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**VOLTAMMETRIC TECHNIQUES FOR  
COMPLEXATION MEASUREMENTS IN  
NATURAL AQUATIC MEDIA**

**Role of the Size of Macromolecular Ligands  
and Dissociation Kinetics of Complexes**

Prepared for publication by

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# Voltammetric techniques for complexation measurements in natural aquatic media: Role of the size of macromolecular ligands and dissociation kinetics of complexes

**Abstract** - The use of voltammetric techniques for the study of complexation between metal ions and synthetic or natural macromolecular ligands is critically reviewed. In particular attention is paid to the effects of (i) finite rates of association/dissociation of complexes and (ii) the differences between the diffusion coefficients of the various metal species. The present state of the theory of voltammetry for metal complex systems with unequal diffusion coefficients is reviewed and necessary extensions are indicated. Relevant experimental data from literature are compiled and classified according to the mode of interpretation. On the basis of the theoretical and experimental results, recommendations are presented for the optimal application of voltammetric methods (both direct and indirect methods) to metal complex studies. These recommendations include the selection of suitable experimental conditions and the required mode of interpretation of the data, as well as their mutual relation. Remaining uncertainties, e.g. those due to distribution of kinetic parameters or by diffusion coefficients, are indicated.

## I. INTRODUCTION

Voltammetric methods are frequently used for heavy metal speciation studies in natural waters. In the seventies and early eighties, voltammetry has actually developed into a very popular method in this field (e.g. ref. 1,2). A great deal of the speciation work has been devoted to the binding of metal ions by macromolecular ligands such as polysaccharides, nucleic acids, proteins, humic acids, synthetic polymers, etc. These ligands are much larger than the hydrated metal ions or the simple complexes with small ligands (halides, cyanides, glycinate, etc.). For interpreting the voltammetric results of a metal complex system, this observation is of great relevance since the complex may influence the voltammetric signal not only via the association/dissociation reaction, but also by contributing to the transport of the electroactive metal towards the electrode surface. Taking these two effects into proper account is therefore necessary for a correct determination of the properties of the studied complexes, and ultimately of their environmental role.

Traditional voltammetric speciation has been based primarily on the exploitation of **potential** characteristics of the polarographic or voltammetric waves or peaks of labile complexes. Lingane or DeFord-Hume types of analysis of half-wave potential or peak potential shifts (see section III.1) have been used to evaluate numerous stability constants of metal complexes [3,4]. In many of the classical studies, the size of the ligand was small and therefore played only a minor (often negligible) role, since for labile complexes the potential shifts involved are predominantly governed by the stability constants, and their dependence on diffusion coefficient variations is small. However, by decreasing the strength of the complex as well as the diffusion coefficient ratio of complexed to free metal ion (as it is the case with natural macromolecular or colloidal complexing agents [5]) potential shifts may become small and the evaluated speciation parameters become inaccurate. Moreover, the interest in studying metal ions at very low concentration levels has led to the use of multi-step methods such as anodic stripping voltammetry, for which the relation between potential characteristics and complexation in the bulk solution is much more involved than with direct methods.

These developments have led to the utilization of **current** characteristics for metal speciation purposes. Unfortunately however, the electroanalytical discipline did not provide an appropriate theoretical background so far. The reasons for this are that:

(i) the practical interest for metal speciation in systems with large size ligands is relatively new;

(ii) the abandoning of the condition  $D_M = D_{ML}$  ( $D_M$  and  $D_{ML}$  are the diffusion coefficients of the free metal ion and the complex respectively; see also Note) greatly complicates the theory for mass transport towards the electrode. E.g. for expanding mercury drop electrodes,

the theory of polarography of metal complexes is sufficiently complicated by itself, and the introduction of the inequality of  $D_M$  and  $D_{ML}$  leads to insurmountable difficulties, at least on the analytical level. This explains why the early efforts by Koutecký [6] had to wait so long for continuation and extension.

In the practice of voltammetric speciation, most investigators did not wait for the development of a suitable theory. They used a voltammetric method (most DCP, DPP or ASV techniques) with the measured current signal,  $I$ , as the basic speciation parameter (see Table I in sect. IV for a compilation). The method used to transform  $I$  into the complexation parameters was rarely tested, despite the fact that  $I$  may be influenced by many different factors, particularly in complicated media [1,5].

In the following, we shall use the term 'labile' for those complexes where the rate constants for association/dissociation reactions have such high values that they do not limit the current. The notion of 'mobility' will be used for the rate of physical transport: it is related to the value of  $D_{ML}/D_M$ , the mobility of complexes increasing with this ratio.

In this context, the following categories of interpretation of  $I$  can be used and will be discussed below in some detail:

- (i) the complexes are chemically inert.  $I$  is then directly proportional to the concentration of the free (hydrated) metal ion;
- (ii) the complexes are labile and  $D_M = D_{ML}$ .  $I$  is then a measure of the sum of all the metal species (free + complexed);
- (iii) the complexes are labile, but with  $D_M \neq D_{ML}$  (normally  $D_{ML} < D_M$ ).  $I$  is then a weighted average of the diffusion of all metal species (free and complexed);
- (iv) the complexes are neither totally inert nor fully labile (chemically slow). In this case,  $I$  depends on both diffusion and dissociation/association kinetics. This is the general case.

Recently, theoretical attention has been given to the general case, which corresponds to a situation often encountered in environmental and biological systems. The first three categories are just limiting cases, as was explained in detail [7,8].

The present report is intended to:

- (i) outline the status of the theory for voltammetry of metal complex systems with different diffusion coefficients of the species involved;
- (ii) offer recommendations, based on the above theory, for the optimal use of voltammetry in environmental and biological samples by properly choosing both suitable experimental conditions and interpretation mode of the data;
- (iii) to provide the reader with a critical compilation of the pertinent voltammetric literature to allow an easy comparison of the methods used up to now, and of their theoretical foundations.

## II. CURRENT STATUS OF THE THEORY

The earliest attempts to incorporate different mobilities of simultaneously diffusing species into voltammetric theory are due to Hanus & Brdicka [9] and Kacena & Matousek [10]. In different contexts, they put forward the concept of some averaged diffusion coefficient which is applicable when the different diffusing species may be converted into each other. Their findings were followed by the development of theoretical backing from Koutecký [6] and Dogonadze [11]. These authors analyzed the polarographic waves produced at the DME [6] and the RDE [11] by a depolarizer (for the present purpose a metal ion) which can react to form an electroinactive compound (the complexed species) with a different diffusion coefficient. Their treatments are limited to cases where the reaction rate constants are high with respect to the reciprocal time-scale of the experiment. More recently Lovric & Ruzic [12] presented a treatment for the planar electrode, the applicability of which is however limited to large stability constants of complexes (corresponding to the situation where the bound metal is the only metal species present in significant concentration) and similar values of  $D_M$  and  $D_{ML}$ . Koutecký's approach [6], including its restrictions, has been reformulated in terms of the scheme of a metal complex system [13]. A rigorous treatment, valid for any set of rate constants and diffusion coefficients for the case of ligand excess, is at hand in the form of an exact solution in the Laplace domain [7]. Further work, including numerical analysis of the problem is urgently required. The theoretical discussion in section II is mostly based on refs. 6, 7, 8 and 13. Unless indicated otherwise, the existence of only two metal species, M and a single complex ML, and only the factors influencing the limiting current are considered. These basic considerations are expanded to natural systems and to the use of potential shifts in section III.

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Note: A list of symbols and abbreviations is given at the end of the report.

## II.1. General principles

Let us consider the basic scheme of an electroactive metal ion M and a ligand L which may associate to give the electroinactive complex ML:



where  $M^0$  denotes the metal atom. For the sake of simplicity, charges of ionic species are omitted. The rate constants for association and dissociation of ML,  $k_a$  and  $k_d$  respectively, are coupled by the stability constant K:

$$K = k_a/k_d \quad (2)$$

The following basic assumptions will be used later on (assumptions no. (i) and (iv) being implicit in the scheme):

- (i) the electron transfer reaction between M and  $M^0$  is sufficiently fast to render the system electrochemically reversible;
- (ii) ML is electroinactive;
- (iii) the ligand is in large excess compared to total M concentration;
- (iv) L reacts only with M, and only the 1:1 ML complex is formed.

