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COMBINING FIELD MEASUREMENTS FOR SPECIATION IN NON PERTURBABLE WATER SAMPLES

(Application to the Iron and Sulfide Cycles in a Eutrophic Lake)

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1. INTRODUCTION

During the last two decades, most of the effort in the development of analytical methods has been devoted to the improvement of sensitivity, automation, and miniaturization of instruments, i.e. essentially to technical problems. Apart from a few exceptions, such as chromatography, most of the techniques have been developed for total concentration determinations, and little attention has been paid to develop sensitive species selective methods, in particular, in the field of inorganic compound analysis. It is, however, well known that the behavior of elements and organic compounds in ecosystems is largely dependent on the nature and properties of the chemical forms in which those are present [1,2,3].

It is becoming increasingly apparent that the analytical chemistry challenge is no longer in only continuing to develop sensitive methods and automated systems (although there is still much to be done in this field), but also and perhaps, above all, to apply the existing instrumental methods to the measurement of chemical reactivity in

Metal Speciation: Theory, Analysis and Application. Edited by James R. Kramer and Herbert E. Allen.

complicated media like environmental biological or industrial samples.

The importance of chemical reactivity in analytical chemistry was obvious 30 or more years ago while dealing with gravimetry, volumetry and colored reaction based organic and inorganic qualitative analyses. With the development of instrumentation, analytical chemistry has been transformed progressively towards chemical analysis (i.e. determination of compounds without considering their reactivity) and even simply to analysis (since now many of the methods are mostly based on physical concepts).

It is time that the large numbers of very sensitive and sophisticated instrumental methods available be used for the real needs of environmental quality studies. In this field analytical problems to be solved are less and less concerned with speed and sensitivity of total concentration measuring techniques. Environmental quality control is presently much more limited by the lack of methods enabling one to determine the nature and physico-chemical properties of particular chemical species directly in natural medium conditions, i.e. for test species i) present at low concentration (often below 10^{-6}-10^{-5} M), ii) sometimes very reactive, and iii) often being a minor component of a very complicated mixture.

2. FIELD MULTIMETHOD APPROACH TO SPECIATION

2.1 Statement of the Speciation Problem

The various approaches which may be used for speciation measurements in natural waters have been discussed elsewhere [2,4]. They depend on the particular purpose and on the reactivity of the test species. This paper discusses an approach that is applicable to very reactive sulfide, iron and manganese species formed in the water column of stratified eutrophic lakes. Figure 1 schematically shows the relevant reactions which occur in Lake Bret (Vaud, Switzerland). This lake receives a large amount of phosphate mostly of agricultural origin, which gives rise to high primary productivity in spring and summer. During this period, the lake stratifies and develops a severe reducing zone at the bottom [4,5], where Fe(II), Mn(II) and S(-II) are produced by the reduction of sedimenting Fe(III) and Mn(IV) oxyhydroxides and of dissolved SO_4^{2-} respectively. The reduced species diffuse and may reoxidize at the boundary between the oxic and anoxic zones. During

