77*). Complexes of fulvic and humic acids with Cd(II) are generally labile by DGT, PLM, SV, and SCP (*69*, *78*), while those with Cu(II) are considerably less labile under similar experimental conditions (*69*).

Mobility Characteristics of Metal Complexes. The physical mobility of metal complexes must also be considered for interpretation of the operationally dynamic species (*52*, 79−*82*). For heterogeneous fulvic and humic ligands, the effective diffusion coefficient of their complexes is significantly lower than that of the free M, and this applies to an even greater extent for the usually larger inorganic colloidal ligands. Diffusion coefficients for macromolecular ligands are pH dependent: this effect is only slight for fulvic and humic substances unless aggregation phenomena are involved (*82*, *83*), but may be significant for other macromolecules. A relatively low flux of metal can arise from low mobility, independent of dissociation kinetics. Thus the concept of dynamic species involves both mobility and lability (*84*, *85*).

An example of the impact of mobility of metal species on the measured flux is given in ref *85* where the speciation of Zn(II), Cd(II), Pb(II), and Cu(II) has been studied by direct square wave stripping voltammetry (SWSV) measurements with GIME in the river Arve (Switzerland). This river contains a high load of aluminosilicate (clay) particles but not much dissolved complexing organic compounds. Modeling and experimental studies have shown that a small proportion of the metals is in the free form or bound in weak, so-called

TABLE 3. Voltammetric Determination of the Total, $c^*_{M_T}$, and Mobile, c_{M_m}, Metal Species in the River Arve at pH 2 and Natural pH (7.6), Measured by SWSV at a GIME (*85*)

metal	c_{M_m}/nmol dm^{-3}	$c^*_{M_T}$/nmol dm^{-3}	$100c_{M_m}/c^*_{M_T}$ %
Zn(II)	9 ± 1	28.8 ± 3.2	31
Cd(II)	0.1 ± 0.02	0.27 ± 0.25	37
Pb(II)	0.7 ± 0.1	3.9 ± 0.6	18
Cu(II)	1.5 ± 0.2	16.1 ± 2.1	10

inorganic, complexes with OH^-, CO_3^- , and SO_4^{2-} . These species have mobilities similar to that of the free metal ions and will penetrate the GIME gel layer. The rest of the metals is bound to the particles which have sizes larger than ca. 5−10 nm. The contribution of particulate metal species to the flux measured by SWSV will be small, because (*i*) the particles with diameter >70 nm are excluded from the gel of the GIME, and/or (*ii*) the mobility of the smaller particles able to penetrate the gel is inherently small. Table 3 shows the concentrations of dynamic species (free ion + inorganic complexes that are both mobile and labile) measured at natural pH (7.6) and after acidification to pH 2. At the latter pH all complexes are dissociated and the measured dynamic metal concentration corresponds to the total metal concentration. Table 3 shows that under natural conditions, the fraction of species with mobility too low to be measured by SW-SV is quite significant (60−90% depending on the metal).

Role of the Diffusion Layer Thickness and Diffusion Time Scale. Under planar diffusion conditions ($r_0 \gg \delta$), the lability of metal complexes is directly related to either the diffusion layer thickness, δ, or the diffusional time scale, t (see expressions for L in Table 1), t being directly linked to δ through $\delta = (\pi \bar{D} t)^{1/2}$. The fact that lability increases with δ can be understood by considering that $L = J_{kin}/J_{dif}$. The maximum diffusion flux, J_{dif}, is proportional to the concentration gradient in the diffusion layer, which decreases when the thickness of this layer increases, that is with increasing time. That is, for larger δ, the time scale on which complex dissociation may occur is greater. The relationship between a steady-state δ and a transient $(\pi \bar{D} t)^{1/2}$ is that the former is achieved in a typical time $\delta^2/\pi\bar{D}$. Thus in a steady-state accumulation process, it is not the accumulation time, but rather the $\delta^2/\pi\bar{D}$ that determines lability. When comparing different techniques, a key aspect to consider is thus to compare their diffusion layer thicknesses or time scales. The best dynamic techniques are those for which δ is well controlled or can be manipulated, e.g., as can be achieved by variation of δ_g in DGT, or δ_s in SV and PLM. For example, a flow-through PLM cell allows manipulation of δ_s by controlled variation of the flow-rate of the test solution which circulates as a laminar flow along the membrane (*39*). Using this cell, the normalized flux, J_0/J, in the presence of Cu(II)−sulfosalicylate complexes was observed to depend not only on the degree of complexation, $\alpha = c^*_{Cu_T}/c_{Cu^{2+}}$, but also on δ_s, i.e., increasing significantly as δ_s increases (Figure 4).