On the other hand, no assumption will be made about the dimensions of the ligand, i.e. the diffusion coefficient of the complex ( $D_{ML}$ ) can take any value, equal or different, normally lower, than that of the uncomplexed metal ( $D_M$ ). Assumption no. (i) is only relevant when the current-potential-time curves are discussed, and not for the interpretation of the limiting current. Assumption (ii) is not very restrictive in that ML, if electroactive, is generally reduced at more negative potential than M, so that it is often possible to find a potential range where ML is not reduced. Assumption (iii) will be discussed below. Assumption (iv) is used for the sake of simplifying the discussion. It must be noted that it can be readily extended to the case where L may participate in very fast reactions with other compounds, X, the concentration of which is constant. This situation often occurs in natural systems where X is either a major (e.g.  $Ca^{2+}$ ) or a buffered component (e.g.  $H^+$ ). In the following equations  $c_L^*$ , the ligand concentration in the bulk, must then be replaced by the concentration of free L, which, under the above conditions, is proportional to  $c_L^*$ .

For diffusion towards a stationary planar electrode surface, the basic differential equations for M and ML are:

$$\frac{\partial c_M}{\partial t} = D_M \frac{\partial^2 c_M}{\partial x^2} + k_d [c_{ML} - K c_M c_L^*] \quad (3a)$$

$$\frac{\partial c_{ML}}{\partial t} = D_{ML} \frac{\partial^2 c_{ML}}{\partial x^2} - k_d [c_{ML} - K c_M c_L^*] \quad (3b)$$

where c denotes concentrations. For the expanding drop electrode, these equations have to be extended with the well-known convective term  $(2x/3t)(\partial c/\partial x)$  [3]. Likewise, for a stationary electrode in a stirred solution the right hand sides of eqs. (3a,b) are extended with the proper convective term [11]. In the limiting current region, the case of semi-infinite linear diffusion is subjected to the boundary conditions:

$$\left. \begin{array}{l} t = 0, \quad x \geq 0 \\ t > 0, \quad x \rightarrow \infty \end{array} \right\} \quad c_M = c_M^*, \quad c_{ML} = c_{ML}^* \quad (4a)$$

$$t > 0, \quad x = 0: \quad c_M = 0, \quad \partial c_{ML}/\partial x = 0 \quad (4b)$$

where concentrations with and without superscript \* denote bulk and surface concentrations respectively. Eqs. (3a,b) show that the ligand concentration,  $c_L$ , is in principle a variable since it is involved in the interconversion of M and ML, and therefore if the ligand is not in excess compared to M, a rigorous treatment requires an extra differential equation for L. Unfortunately, theoretical work on this situation of no excess of ligand lacks completely for the case of unequal diffusion coefficients. The situation simplifies greatly by introducing the following assumption:

$$c_L^* \gg c_M^* + c_{ML}^* \quad (5)$$

Under this condition,  $c_L \approx c_L^*$  = constant at any time and distance, so that we may define the quasi-monomolecular parameters:

$$k'_a = k_a c_L^* \quad , \quad K' = k'_a / k_d \tag{6}$$

Now the kinetic terms in eqs. (3a,b) are simplified to:

$$\frac{\partial c_M}{\partial t} = D_M \frac{\partial^2 c_M}{\partial x^2} + k_d (c_{ML} - K' c_M) \tag{7a}$$

$$\frac{\partial c_{ML}}{\partial t} = D_{ML} \frac{\partial^2 c_{ML}}{\partial x^2} - k_d (c_{ML} - K' c_M) \tag{7b}$$

Eq. (7) shows an important difference between two limiting cases corresponding to (i)  $k_d = 0$  (chemically inert complexes) and (ii)  $D_{ML} = 0$  (immobile complexes). It is seen that by far the most simple case corresponds to that of chemically inert complexes. Then, since ML is electroinactive, there is no gradient of ML and eq. (7a) can be solved without consideration of the equilibrium existing between M and ML in the bulk solution. On the other hand, if  $k_d \neq 0$ , eqs. (7a,b) must be combined, leading to more complicated mathematics, even in the case where  $D_{ML} = 0$ .

After transformation into the Laplace domain, eqs. (7a,b) may be written as:

$$\frac{d^2 \bar{c}_M}{dx^2} = \frac{s + k'_a}{D_M} \bar{c}_M - \frac{k_d}{D_M} \bar{c}_{ML} - \frac{c_M^*}{D_M} \tag{8a}$$

$$\frac{d^2 \bar{c}_{ML}}{dx^2} = - \frac{k'_a}{D_{ML}} \bar{c}_M + \frac{s + k_d}{D_{ML}} \bar{c}_{ML} - \frac{c_M^*}{D_{ML}} \tag{8b}$$

where  $s$  is the Laplace transform parameter and the super bar denotes a transformed variable.

Using the methodology of d'Alembert, it is possible to solve the set of equations without any approximation [7]. The result is found in the form of an expression for the gradient at the electrode surface:

$$\left( \frac{d \bar{c}_M}{dx} \right)_{x=0} = - \frac{c_M^* \left( d_- + d_r + K' \epsilon^{-1} \right) \left( d_+ + d_r \right)^{\frac{1}{2}} - \left( d_- - d_r + K' \epsilon^{-1} \right) \left( d_+ - d_r \right) \left( d_+ + d_r \right)^{-\frac{1}{2}}}{D_M^{\frac{1}{2}} k_d^{\frac{1}{2}} \left( d_+ - d_r \right) \left( d_- - d_r \right) - \left( d_- + d_r \right) \left( d_+^2 - d_r^2 \right)^{\frac{1}{2}}} \tag{9}$$

where

$$d_{\pm} = \frac{1}{2} \left[ \frac{s}{k_d} \left( \epsilon^{-1} \pm 1 \right) + \epsilon^{-1} \pm K' \right] \tag{10}$$

$$d_r = \left( d_-^2 + \epsilon^{-1} K' \right)^{\frac{1}{2}} \tag{11}$$

$$\epsilon = D_{ML} / D_M \tag{12}$$

Back-transformation into the time domain yields the gradient of M (proportional to the current) as a function of time. In this way a number of limiting cases may be solved analytically. We shall discuss here two important cases:

(i) the case with high chemical rate constants, that we shall call the **dynamic** situation where a thermodynamic equilibrium is always established between M and ML:

$$k'_a \tau, k_d \tau \gg 1 \tag{13}$$

with  $\tau$  being the characteristic time-scale of the method employed e.g. pulse duration in pulse polarography, drop time in d.c.- and reciprocal frequency in a.c.-polarography;

(ii) the case with very low diffusion coefficients of L and ML, i.e. the system with an **immobile** complex, for which  $D_{ML} / D_M \rightarrow 0$ .

## II.2. The dynamic case

The conditions in eq. (13) imply that, during the transport of a metal ion inside the diffusion layer, it frequently flip-flops between the free state M and the bound state ML. In this dynamic situation there is another condition that needs to be considered, i.e. the condition of lability. It may be shown [1,8,19] that in the case of unequal diffusion coefficients, a complex is labile if:

$$k_d \left( \frac{D_M}{D_{ML}} \right)^{\frac{1}{2}} \left( \frac{\tau}{k'_a} \right)^{\frac{1}{2}} \gg 1 \quad (14)$$

It should be noted that the conditions in eqs. (13) and (14), though conceptually related, do not necessarily run parallel. The lability criterion, eq. (14), compares the reduction current controlled by chemical kinetics with that purely controlled by the diffusive transport of ML: fulfillment of the lability criterion means that the production flux of M (represented by  $k_d(\tau/k'_a)^{\frac{1}{2}}$ ) which results from the dissociation of ML in a given time  $\tau$  is much larger than the diffusive flux of ML relative to that of M (represented by  $(D_{ML}/D_M)^{\frac{1}{2}}$ ). Another way of looking at condition (14) is to say that a complex ML is labile when the rate of production of M from dissociation (determined by  $k_d c_{ML}^*$ ) and the mean free path of the produced M (proportional to  $(D_M/k'_a)^{\frac{1}{2}}$ ) are so large that a diffusion controlled consumption of ML develops at the electrode surface. With higher  $k'_a$  values only those metal ions which are produced by dissociation inside a very thin solution layer adjacent to the electrode surface (the so-called reaction layer) can be reduced before being recomplexed again. The complete conversion of ML is then only achieved with very high  $k_d$  values.