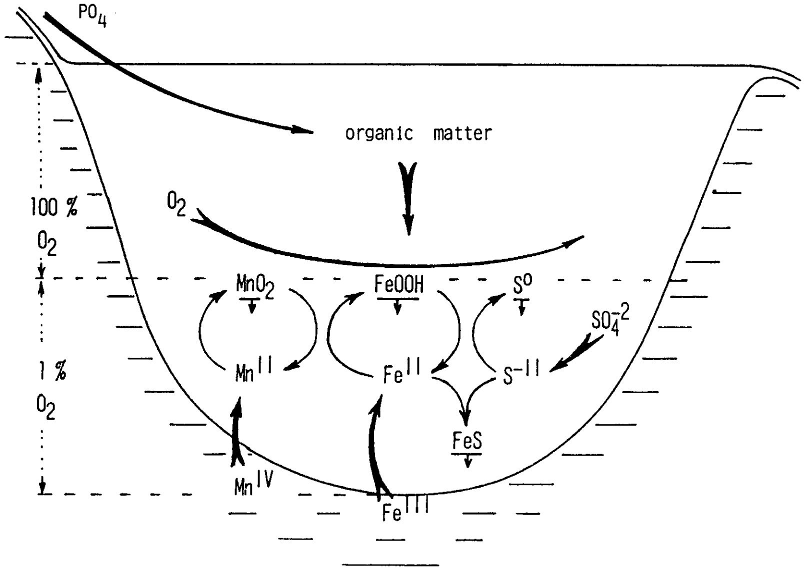

FIGURE 1. Schematic Representation of the Stratification and Chemical Cycles of Mn, Fe and S in Lake Bret (Vaud, Switzerland) During the Productivity Period (May to October).

this process, several colloidal, very reactive compounds are formed (Mn(IV) and Fe(III) oxyhydroxides, iron phosphate, iron sulfide, elemental sulfur) which in principle, may play an important role in controlling the trace metals and phosphate contents in water due to their high adsorption properties as well as their capability to coagulate and to sediment. The exact quantitative importance of this role, however, depends on a) colloid maximum adsorption capacity, b) in the case of Mn(IV) and Fe(III) oxyhydroxides, their sedimentation flux relative to their reduction rate when going through the reducing zone, since if the oxide particle is reduced again before reaching the sediments, the adsorbed metals or phosphate will be released in the water column, and c) in the case of iron phosphate or sulfide, their nucleation rate which may be slow enough to hinder the formation of solid phase despite the oversaturation of the solution. Consequently, in order to determine the precise role of these compounds in trace metal cycling, methods must be available to determine not only their total iron or manganese contents, but also their chemical nature, reactivity, and their size distribution.

2.2 Principle of the Multimethod Approach

The choice of these methods is very limited, precisely because of the high reactivity of the test compounds. All colloids can coagulate or be adsorbed on the walls of the sampling vessels; Fe(II) and S(-II) are easily oxidized e.g. by trace O_2 contamination; Fe(III) or Mn(IV) oxyhydroxides are metastable and may be either reduced by S(-II) (if it is present) or transformed into a more inert oxide form by self-dehydration during storage; degassing of H_2S or CO_2 may occur due to pressure changes during sampling, resulting in changes in FeS saturation index and pH which may in turn affect all speciation reactions. These considerations have the following implications:

- *storage* must be avoided; instead the analysis of the test species must be done *in the field.* If possible sampling is best done *at the desired depth* to minimize sampling perturbation (particularly degassing or O_2 contamination)

- *preconcentration steps* are usually unusable cases; the test colloids coagulate or are adsorbed on vessel walls. Consequently only *sensitive detection techniques* can be used (about 10^{-7} to 10^{-5} M for the compounds studied here).

- classical *separation* methods must be avoided as much as possible for the same reason as given under "preconcentration". The problems encountered are generally even worse when separation techniques like chromatography are used because there is a high probability of irreversible adsorption of the test colloid, owing to the large surface to volume ratio of the separation phase (resin, dextran gel, silica or alumina). Instead two approaches preferably in combination can be used:

a) *natural fractionation processes*: The physico-chemical processes occurring in the lake result in a chemical stratification with several layers, each of them being richer in a particular compound than the others (Figures 3, 9). A preliminary determination of the concentration profile of the test compound enables one to locate the depth and thickness of the corresponding layer where the relative concentration of that compound will be at its maximum. Further studies on this compound can then be done more readily by sampling at this depth.

b) *combination of several selective detection methods:* The selectivity of any analytical instrumental technique for a given element depends on a particular property of the compound bearing this element (e.g. its size and diffusion properties in voltammetries, its ability to form colored complexes in colorimetry). Therefore in a given