The influence of δ, or the corresponding diffusion time, is well-known in voltammetry; for instance the variation of the pulse duration in normal pulse polarography has been used to illustrate the partial lability of the Cd(II)−EDTA system at low pH (*86*). For the techniques discussed herein one can mention in particular: (*i*) the PLM example given in Figure 4, (*ii*) the reduction in lability of Cd(II) complexes with NTA and pyridine-2,6-dicarboxylic acid at a microelectrode as compared to a conventional one (*62, 63, 87*), and (*iii*) the increase in lability of Ni(II)NTA complexes with increasing thickness of the gel layer in DGT (*66*).

Intercomparison of Experimental Data. The relationship between the corresponding lability criteria and the measured operationally dynamic fraction is shown in Figure 5 for various analytical techniques. Figure 5 illustrates the validity of the dynamic concepts for interpretation of the analytical signals for these techniques. There is qualitative agreement between the operationally dynamic fraction and the lability criterion: a greater proportion of the metal species are measured for greater L. Still, there are differences between theory and experiment which may arise from (*i*) experimental error, and (*ii*) uncertainty on the calculated lability criterion due to considerable uncertainties on k_{-w}, K_{os}, and K.

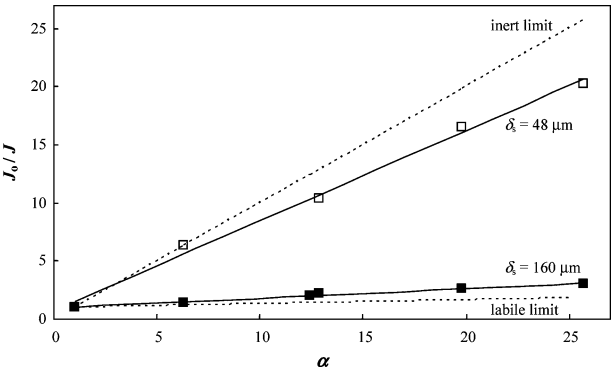

FIGURE 4. Effect of δ_s on the ratio of the PLM flux in the absence, J_0, and presence, J, of ligand, for variable degree of complexation, α, of Cu(II) by sulfosalicylate at pH 6 (*39*). The theoretical predictions for the fully labile and fully inert limiting cases are indicated with dashed lines.

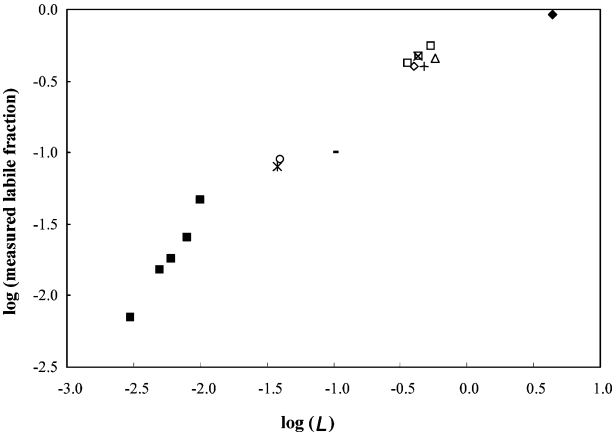

FIGURE 5. Relationship between the lability criterion, L, (Table 1) and the measured operationally dynamic metal fraction. DGT (*66*): Ni(II)NTA, ■; Pb(II)NTA, ◆. SSCP at a conventional electrode (*63*): Cd(II)NTA, ◇; Pb(II)NTA, ○; Cd(II)triazacyclononane, △; Cu(II)alanine, □. SSCP at a microelectrode (*63*): Cd(II)NTA, −; SV at a conventional electrode: Cd(II)NTA: + (*88*), * (*89*); Pb(II)NTA: × (*90*)). L calculated taking $D_M = D_{ML}$ ($D_{Pb} = 8.3 \times 10^{-10}$ m^2 s^{-1} (*91*), $D_{Cd} = 7 \times 10^{-10}$ m^2 s^{-1} (*91*), $D_{Cu} = 7.2 \times 10^{-10}$ m^2 s^{-1} (*91*)); DGT, $\delta_g = 1.6 \times 10^{-4}$ to 2×10^{-3} m; conventional electrode, $\delta_s = 2 \times 10^{-5}$ m; microelectrode, $r_0 = 4.5 \times 10^{-6}$ m.