If both conditions (13) and (14) are satisfied, the gradient is readily obtained by introducing:

$$c_T = c_M + c_{ML} \quad (15)$$

so that eqs. (7a,b) reduce to:

$$\frac{\partial c_T}{\partial t} = \bar{D} \frac{\partial^2 c_T}{\partial x^2} \quad (16)$$

with the boundary conditions valid for the limiting current regime:

$$\left. \begin{array}{l} t = 0, \quad x \geq 0 \\ t > 0, \quad x \rightarrow \infty \end{array} \right\} \quad c_T = c_T^* \quad (17a)$$

$$t > 0, \quad x = 0: \quad c_T = 0 \quad (17b)$$

In eq. (16), the diffusion of both M and ML is taken into account by means of the mean diffusion coefficient  $\bar{D}$ , defined by:

$$\bar{D} = \frac{c_M^*}{c_T^*} D_M + \frac{c_{ML}^*}{c_T^*} D_{ML} \quad (18)$$

The solution of eq. (16) is classical [3] and gives the well-known Cottrell-like expression for the limiting current density  $i_l$ :

$$i_l = - \pi^{-\frac{1}{2}} n F D^{\frac{1}{2}} c_T^* \tau^{-\frac{1}{2}} \quad (19)$$

As expressed by eq. (18),  $\bar{D}$  is an average of the diffusion coefficients of the different metal species, weighted by their particular proportion relative to  $c_T^*$ . As long as conditions (13) and (14) are satisfied, the averaging of the diffusion coefficients applies to any complex species in the system. Equation (18) can then be generalized to:

$$\bar{D} = \frac{\sum_i \frac{c_i^*}{c_T^*} D_i}{\sum_i \frac{c_i^*}{c_T^*}} = \frac{\sum_i c_i^* D_i}{\sum_i c_i^*} \quad (20)$$

where the summation includes all the species obeying (13) and (14).

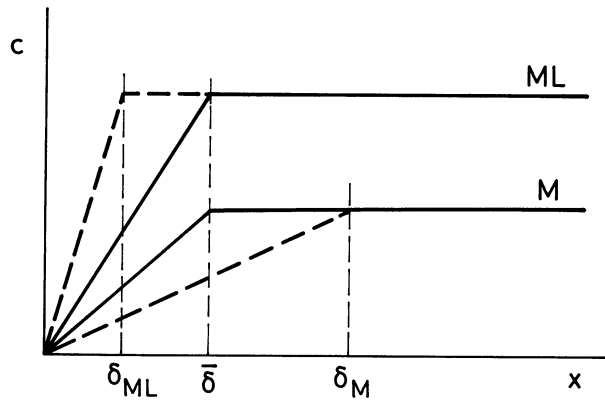


Fig. 1. Schematic concentration profile for a metal complex system (M, ML) under limiting current conditions. The dotted curves: M and ML are assumed to be fully independent of each other (e.g. ML is an electroactive fully inert complex) and diffuse separately, each with its specific diffusion layer thickness,  $\delta_{ML}$  and  $\delta_M$ . The full curves: ML is a labile complex in instantaneous equilibrium with M and L. M and ML diffuse simultaneously; under conditions where  $\bar{\delta}$  is operative (and  $D_{ML} < D_M$ ), so that the resulting diffusion layer has the corresponding intermediate thickness  $\bar{\delta}$ .

It should be emphasized that, according to eq. (19), the measured signal is **not equal to the sum of the separate responses** which would be obtained for each particular complex species without their mutual interactions. Each of these separate responses would be proportional to  $D_i^{\frac{1}{2}}c_i^*$ , so that their sum would depend on:

$$\sum_i D_i^{\frac{1}{2}}c_i^* \tag{21a}$$

whereas the corresponding term in eq. (19) is:

$$\bar{D}^{\frac{1}{2}}c_T^* = \left[ \sum_i c_i^* \sum_i D_i c_i^* \right]^{\frac{1}{2}} \tag{21b}$$

An example may clarify this noteworthy point. For a system M + ML where  $c_M^*/c_T^* = 0.1$  and  $D_{ML}/D_M = 0.01$ ,  $\bar{D}^{\frac{1}{2}}c_T^*$  equals  $0.33 \cdot D_M^{\frac{1}{2}}c_T^*$ , whereas  $(D_M^{\frac{1}{2}}c_T^* + D_{ML}^{\frac{1}{2}}c_{ML}^*)$  equals  $0.19 \cdot D_M^{\frac{1}{2}}c_T^*$ . The physical significance of this is further illustrated in Fig. 1: as a result of its faster diffusion, there is a tendency to a net depletion of M compared to ML within the diffusion layer. To maintain the equilibrium concentrations, ML will be forced to dissociate resulting in both a local decrease in ML concentration and increase in M concentration. The extent of this additional dissociation of course varies with x. As compared with the case where ML and M are independent of each other, the net result is an extension of the diffusion layer of ML and the opposite for M. The eventual result is a **common** diffusion layer with a thickness  $\bar{\delta}$ , lying between  $\delta_M$  and  $\delta_{ML}$ . The value of  $\bar{\delta}$  is related to  $\bar{D}$  in the conventional way, e.g. by  $\bar{\delta} = (\pi \bar{D} \tau)^{\frac{1}{2}}$  for the case of semi-infinite diffusion to a plane. In the situation where eqs. (9) and (10) are satisfied, the exact gradient of  $c_{ML}$  very close to the surface is not important any more. The boundary condition (4b), with  $\partial c_{ML} / \partial x = 0$ , is overruled by the dynamics of the ML  $\leftrightarrow$  M interconversion. The final result in this case is identical to what is obtained for the alternative boundary condition  $c_{ML} = 0$  at  $x = 0$ , i.e. for an electroactive complex [6].

### II.3. The case of an immobile complex: $D_{ML}/D_M \rightarrow 0$

It is already clear from the above discussion that, under certain kinetic conditions, completely immobile complexes give rise to a contribution to a measured current. For labile complexes, for instance, eq. (18) shows that if  $D_{ML} = 0$ ,  $\bar{D}$  may be small (if  $c_M^*/c_T^*$  is small) but is never zero. This is due to the flip-flop effect explained above, which explains that every metal ion, even if predominantly bound, spends a certain part of time as free M during which it diffuses towards the electrode surface. Therefore the neglect of contributions to

voltammetric signals from M bound to high molecular weight complexants is generally incorrect. This is also clearly pointed out by the numerical example given above: even for the stable ( $c_M^*/c_T^* = 0.1$ ) and rather immobile ( $D_{ML}/D_M = 0.01$ ) complex considered, the observed current (proportional to  $D_M^{-\frac{1}{2}}c_T^* = 0.32 * D_M^{\frac{1}{2}}c_T^*$ ) would still be 32% of the current which would be observed if  $D_{ML}$  and  $D_M$  had equal values (current proportional to  $D_M^{\frac{1}{2}}c_T^*$ ).

These considerations also apply to disperse systems such as colloids where electroactive metal ions are adsorbed on particles. Unless, for kinetic reasons, no appreciable desorption takes place on the time-scale of the voltammetric experiment, some contribution from the adsorbed metal should be expected. Unfortunately, the field of voltammetry of electroactive colloids and colloids which interact with electroactive species is almost totally unexplored.

The theory for the case of an essentially immobile ligand may be derived as a simple case of eq. (8). For  $D_{ML} = 0$ , eq. (8b) simplifies to:

$$-k'_a \bar{c}_M + (s + k_d) \bar{c}_{ML} - c_{ML}^* = 0 \quad (22)$$

and this allows the direct substitution of  $c_{ML}$  in eq. (8a), yielding

$$\frac{d^2 \bar{c}_M}{dx^2} = \frac{1}{D_M} \left( \frac{s(s + k'_a + k_d)}{s + k_d} \right) \bar{c}_M - \frac{c_M^*}{D_M} \left( \frac{s + k'_a + k_d}{s + k_d} \right) \quad (23)$$

Taking into account the boundary conditions in eqs. (4,b), eq. (23) can be solved into the gradient at the electrode surface:

$$\left( \frac{d\bar{c}_M}{dx} \right)_{x=0} = \frac{c_M^* (s + k'_a + k_d)^{\frac{1}{2}}}{D_M^{\frac{1}{2}} (s + k_d)^{\frac{1}{2}} s^{\frac{1}{2}}} \quad (24)$$

From this an analytical expression for the current can be derived [7], valid for any value of  $k'_a$  and  $k_d$ . However, that expression is rather complicated.

An interesting limiting case is that corresponding to a dynamic system (where eq. (13) applies), for which ML is electrochemically labile (eq. (14) applies). Then eq. (24) gives:

$$i_l = -\pi^{-\frac{1}{2}} n F D_M^{\frac{1}{2}} (c_M^* c_T^*)^{\frac{1}{2}} \tau^{-\frac{1}{2}} \quad (25)$$

Note that this expression is also obtained by combining eqs. (18) and (19) with  $D_{ML} = 0$ . For the case where, e.g.  $c_M^*/c_T^* = 0.1$  one finds that  $D_M^{\frac{1}{2}} (c_M^* c_T^*)^{\frac{1}{2}}$  is about a factor of 3 higher than  $D_M^{\frac{1}{2}} c_M^*$ , illustrating again that the contribution from the immobile complex is by no means negligible.

### III. ENVIRONMENTAL SAMPLE VOLTAMMETRIC DATA INTERPRETATION

When voltammetric techniques are applied to study metal complexation by natural complexing agents one can distinguish three types of problems with different levels of complexity in data interpretation [1]:

- (i) the first difficulty, which exists with any type of ligand (even those with the simplest structure), is related to the determination of the **nature of the electrode process** which controls the overall reduction rate (in particular discrimination between dissociation and diffusion). This is necessary to transform correctly the measured current into bulk solution complexation parameters;
- (ii) a second difficulty is linked to the fact that, with colloidal or macromolecular complexing agents, not only (as discussed in section II and shown in Table 1), their diffusion coefficient is significantly smaller than that of free metal ions, but they also possess more **complicated behaviour** compared to 'simple' ligands, such as polyelectrolytic, gel or aggregation properties which must all be considered when interpreting their complexation role;
- (iii) a third aspect results from the fact that natural media always include a very complicated mixture of ligands which can be, at best, fractionated into groups of homologous compounds [1] but from which the isolation of a pure natural complexant is only rarely possible. Consequently, any voltammetric signal measured on such samples is always an average of the contributions of all species, weighted in an often complicated manner because of their different chemical equilibria, chemical kinetics and diffusional rate transport [1]. This is referred to below as the **mixture effect**.



The problems related to point (ii) are dependent on the properties of the system and not on the technique used. They are treated in details in several reviews or monographs (e.g. refs. 5,15) and will not be discussed here. Refs. 1 and 2 also give guidelines to discriminate between the most important factors which may affect the voltammetric signal, namely chemical kinetics, diffusion controlled transport, ligand and complex adsorption, non-excess of ligand and mixture effect. The purpose of this section is to discuss specifically:

(i) the criteria which can be used in practice to discriminate between chemical and diffusion rate control;

(ii) the conditions to use, and the limitations to take into account to enable an appropriate interpretation of the kinetic nature of voltammetric signals obtained with natural samples, in spite of their complexity.

Note that in the following the current densities in presence,  $j^L$ , and absence,  $j$ , of ligand L are compared. By definition,  $j = I/A$ , where  $I$  is the current, and  $A$  is the surface area of the electrode.

### III.1. Interpretation of voltammetric curves obtained with pure metal-ligand systems

III.1.1. Criteria for discrimination between chemical and diffusional rate control. These criteria can be readily found from the expressions of the ratio of the limiting current densities in the absence ( $j_l$ ) and presence ( $j_l^L$ ) of ligand L, for the two limiting cases where the current is controlled either purely by chemical kinetics or only by diffusional transport. These expressions directly follow from the lability criterion, see eq. (14), and under the condition:  $c_L^* \gg c_T^* \approx c_{ML}^* \gg c_M^*$ , it can be shown [1] that:

-  $j_l^L$  controlled by diffusion transport only ( $k_d(\tau/k'_a)^{\frac{1}{2}} \gg (D_{ML}/D_M)^{\frac{1}{2}}$ ):

$$\frac{j_l^L}{j_l} = \frac{\delta_M \bar{D}}{\bar{\delta} D_M} \tag{26a}$$

-  $j_l^L$  controlled by dissociation/association kinetics of ML ( $k_d(\tau/k'_a)^{\frac{1}{2}} \ll (D_{ML}/D_M)^{\frac{1}{2}}$ ):

$$\frac{j_l^L}{j_l} = \frac{\delta_M}{(D_M)^{\frac{1}{2}}} \left( \frac{k_d}{Kc_L^*} \right)^{\frac{1}{2}} \tag{27a}$$

where  $\bar{\delta}$  and  $\delta_M$  are the diffusion layer thicknesses in the presence and absence of L respectively. Equations (26a) and (27a) can be used for stationary electrodes in unstirred systems (by replacing  $\delta$  by  $\delta = (\pi D \tau)^{\frac{1}{2}}$ ), for DME ( $\delta = (3\pi D \tau / 7)^{\frac{1}{2}}$ ), and for rotating electrodes ( $\delta = \text{constant} * D^{\frac{1}{2}}$ ) or for HMDE in stirred solution. In this latter case, it has been found experimentally that, during the reduction step of ASV,  $\delta \approx G D^{\frac{1}{2}}$  [16], where  $G$  is a constant which depends on the stirring mode and the geometry of the cell.

It can be seen that in all cases eq. (26a) is independent of hydrodynamic conditions:

$$\frac{j_l^L}{j_l} = (\bar{D}/D_M)^{\frac{1}{2}} \tag{26b}$$

In contrast, eq. (27a) depends on hydrodynamic conditions, for instance:

- stationary electrode ( $\delta = (\pi D \tau)^{\frac{1}{2}}$ ):

$$\frac{j_l^L}{j_l} = (\pi \tau)^{\frac{1}{2}} \left( \frac{k_d}{Kc_L^*} \right)^{\frac{1}{2}} \tag{27b}$$

- HMDE in stirred solution ( $\delta = G(D)^{\frac{1}{2}}$ ):

$$\frac{j_l^L}{j_l} = G \left( \frac{k_d}{Kc_L^*} \right)^{\frac{1}{2}} \tag{27c}$$

Therefore in general, eqs. (26) and (27) show that, for ML complexes formed with a pure simple ligand L, the relative importance of chemical kinetic control and diffusion control can be established by changing either the diffusion layer thickness or the complexing conditions. The first factor can be changed by modifying the conditions of the technique used: either the measuring time,  $\tau$ , (drop time in DC polarography, pulse duration in NPP or DPP) or the stirring mode (factor  $G$ ) in the reduction step of ASV. The complexing conditions

can be changed by modifying the ligand concentration, or the pH which influences the free ligand concentration and the conditional equilibrium constant  $K$ . From eqs. (26) and (27), one can predict that:

(i) for **diffusion controlled systems** (eq. (26b))  $i_{\ell}^L/i_{\ell}$  is independent of  $\delta$ , and thus it depends neither on  $\tau$  nor on the stirring rate. It is also independent of the complexing conditions ( $Kc_L^*$ ) in the range where  $c_M^*/c_T^*$  and  $D_{ML}/D_M$  are such small that  $\bar{D} \approx D_{ML}$ . Then

$$i_{\ell}^L/i_{\ell} = (D_{ML}/D_M)^{\frac{1}{2}};$$

(ii) for systems **controlled by association/dissociation kinetics** (eq. (27a))  $i_{\ell}^L/i_{\ell}$  decreases with  $\delta_M$  (corresponding to a increase in  $1/\tau$  or in the stirring rate) or increases with  $Kc_L^*$ .

The above criteria are based only on the current obtained in limiting conditions (i.e. at sufficiently negative potential to give  $c_M = 0$  at the electrode surface). It must be noted

however that the whole  $j=f(E)$  curves (and even better for stationary electrodes in quiescent solutions, their time dependence, i.e. the  $j = f(E,t)$  curves) contain much more information on the nature of the rate controlling electrode process than just the limiting current density. General  $j=f(E,t)$  relationships have been derived recently [75], but until now, interpretation of the complete  $j=f(E,t)$  curves in the case  $D_{ML} < D_M$ , has been done only for chemically dynamic systems, with electrochemically labile complexes. It is the so-called DeFord-Hume treatment (e.g. ref. [3]) which enables the computation:

of  $\bar{D}$  by:

$$\frac{i_{\ell}^L}{i_{\ell}} = \left( \frac{\bar{D}}{D_M} \right)^{\frac{1}{2}} \quad (28)$$

of  $f = c_M^*/c_T^*$  by:

$$\ln f = \frac{nF}{RT} \left( E_{\frac{1}{2}}^L - E_{\frac{1}{2}} \right) + \ln \left( i_{\ell}^L/i_{\ell} \right) \quad (29)$$

and of  $D_{ML}$  by (see eq. (18)):

$$\bar{D} = D_{ML}(1 - f) + D_M f \quad (30)$$

It is important to emphasize that, for labile complexes, the shift in half-wave potential (or peak potential, depending on the technique used) depends not only on the fraction of uncomplexed metal ion,  $f$ , but also on the inequality between  $\bar{D}$  and  $D_M$ , and must therefore be corrected for that effect, to give correct results of  $f$ . It is also interesting to note that  $f$  (and thus  $K$ ) can be computed either from the corrected potential shift (eq. 29) or from only limiting current measurements (eqs. (28) and (30)). The choice of an approach depends on the size of the complexant, i.e. the value of  $D_{ML}$ : for very low  $D_{ML}$  values, the absolute value of the second term in the right hand side of eq. (29) is large. Consequently, for a given value of  $f$  in that case, the corresponding  $E_{\frac{1}{2}}$  shift may be small and its determination is not accurate. On the contrary, in this situation,  $i_{\ell}^L$  is very different from  $i_{\ell}$ , and a precise measurement of their ratio is possible. When  $D_{ML}$  is not very different from  $D_M$  the reverse situation holds, and computation of  $f$  from eq. (29) is preferable. The usefulness of recording both current and potential data is also illustrated in sec. III.1.3., where the interpretation of the complexation titration curves is discussed.