In Situ Applications. SV and DGT have been widely applied to in situ measurements on natural waters. However, there have been few attempts to interpret the results in terms of the dynamic nature of the metal species measured. SV measurements with GIME in river water with a high concentration of inorganic colloids provided a measure of metal species with size less than a few nanometers, which were ascribed to mostly carbonate complexes (*85*). Consistent with its longer effective time scale, DGT generally measures a greater amount of dynamic species (*92*−*94*). DGT devices with very small gel pore sizes have been claimed to largely exclude metal humic complexes (*78, 95, 96*). Gels with pores not larger than the radii of fulvics and humics (<3 nm, (*82*)) should however show very low permeability. The influence

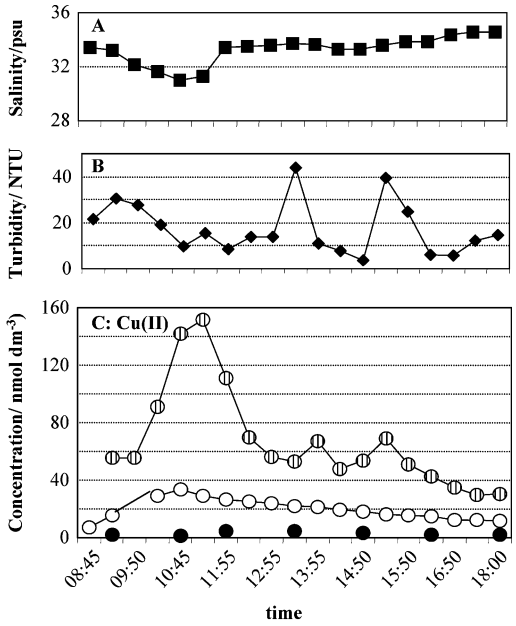

FIGURE 6. Time evolution of (A) salinity (■), (B) turbidity (◆), and (C) Cu(II) species: total (lined circle), dynamic (○), and free (●), in the Fal estuary (UK) (*100*).

of the physicochemical properties of gels, such as steric effects and pore size distribution, hydrodynamic features, electrostatic effects, or specific complex formation, on the speciation measured by GIME and DGT have been studied in some detail (*22*, *23*, *34*, *35*), but still need further investigation.

A few studies have compared measurements by different techniques on natural waters and soil solutions (*78*, 97−99). Deployment of a combination of sensors provides a complementary suite of information. Techniques allowing quick data acquisition, such as GIME with a relatively thin gel layer, can be used for monitoring speciation variations with high temporal resolution. This may be of major advantage as exemplified in ref *100*, where time evolution of (*i*) total concentrations of Cu(II), Pb(II), and Cd(II), (*ii*) the concentrations of their dynamic species, and (*iii*) the free metal ion concentrations, were measured simultaneously in situ by voltammetry in the Fal estuary (UK). Following the time evolution is of great help in the biogeochemical interpretation of metal circulation. For instance, Figure 6 (*100*) clearly shows the different behavior of dynamic species as compared to total Cu(II): total Cu(II) is directly linked to tide (salinity) and turbidity, whereas dynamic complex and free ion concentrations are much more constant, probably due to buffering reactions. Methods with intermediate or long data acquisition times (PLM, hours; DGT, days) measure concentrations averaged over accumulation time. Obviously, the dynamic requirements on a speciation sensor depend on the nature of the question posed. In any case, comparing curves such as those in Figure 6 to data acquired over longer time periods enables a better understanding of the nature of the average signals obtained with "'slow" techniques.