The above examples however are only simple limiting cases. There is presently no theory available for whole voltammetric curves corresponding to more complicated cases. This suggests (as do considerations of sec. III.1.2.) that the informative content of the current-potential-time voltammetric curves is probably much underused. In that respect there is a strong need for a general theory, or at least for numerical solutions of the current-potential-time voltammetric curves of systems where both homogeneous dissociation kinetics and diffusion are considered.

III.1.2. Summary of the interpretation capabilities for the various system types. Based on the present state of the theory, the various system types may be classified as follows:

(i) the simplest situation is met when all the complex species are chemically inert, i.e. when the association/dissociation rate parameters are so low that the complexes do not contribute significantly to the signal. The voltammetric curve and the corresponding parameters are then directly related only to the free metal ion concentration in the bulk solution and no information can be gained about the complexation parameters ( $D_{ML}$ ,  $K$ ,  $k_d$ );

(ii) the other extreme simple case is when the association/dissociation kinetics of all the complexation reactions are not rate limiting for the voltammetric signal. The measured current is then controlled by a mean diffusion coefficient which takes into account the contribution of each complex weighted for its own proportion and diffusion coefficient. This mean diffusion coefficient is therefore the basic speciation parameter in analysing voltammetric current data. In these limiting cases the mathematical expression of the whole current-potential-time curve is also available (DeFord-Hume treatment). Complexation parameters ( $D_{ML}$  and  $K$  but **not**  $k_d$ ) can therefore be obtained either from potential or current measurements (eqs. (28-30));

(iii) the intermediate cases cover kinetic regimes where the association/dissociation rate parameters combine with diffusion to control the voltammetric signal. The different possible situations within that regime are often complicated and a complete analysis is under development [7,8].

III.1.3. Interpretation of complexation titration curves. Complexation titration curves are obtained by varying the metal to ligand concentration ratio and recording the voltammetric curve (in particular their characteristic parameters  $i_p^L$  and  $E_p^L$ ) corresponding to each titration point (Fig. 2). Such curves are interesting for two reasons: firstly it has been seen that varying the complexation characteristics of the solution ( $Kc_L^*$ ) is a good criterion for testing the lability or inertness of the complexes. Secondly, these types of curves are largely used in the literature dealing with natural waters, for the determination of the total ligand concentration,  $|L|_T$  also called the 'complexation capacity'. The principle of this measurement is based on the assumption that in solutions containing only the free ion  $M$  plus inert complexes  $ML$ ,  $i_p^L$  (or  $i_p^L$ ) is just a measure of the free metal ion concentration  $c_M^*$ . In the most simple case of very stable  $ML$  complexes (Fig. 2a, curve 1)  $i_p^L = 0 (= c_M^*)$  for any value of  $c_T^*$  smaller than  $|L|_T$ . After that point  $i_p^L$  increases with  $c_T^*$  according to a

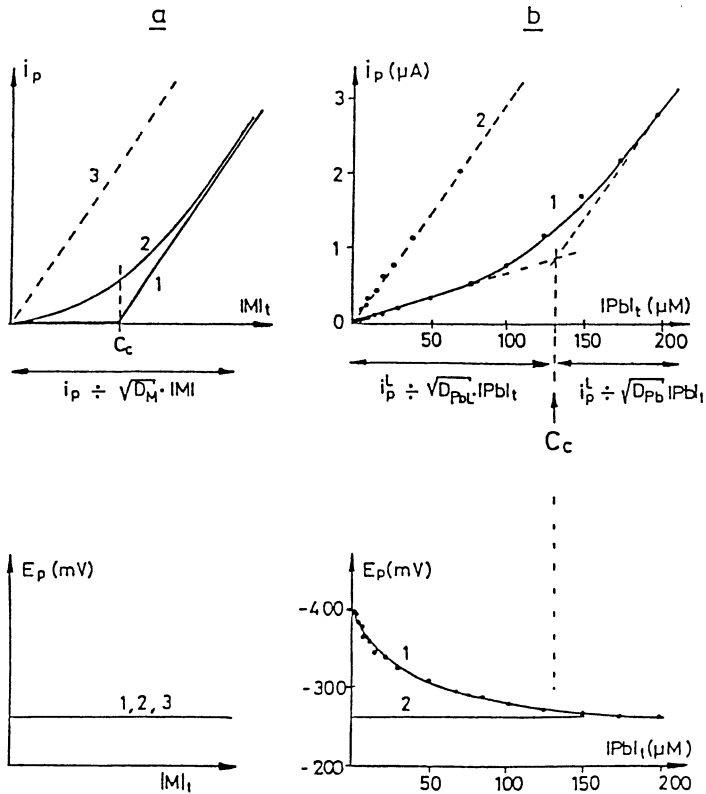


Fig. 2. Shapes of titration curves of complexing media by  $M$  (from ref. 1). (a) Inert complex ( $k_d \rightarrow 0$ ): (1) very stable ( $K|L|_T > 1000$ ); (2) moderately or slightly stable ( $K|L|_T < 100$ ); (3)  $|L|_T = 0$ . (b) Labile system ( $k_d \rightarrow \infty$ ) with  $D_{ML} < D_M$  (case of Pb-fulvic compounds using DPP; PROM: (1) 36 mg/l, (2) 0 mg/l; pH = 6).  $|L|_T$  represents the total concentration of ligands or complexing sites, and  $i_p^L$  the peak current density measured in presence of the ligand.

curve parallelling the calibration curve, and  $|L|_T$  is readily obtained at the break of the  $j_p^L = f(c_T^*)$  curve. In practice however one observes more often curves resembling Figs. 2a (curve 2) or 2b. Although these two curves are similar to each other, they must be clearly discriminated because their interpretation differs [1]:

- (i) in Fig. 2a (curve 2), ML is inert but of low thermodynamic stability. The difference in curve 2 relative to curve 1 arises from the fact that in the vicinity of the point  $c_T^* = |L|_T$  the free concentration of M in the bulk solution,  $c_M^*$ , is not negligible because of the relatively low value of the equilibrium constant of ML;
- (ii) in Fig. 2b, ML is labile and thermodynamically stable, but  $D_{ML} < D_M$ . Then:  $j_p^L/j_p = (\bar{D}/D_M)^{1/2} < 1$  for  $c_T^* < |L|_T$ . In this case the curve never tends to a horizontal line, even at very low  $c_T^*/|L|_T$  value.

To establish correct values of  $|L|_T$ , it is important to discriminate between these two cases. Indeed, in the first case a rigorous value of  $|L|_T$  is always obtained by extrapolation of the linear part of the curve to  $j_p^L$  (or  $j_p^L$ ) = 0, irrespective of the curvature in the vicinity of  $|L|_T$ . On the other hand in the second case, it is the change in slope which reflects the ligand saturation and  $|L|_T$  is obtained from the intersection of the two linear segments. Discrimination between inert and labile complexes is therefore important, but, as Figs. 2a and 2b show, this is **not** possible just by examining the shape of the  $j_p^L$  vs.  $c_T^*$  curve. This however can be done by recording  $E_{1/2}^L$  (or  $E_p^L$ ) vs.  $c_T^*$  (Figs. 2a, 2b): for the formation of inert complexes,  $E_{1/2}^L$  is independent of  $c_T^*$  and equal to the value  $E_{1/2}^L$  obtained in the absence of ligand, whereas for the formation of labile complexes  $E_{1/2}^L$  is always **more negative** than  $E_{1/2}^L$  but tends towards  $E_{1/2}^L$  as  $c_T^*$  increases, since  $f$  and  $j_p^L/j_p$  tend to 1 (eqs. (28-30)).