Dynamic Concepts for Prediction of Bioavailability. The features of dynamic sensor signals have obvious parallels with those of bioavailability, which refer to the fraction of the total metal concentration that may be taken up by an organism (*5*); Figure 2. For instance, there is some analogy between diffusion in gels (GIME, DGT) and microorganism cell walls. There is also an analogy between the carrier transport through PLM and certain transport mechanisms through plasma membranes. The most elementary analogy is that both organisms and sensors include a reactive interface (interface between solution and plasma membrane, Hg electrode in SV or GIME, resin surface of DGT, membrane/solution interface of PLM and DMT) separating a sink (cytoplasm, Hg, resin, acceptor/strip solutions) in which the analyte metal is accumulated from the external (environmental) medium where the common reaction/transport processes occur. Thus, the dynamic sensors discussed above can be seen as "bioanalogical sensors" (*16*) and the concepts discussed in this paper for sensors are to some extent also applicable to microorganisms. In general terms, description of selective consumption of a certain metal species in an interfacial process (biouptake or analytical sensor) requires a rigorous analysis of the dynamics of the various complexation equilibria and their coupling with mass transport from the bulk medium. Indeed, like the signals of sensors, the bioaccumulation depends on the flux of dynamic species, with the same equations applying outside the sensors and microorganisms. Mostly the boundary conditions at the reactive interface are different due to the different nature of the processes involved.

As explained above, the available suite of dynamic analytical speciation techniques spans a range of diffusion time scales. Microorganisms have their own diffusion time scale as defined by their geometry. The relationship between bioavailable species and the dynamic species measured analytically will be based on the comparison of the corresponding time scales (*8, 101*). To simultaneously reproduce in an analytical sensor the reaction/diffusion conditions characteristic of a given organism, and the exact nature of processes at the membrane surface, would be an extremely challenging task, and not a very practical one. Not only do the uptake rate parameters (number and affinities of transporter sites, rate of transfer of metal by the transporter) vary from one type of biological species to another, they also generally vary with conditions in the medium (biological "adaptation"). To develop a biomimetic sensor for all organisms in any condition therefore seems unfeasible and even a futile endeavor. The better option to tackle the bioavailability issue seems to be to measure a kinetic speciation spectrum of metal species in the test medium, either by combining a suitably chosen combination of dynamic speciation techniques (bioanalogical sensors) or by deliberate controlled variation of the flux of metal at the reactive interface (e.g., by varying the carrier concentration in PLM (*41*)). This allows the computation of metal bioavailability for any set of uptake fluxes at organisms. For example, for carp the relevant spatial scale is related to the distance between the gill microlamellae (*6*).

It should be clear by now that a labile metal species is not *necessarily* bioavailable. Rather, it is *potentially* bioavailable, depending on the magnitude of the metal flux through the plasma membrane. The larger this flux, the larger will be the fraction of dynamic species consumed. Therefore, the notion of bioavailability is of an organism-specific nature, and meaningless if the pertaining organism is not specified.

A dynamic analysis establishes the conditions under which complex species will contribute to biouptake, i.e., it identifies the domain of validity of equilibrium-based models such as the free ion activity model (FIAM) or the biotic ligand model (BLM) (*102*). Numerous exceptions to the equilibrium-based biouptake models have been reported (*103*−*105*), and a more comprehensive dynamic framework allows these results to be interpreted in the appropriate context. For example, the FIAM is restricted to cases where mass transfer to the biointerface is not flux-determining and the unsupported diffusion flux of the free metal alone is much larger than the maximum biouptake flux (*11*).

The relevant expressions can be formulated in a manner analogous to that presented above for the analytical sensors. The rate of biouptake of metal ions can usually be described by a Michaelis−Menten type steady-state flux, i.e., Lang-

muirian adsorption followed by first-order rate-limiting internalization. The biouptake flux, J_u, is given by (*11*, *56*)

$$J_u = J_u^* \frac{c_M^0}{K_M + c_M^0} \quad (8)$$

where J_u^* is the limiting uptake flux, c_M^0 is the concentration of the bioactive species M *at the biointerface*, and K_M is a bioaffinity parameter (corresponding to c_M^0 for $J_u = {}^1/_2 J_u^*$). Description of the kinetic features of the biouptake process requires coupling of the biouptake flux, eq 8, with the supply of M from the bulk medium. The result takes the form of the so-called Best equation (*11*, *56*, *106*, *107*). For a fully labile metal complex system, the diffusive flux of metal from the medium is solely determined by the coupled diffusion of M and ML and is described by the steady-state expressions in Table 1. Nonlabile systems require coupling of the appropriate kinetic flux with J_u, and various expressions have been derived (*11*, *56*, *108*). The extent to which complex species in the medium contribute to biouptake depends on the interplay between two fundamental quantities: the relative bioaffinity and the ratio between the limiting biouptake flux and the limiting supply flux from the medium. The two extreme situations are (*i*) the uptake demand is much less than the limiting flux of free metal ion alone, and there is no appreciable concentration gradient of M in the supplying medium, and thus no need for contribution from ML species, and (*ii*) the uptake demand is very high, and full contribution of all ML complexes is called into play to satisfy the organism. As said before, the relevant dimensions of an organism can have a dramatic influence on the rate of biouptake as well as the extent of lability, and consequent bioavailability, of metal complex species.