Without going into details, it must be emphasized here that in practice the simple interpretations given above are rarely applicable to complexation titration curves obtained from environmental or biological systems. Indeed several problems often severely limit the validity of these simple interpretations and consequently the usefulness of such titration curves for simple measurements of  $|L|_T$ :

- (i) irrespective of the nature of the rate controlling process, it is generally observed that the curvature of the  $j_p^L$  vs.  $c_T^*$  curve extends very far from  $|L|_T$  which generally makes an accurate extrapolation difficult in practice (see refs. 1,2 for details);
- (ii) the interpretation of complexation data obtained from the titration of macromolecular or colloidal complexants must take into account the possible physical changes (e.g. electric charge, shrinking or swelling, conformation) of the complexant molecule during the titration [15]. This may result in changes of  $D_{ML}$ ,  $K$  and  $k_d$  along the titration, resulting in a change in  $j_p^L$  which may just mimic that of curves in Fig. 2a and 2b (e.g. see ref. 17 for the influence of polyelectrolytic effect on voltammetric current). Additional non-voltammetric data are then necessary to determine the importance of these effects.
- (iii) in the case of labile complexes, it must be emphasized that the value of  $|L|_T$  obtained as indicated in Fig. 2b is only an approximate value of the total ligand concentration. A necessary condition for a simple and rigorous interpretation of the current due to labile complexes is an excess of ligand relative to M, as discussed in sect. II. This condition is obviously not fulfilled in the saturation zone, i.e. in the vicinity of  $|L|_T$ ;
- (iv) finally a similar but even more important problem occurs when anodic stripping techniques are used for determining the complexation titration curves [1,18,19]: since the metal is pre-concentrated in the electrode during the initial reduction step, the metal to ligand concentration ratio during the stripping is much larger (often  $\approx 100$  times) at the electrode surface than in the bulk solution. As a consequence, for labile complexes, the ligand may be saturated at the surface even if it is in excess in the bulk solution and both current and potential peaks are strongly affected [18]. It may be shown [18-20] that varying the deposition time serves as a powerful criterion to point out the importance of that effect. In this way, ASV can be used to estimate  $D_{ML}$ , the nature of the electrode process, and the complexation characteristics of complicated metal-macromolecular ligand systems like the  $Pb^{II}$ -fulvic acid system [20].

### III.2. Application limits and conditions to environmental and biological samples

As mentioned in the introduction of this section, one of the important complications of natural samples is that they include a (often complicated) mixture of complexants (which can generally not be isolated without perturbations), each of them having different  $D_{ML}$ ,  $K$  and  $k_d$  values. Therefore a rigorous interpretation of voltammetric data obtained from an unknown and unfractionated natural sample is presently virtually impossible. However, some 'simple' cases may be considered:

(i) **all complexes of the test medium are inert:** this may be the case for some trace metal complexes (transition metals, heavy metals) with organic or inorganic colloid-rich natural water. These compounds often include complexing sites in low content but forming highly stable and very slowly dissociable complexes (minutes to days, therefore polarographically inert complexes) [1]. If the metal content is low, these strong sites are not saturated and the metals are preferentially bound to them, even if the medium contains larger concentrations of other weaker ligands. Under such conditions, the medium only contains inert complexes and  $i_L^L$  is proportional to  $c_M^*$  (sec. III.1.2.). Then,  $f$  is easily obtained by:

$$f = i_L^L / i_L \quad (31)$$

(ii) **all complexes are labile with  $D_{ML_i} = D_M$  for all ligands  $L_i$ :** in this case, the media contain only small sized ligands (e.g. mineral waters where the  $L_i$  are only simple inorganic anions such as  $Cl^-$ ,  $CO_3^{2-}$ ,  $SO_4^{2-}$  or open sea waters containing a very low content of colloids or organic compounds). Now,  $\bar{D} = D_{ML_i} = D_M$  and the fraction  $f$  is given by eq. (29) with  $i_L^L / i_L = 1$ ;

(iii) **all complexes are chemically labile but physically immobile ( $D_{ML_i} \rightarrow 0$  for all  $L_i$ ):** This would be the case of a natural (more often fresh) water whose predominant complexing agents are large macromolecules or colloids under such conditions that the metal is predominantly bound to surface sites forming labile complexes. All the terms in eq. (20) drop out except that corresponding to free  $M$ , i.e.  $\bar{D} = D_M f$ . Then,  $f$  can be obtained by:

$$f^{1/2} = i_L^L / i_L = (\bar{D} / D_M)^{1/2} \quad (32)$$

For spherical particles, the limit of the radius  $r$ , below which this simplification does not hold can be estimated by considering that, for  $r = 50$  nm,  $D_{ML} \approx 5 \cdot 10^{-8}$  cm<sup>2</sup>/s, or  $(D_{ML} / D_M)^{1/2} \approx 1/12$ , i.e. the contribution to the current, due to diffusion of  $ML$ , is still 8% of the limiting current which would be observed in the absence of  $L$  [1]. Incidentally, it can be emphasized that chemically inert and physically immobile complexes are not equivalent since their limiting current dependence on  $f$  is different;

(iv) **labile complexes  $ML_j$  with  $D_{ML_j} = D_M$  and inert complexes  $ML_i$ :** the complexes formed in the system may be divided into two groups: labile complexes formed with  $L_j$  ( $j$  variable) with  $D_{ML_j} = D_M$  irrespective of  $L_j$ , and inert complexes formed with  $L_i$  ( $i$  variable). This would be the case, for instance, of a sea water containing small simple inorganic anions (forming the pool of ligands 'j') together with a few natural or anthropogenic organic ligands forming inert complexes (forming the set of ligands 'i'). In such a case, any potential shift is due to the formation of labile complexes, and any change in  $i_L$  results from the formation of inert complexes. Therefore the fraction of labile complexes,  $f_1 = \sum_j |ML_j| / c_T^*$  is given by eq. (29) where the second term in the right hand side is equal to 0, whereas the fraction of inert complexes,  $f_i = \sum_i |ML_i| / c_T^*$ , is given by  $(i_L - i_L^L) / i_L^L$  [1,2]. Such a case was studied, for instance by Raspor et al. [21].

In many cases [1], natural media include compounds forming complexes which are neither fully inert or fully labile (i.e. in many cases:  $0.1 < k_d \{ (D_M / D_{ML}) (\tau / k'_a) \}^{1/2} < 10$ ), nor physically immobile (i.e. in many cases their size is in the range  $0.4 < r < 50$  nm). Furthermore, the thermodynamic and kinetic complexation properties of natural macromolecules can generally not be described by a few constants characteristic of the compound. Because of their polyfunctionality (large number of different complexing sites on the same molecule), their polyelectrolytic properties (large electric charge density) and their high capabilities of conformational changes, the stability and dissociation/association rates of the complexes are strongly dependent on the metal/ligand concentration ratio. It has been mentioned (sec. III.1.3.) that this is also true for the diffusion coefficient. This is schematized in

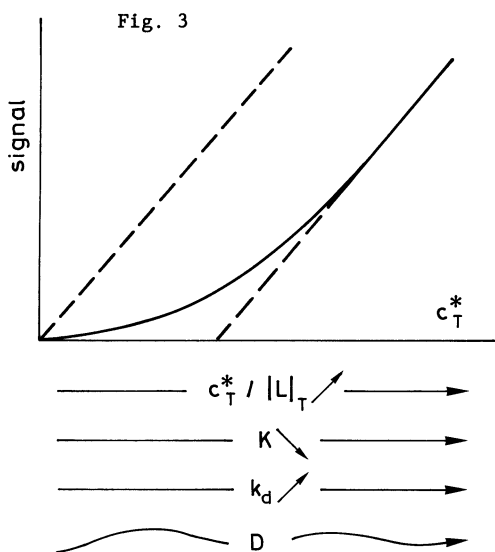


Fig. 3. Variation of main complexation parameters during titration of a homologous complexant by M.

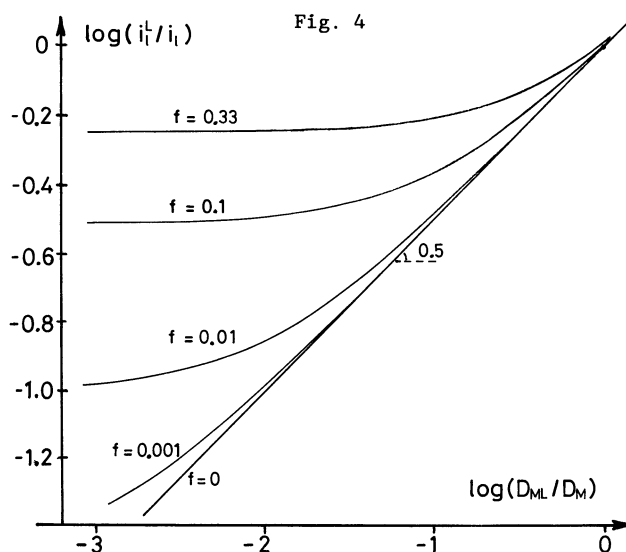


Fig. 4. Theoretical change in  $i_l^L / i_l$ , as a function of  $\bar{D}_{ML} / D_M$  for various values of the net degree of complexation of M. All complexes are assumed to be labile with the same diffusion coefficient,  $\bar{D}_{ML}$ . Values of  $f = c_M^* / c_T^*$  are indicated on the curves.

Fig. 3 which shows that, during a complexation titration curve, three parameters may vary, making the interpretation of the voltammetric signal very difficult:

(i) the thermodynamic stability of the complexes (represented by K) decreases because the strongest sites are saturated first and the electrostatic contribution to the binding decreases;

(ii) the dissociation rate constant is expected to increase, since, as a first approximation, the stronger the complexes, the slower their dissociation rate;

(iii) the diffusion coefficient may vary in an unpredictable manner, since there is no relationship between the stability of the complex formed between a site and a metal ion M, and the size of the molecule bearing this site. Only if the medium is a pure solution of a macromolecular complexant, one can expect D to increase (although to an extent difficult to predict) because an increase in site saturation generally also corresponds to a decrease in the overall charge of the macromolecule and in its degree of expansion (therefore  $r$  tends to decrease). Besides, in the case of formation of  $ML_2$ , intramolecular bridging may occur, also resulting in shrinkage of the macromolecular coil.

Because of this very large number of possible physico-chemical reactions, the thermodynamic, kinetic and diffusion properties of a metal ion M in a natural water or biological system can no longer be represented by a few single constants ( $K, k_d, D_{ML}$ ), but must be described by corresponding distribution functions [1]. Rigorously they should replace the corresponding constants in eqs. (3a,b). This would however lead to untractable mathematical expressions. The only present solution to this difficult problem is to size fractionate the sample and perform voltammetric measurements on each fraction. There are not many size fractionation methods applicable to environmental samples in the useful range ( $0.4 < r < 50$  nm). One can cite however (together with their main limitations within parentheses): gel filtration (application difficult to natural water systems because of secondary effects like irreversible adsorption), field flow fractionation [22] (needs further developments and is presently limited to analytical, not preparative applications), and cascade ultrafiltration (ill-controlled membrane effects and resulting fractions whose particles are distributed in a relatively wide size range).

For natural water studies, size fractionation is interesting in two respects: not only it facilitates the voltammetric interpretation (see below), but simultaneously it may be shown [1] to permit obtaining fractions possessing a certain chemical homogeneity, provided the cut-off limits are correctly chosen. From a voltammetric point of view, it is interesting to note that, due to the characteristics of these techniques, a relatively rough fractionation may produce large improvement in the interpretation. For instance, by cascade ultrafiltration one typically obtains fractions whose compounds have molecular weights ranging within one decade ( $\Delta \log M_w = 1$ ). This corresponds to a diffusion coefficient range

of  $\Delta \log D \approx 0.5$  [1]. Fig. 4 based on eq. (34) (see below) shows that the corresponding range of limiting current densities (expressed by  $\Delta \log(i_L^L/i_L^R)$ ) is between 0 and 0.25 depending on the value of  $f$ . This is of course a much better situation than the range of 1 or 2 decades which would correspond to an unfractionated sample containing macrocompounds with size ranging from 1 nm to 1  $\mu\text{m}$ , i.e. diffusion coefficients ranging from  $10^{-8}$  to  $10^{-5}$   $\text{cm}^2/\text{s}$ , as it is generally found in natural waters [1] (see also Table 1). Equations (26), (27) and (30) clearly show the great simplification introduced by size fractionation for interpretation of the voltammetric data. For instance, in the case where all the complexes,  $\text{ML}_i$ , are labile, both with  $D_{\text{ML}_i} \neq D_M$ , eq. (20) is applicable and may be written as:

$$\bar{D} = D_M \frac{c_M^*}{c_T^*} + \sum_i D_{\text{ML}_i} \frac{|\text{ML}_i|}{c_T^*} \quad (33)$$

In a fractionated sample,  $D_{\text{ML}_i} \approx \bar{D}_{\text{ML}} \approx \text{constant}$ , irrespective of the nature of  $L_i$ .

Therefore, by also considering that  $c_M^*/c_T^* = f$ , eq. (33) simplifies to:

$$\bar{D} = D_M f + \bar{D}_{\text{ML}}(1 - f) \quad (34)$$

$f$  and  $\bar{D}_{\text{ML}}$  can therefore be obtained readily from eqs. (28-30), as for systems containing only one complex species ML. From the change of  $f$  with experimental conditions (in particular metal to ligand ratio), the distribution spectrum of complex thermodynamic stability can be computed as discussed e.g. in ref. 1.

#### IV. CRITICAL COMPILATION OF LITERATURE DATA

Voltammetric studies dealing with the interaction of metal ions with the naturally occurring macromolecules humic and fulvic acids are abundant, but the macromolecular nature of these ligands is often not considered in the interpretation. Furthermore, studies on other macromolecular environmental compounds are scarce. Table 1 presents a literature survey of publications dealing with voltammetric studies of metal/macromolecular ligand systems in the period 1950-1987, not limited to environmental media. Its main goal was to be critical with respect to the inventory of the concepts for the treatment of voltammetric data applied up to now to metal/macromolecular ligand systems, but not necessarily exhaustive with respect to all papers dealing with voltammetry of macromolecular metal complexes.

The literature screening was based on the following keywords (in different truncations) in the title: polarography, voltammetry, ASV, NPP, CSV, DPASV, DPP, electrochemical analysis or electrochemical methods, in combination with humic, fulvic, polymer, protein, lignin, polyelectrolyte, or in combination with diffusion coefficient. An extension of the literature screening area, using the same keywords, to the Chemical Abstracts Indexes of the volumes 105-107 did not yield more relevant publications. This survey does not include studies of adsorption effects by macromolecules or surfactants on the voltammetric current. In series of publications reported by a given group of investigators, only those publications have been selected that can be considered as representative of the corresponding series.

Frequently naturally occurring macromolecular ligands are not well-described. Any specific naming used in a publication has been cited as such. For most of the quoted studies, the kinetic characteristics of the complex systems cannot be traced and therefore it is impossible to give an 'a priori' judgement of the correctness of the data treatment. On one hand this is a pity, but on the other it illustrates the need for a theoretically more sound utilization of voltammetric techniques in metal complexation studies.

Table 1 reports on the data treatments used by the authors referred to. The data treatments based on limiting currents or peak currents are symbolized by the letters A to E as defined below. In cases where the half-wave or peak potential shift has been exploited, the letter V is added in the column.

A: the current is assumed to be a direct measurement of the free metal ion concentration. The complexes are considered as **inert**.

B: it is assumed that the current is a measure of the sum of the **free and labile** metal species concentrations and  $D_{\text{ML}} = D_M$ .

C: it is assumed that the current is equal to the sum of those which would be obtained **separately** for M and all the complexes  $\text{ML}_i$ , at the same concentrations as in the test medium and that  $D_{\text{ML}_i} = \bar{D}_{\text{ML}} = D_L$ , irrespective of  $i$ .

- D: the current is considered to be composed of the **diffusion controlled** contribution of the free metal ions **plus a kinetic contribution**, due to the partial dissociation of the complexed metal ions during the time scale of the method.
- E: all metal species are considered to be **labile** and the current is described by a **mean diffusion coefficient**,  $\bar{D}$ , given by eq. (20).
- V: **potential** characteristics are considered to be applicable.

### LIST OF SYMBOLS AND ABBREVIATIONS

A	electrode surface area
$c_T$	total metal concentration
$c_T^*$	total metal concentration in the bulk
$c_i$	concentration of species i
$c_i^*$	concentration of species i in the bulk
$\bar{D}$	mean diffusion coefficient
$D_i$	diffusion coefficient of species i
$d_{\pm}$	parameters defined by eq. (10)
$d_r$	parameter defined by eq. (11)
E	potential
$E_{\frac{1}{2}}$	half-wave potential
$E_{\frac{1}{2}}^L$	half-wave potential in the presence of ligand
$E_p$	peak potential
f	$\frac{c_M^*}{c_T^*}$
G	constant depending on stirring mode and cell geometry
I	current
$I_l$	limiting current
$i_l$	limiting current density
$i_l^L$	limiting current density in the presence of ligand
$i_p^L$	peak current density in the presence of ligand
K	stability constant
K'	$Kc_L^*$
$k_a$	association rate constant
$k'_a$	$k_a c_L^*$
$k_d$	dissociation rate constant
$k_s$	rate constant of the electrode reaction
$ L _T$	total ligand concentration
$M_w$	molecular weight
r	radius
s	Laplace operator
super bar	Laplace transformed variable
t	time
x	distance from electrode surface
$\epsilon$	diffusion coefficient ratio ( $D_{ML}/D_M$ )
$\bar{\delta}$	mean diffusion layer thickness
$\delta_i$	diffusion layer thickness for species i
$\tau$	characteristic time-scale



ASV	Anodic Stripping Voltammetry
ass.	assumed
av.	average
CSV	Cathodic Stripping Voltammetry
calc.	calculated
DCP	Direct Current Polarography
DME	Dropping Mercury Electrode
DPASV	Differential Pulse Anodic Stripping Voltammetry
DPP	Differential Pulse Polarography
FA	Fulvic Acid
FTP	Fourier Transform Polarography
HA	Humic Acid
LSASV	Linear Sweep Anodic Stripping Voltammetry
NPP	Normal Pulse Polarography
P	Polarography
PROM	Pedogenic Refractory Organic Matter
RDE	Rotating Disk Electrode

TABLE 1. Literature survey on voltammetric studies of metal/macromolecular ligand systems.