The validity of these concepts has been established by application to measurements of biouptake fluxes for a range of organisms. Until recently, most studies of metal biouptake that purportedly supported the FIAM were conducted under conditions where the flux of free metal ion alone is sufficient to satisfy the demands of the organism concerned, and the lability of any complexes present in the medium is consequently irrelevant. This has been verified, for instance, for uptake of Pb(II) by algae and bacteria (*40*), and for uptake of Cd(II) (*6*) and Co(II) (*7*) by carp from different complex media. However, several examples have been reported for which biouptake is diffusion-limited and thus the extent of lability of metal complex species must be considered in the interpretation, e.g., uptake of Zn(II) by mussels (*6*) and the algae *Chlorella kesslerii* (*109*), and uptake of Ag(I) by the algae *Chlamydomonas reinhardtii* (*110*) (see ref *111* for other examples and a more detailed discussion of diffusion-limited metal biouptake). In any case, there is a paucity of data that would allow unambiguous demonstration of whether metal biouptake is limited by diffusion, chemical dissociation, adsorption to the biological interface, or transfer through the plasma membrane (*111*). Furthermore, it must be noted that, to facilitate experimental measurements, most laboratory studies have been performed under conditions where diffusion of nutrients in the external medium is not the limiting factor. Such conditions may not be met in most environmental media.

A flux analysis, incorporating the coupled diffusion and dissociation kinetics of the complex species, set against rates of bioconversion, can provide very useful insights into biouptake and bioaccumulation processes. It indicates to what extent the steady-state approximation correctly describes the flux toward the biointerface and the evolution of the accumulated amount of metal (*8,* 112). The dynamic approach has already been extended to consider more than one uptake route (*8*, *113*) and to include depletion of the bulk medium (*114*).

Outlook

There is great interest in establishing sound *predictive* relationships between the speciation of an element and its potential bioavailability. The inherent dynamic nature of environmental systems demands an approach that recognizes the importance of *fluxes* of species, at given spatial and time scales, together with their coupling to the kinetics of chemical conversions of species. To date, kinetics have been considered largely in terms of steady-state conditions. A future challenge is comprehensive understanding of transient phenomena. Quantitative knowledge of transient biouptake processes allows discrimination between the processes of adsorption and internalization, influx and efflux, and various mechanisms of internalization. It can also characterize the relaxation time of the biosystem, i.e., the time for attainment of a steady-state following some perturbation of the medium. The first steps toward modeling of transient fluxes have been presented and shown to provide a good description of time-dependent uptake of Pb^{2+} by the algae *Chlorella vulgaris* (*8*, *112*).

The current suite of analytical techniques, spanning a range of kinetic windows, and the generic dynamic framework for interpretation of their signals lays the foundations for a sophisticated quantitative approach to metal speciation and bioavailability. In situ measurements in natural waters have shown the utility of this strategy (*97*, *115*). The concepts involved are applicable to any analyte, e.g. the nondepletive mode of solid-phase microextraction (*116*) provides similar information on the dynamic features of organic compounds.

Biouptake criteria for microorganisms can be formulated in a manner analogous to that for analytical lability (*8*). The analytical information can thus be translated into predictions of bioavailability by considering the corresponding effective spatial and time scales for the target organism and the nature of the rate-limiting step in its uptake of the target metal. Dynamic analysis at this level of sophistication provides the best estimates of biouptake and allows the limits of applicability of more simple models, such as FIAM and BLM, to be understood within a comprehensive framework (*8*, *117*).

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Supporting Information Available

A list of symbols and acronyms, and the pertaining transport equations and boundary conditions for the situation of a homogeneous solution containing M and ML in contact with a surface (sensor or organism), at which the free metal species M is consumed. This material is available free of charge via the Internet at http://pubs.acs.org.

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