Metal	Macromolecular Ligand	M <sub>w</sub> (av.)	D <sub>ML</sub> 10 <sup>-6</sup> cm <sup>2</sup> /s	Volt. Techn.	Data Treatment	Remarks	Ref.
<u>Synthetic polymers</u>							
- Cu	polyacrylic acid	-	0.1 - 0.2	P	-	reduction irreversible	[23]
- Tl, Cd	polyacrylates	300,000	-	P	A	I <sub>l</sub> is the sum of migration and Faraday currents	[24]
- Mn, Ni, Zn, Cu	Na-polyacrylate	2,700	0.001 - 0.00001	DCP	E*)	*) diffusion along backbone of adsorbed polymer	[25]
- Cu, Zn, Cd	polymethacrylic acid	600,000	0.05	P	C	D <sub>ML</sub> from Stokes equation	[26]
- Cu, Zn	polymethacrylic acid	-	-	P	V	the Cu/polymer system irreversible	[27]
- Ni, Co	polymethacrylic acid (gel)	-	-	P	A	in solution above gel phase	[28]
- Cd, Zn	Na-polyacrylate	2,500	-	DCP FTP	-	k <sub>s</sub> small in polymer system	[29]
- Cd	polyacrylic acid	130,000	-	P	A	I <sub>l</sub> is the sum of migration and Faraday currents	[30]
- Pb, Cd	copolymer of methacrylic acid and its methyl ester	1,000,000	negligible	NPP	C		[31]
- Cd, Pb	polyacrylic acid	50,000	0.2	NPP	E		[32]
- Cd	K-polyacrylate	1,000,000	-	P	A	I <sub>l</sub> : kinetic nature	[33]
- Cu	polymethacrylic acid polyacrylic acid	-	-	DCP	-	ΔE <sub>1/2</sub> accompanies decrease in I <sub>l</sub>	[34]
- Cd, Pb	polymethacrylic acid polyacrylic acid	25,000 50,000	0.2 0.2	NPP NPP	E, V	Stokes-Einstein applicable	[13]
- Cd, Zn	polyacrylic acid	50,000 300,000	0.2 0.1	NPP	E, V		[35]
- Mn, Cu, Ni, Cr, Zn	polyglutamic acid	700-800	0.08 - 0.5	P	C	Stokes-Einstein applicable	[36]
- Cu	polyaziridine	> 10,000	-	P	-	system irreversible, I <sub>l</sub> diffusion controlled	[37]

(Table 1, cont.)

Metal	Macromolecular Ligand	$M_w$ (av.)	$D_{ML}$ $10^{-6} \text{ cm}^2/\text{s}$	Volt. Techn.	Data Treatment	Remarks	Ref.
<u>Synthetic polymers (cont.)</u>							
- Cd	polystyrenesulfonate	1,000,000	-	P	-	$I_{\ell}$ is the sum of migration and Faraday currents	[38]
- In	polystyrenesulfonate	-	-	P	-		[39]
- Pb, Cd	macrocyclic crown ethers	-	0.01 - 1	P	V	some metal-ether systems irreversible	[40]
- Cd	sodium alkyl sulfate micelles	-	0.4 - 1.4	P	B*)	*) all Cd-ions bound	[41]
<u>Biopolymeric material</u>							
- Cd	carboxymethylcelluloses	~ 30,000 - 60,000	-	P	A	$I_{\ell}$ is the sum of migration and Faraday currents	[42]
- Cu	polysaccharides	-	-	DPP	A		
- Cd, Tl	pepsin	37,000	-	P	A	$I_{\ell}$ is the sum of migration and Faraday currents	[24]
- Cu	heparins	4,500 - 15,500	-	P	A	$I_{\ell}$ decreases with increasing $M_w$	[44]
- Zn	alpha-h-corticotropine	-	-	P	V	with correction for irreversibility	[45]
- Cd	albumin	70,000 (calc.)	0.24 (calc.)	P	C	kinetic contributions are considered	[46]
- Zn, Cd	albumin	-	~ 0.72	P	C	several contributions to $I_{\ell}$ are considered	[47]
- Co	albumin	-	0.3 - 1.2	P	C		[48]
- Cu	DNA	-	negligible	-	C		[49]
- Cu, Cd	DNA	10,000,000	~0.001	DCP	E	averaged degree of binding in the diffusion layer	[50] & [51]
- Os	poly-C, poly-U	-	-	DPP	-		[52]
- Cd, Mn	poly-dT RNA, DNA	-	-	DPP	A		[53]
- Cu	poly-U, poly-A transfusion gelatin	75,000	-	DCP	D		[54]
<u>Environmental Organics</u>							
- Cu, Pb	humic acid (K&K Laboratories)	950/eq.	-	DPP DPASV	V	correction for irreversibility	[55]
- Cu, Tl	humic acid (K&K Laboratories)	600 - 1000	-	ASV	B, V	lability decreases: Tl > Cd > Cu	[56]
- Cu	pond water (near Chapel Hill)	-	-	ASV	A		[57]
- Cu	humic and fulvic acid (Black Lake)	-	10	ASV	D	$k_d(\text{CuHA})=0.1 \text{ s}^{-1}$ $k_d(\text{CuFA})=2.7 \text{ s}^{-1}$	[58]
- Cd, Zn	humic acid (forest soil, Darjeeling)	> 12,000	0.28 (calc.)	P			[59]
- Pb	fulvic acid (PROM) (Mare aux Evées, Fontainebleau)	2,200	1 - 3	DPP NPP	V	system is labile & reversible charge transfer	[60] & [61]

(Table 1, cont.)

Metal	Macromolecular Ligand	$M_w$ (av.)	$D_{ML}$ $10^{-6} \text{ cm}^2/\text{s}$	Volt. Techn.	Data Treat-ment	Remarks	Ref.
<u>Environmental Organics (cont.)</u>							
- Zn, Cd Pb, Cu	river water samples (Susquehanna River, St. Lawrence River)	-	-	DPASV	B	$k_d$ is calculated from the labile fraction	[62]
- Cd, Cu Ni, Pb Zn	fulvic acid (New Hampshire)	-	-	ASV DPP	B, V	systems show irre- versibility, ad- sorption effects and non-lability	[63]
- Cd, Cu	fulvic acid (soil derived)	-	0.1 - 1	ASV	D		[64] & [65]
- Al	humic acid (from organic muck soil)	60,000	-	DPP	B		[66]
- Cd, Cu	marine humic and ful- vic acid (Gulf of Mexico)	-	-	DPASV	B		[67] & [68]
- Cd, Pb - Cu	humic acid (Fluka) natural water samples (Bassin d'Arcachon)	- -	0.5 -	NPP DPASV	E A	ass. $k_d$ is small	[32] [69]
- Cd, Pb Zn	fulvic acid (Adriatic and Ligurian Sea, and Lake Ontario)	-	-	DPASV	B		[70]
- Cd, Pb	humic acid (from O- Ah- Bh-Horizons, and from sediments)	-	-	DPP	A	systems non- labile	[71]
- Cu	seawater (North Sea)	-	-	DPASV	B		[72]
- Cu, Pb	natural waters (oceans, coastal- and freshwaters)	-	-	DPASV	B	review article	[73]
- Cd, Pb - Pb	humic acid fulvic acid (PROM) (Mare aux Eves Fontainebleau)	~ 10,000 1,800	0.5 2	NPP LSASV	E, V E		[35] [20]
- Cu, Pb	fulvic acid (Tamar River)	-	10 (ass.)	DPP LSASV DPASV	D	way of calcula- tion kinetic current not given	[74]

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