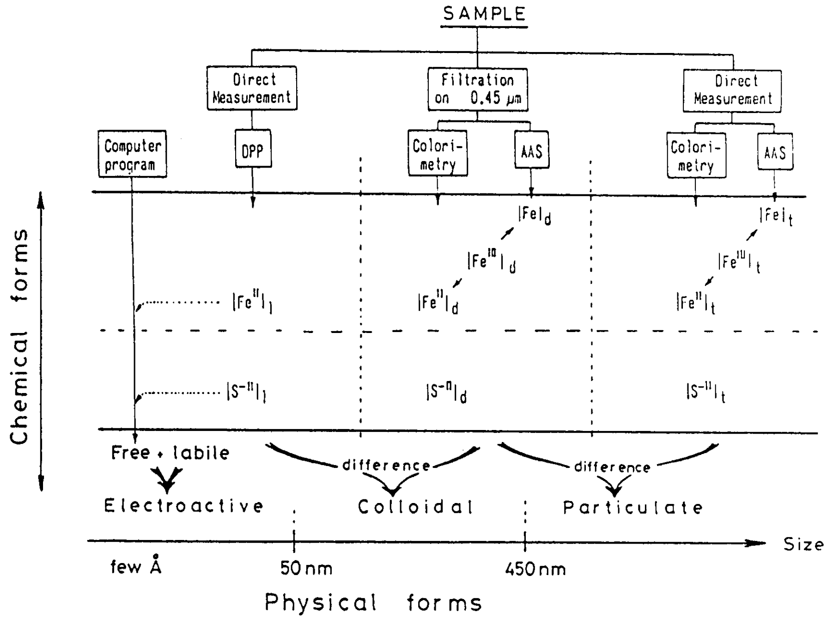

FIGURE 2. Multimethod Speciation Scheme for the Determination of Size Fractions and Redox State of Iron and Size Fractions of S(-II).

unperturbed sample, different methods "see" a different fraction of the element corresponding to different compounds, producing different results. Thus by i) combining the signals of several techniques and ii) knowing the nature and quantitative role of the major physico-chemical factors which influence each signal, it is possible to find the physico-chemical characteristics of the most important species and the distribution of the element between these species.

This approach was applied to Lake Bret for characterizing and following the seasonal trends of the different types of i) natural organic matter [4], ii) Mn species [5], iii) iron species [4,5], and iv) sulfide species [4,5]. It is mostly the two latter species which are discussed below.

2.3 The Multimethod Scheme Used for Iron and Sulfide in Lake Bret

Figure 2 shows a simplified diagram of the multimethod scheme applied to *in situ* speciation of iron and sulfide. More details (including the manganese speciation scheme and a detailed outline of the procedures) are given in [4,5]. All methods except atomic absorption spectrometry (AAS) were employed on a raft moored at the sampling point. AAS was used to measure total Fe or Mn concentrations; the corresponding samples were acidified and stored at 4°C in the dark until analyzed. The colorimetric o-phenanthroline method was used to measure total Fe(II), the difference between this and AAS giving the total Fe(III) concentration. This combination of methods therefore enables one to get an insight on the redox distribution of Fe species.

The size distribution of Fe(II) species was estimated by means of three different measurements. The above analyses were repeated for both unperturbed samples and samples filtered through 0.45 μm, and in addition Fe(II) was analyzed by differential pulse polarography (DPP) under conditions [4-6] very close to those described by Davison (see [7-9] for critical reviews). DPP is a non perturbing measurement (since measurements can be performed on unmodified samples) which measures only small sized species (approximate upper limit of 50 nm; see below and [2,4]). Iron incorporated in these species is referred to below as electroactive iron. Filtration is the only fractionation step that was used here. Its results were found to be affected by several factors other than size. Size limits given for both DPP (50 nm) and filtration (450 nm) must therefore be considered only as orders of magnitude. They are however useful for getting an overall picture of the Fe physico-chemical form distribution. From the concentration of electroactive iron and the tabulated thermodynamic complexation constants, it is also possible to compute the distribution of the various forms of Fe(II) in labile equilibrium, in particular the free (aquated) ion and its complexes with the inorganic anions of the water sample. This scheme therefore allows one to discriminate between particulate forms, colloidal forms, labile species and aquo ion. A similar scheme is applicable to S(-II) (Figure 2), by using DPP and the colorimetric methylene blue method for measuring total S(-II), in either filtered or unfiltered samples.

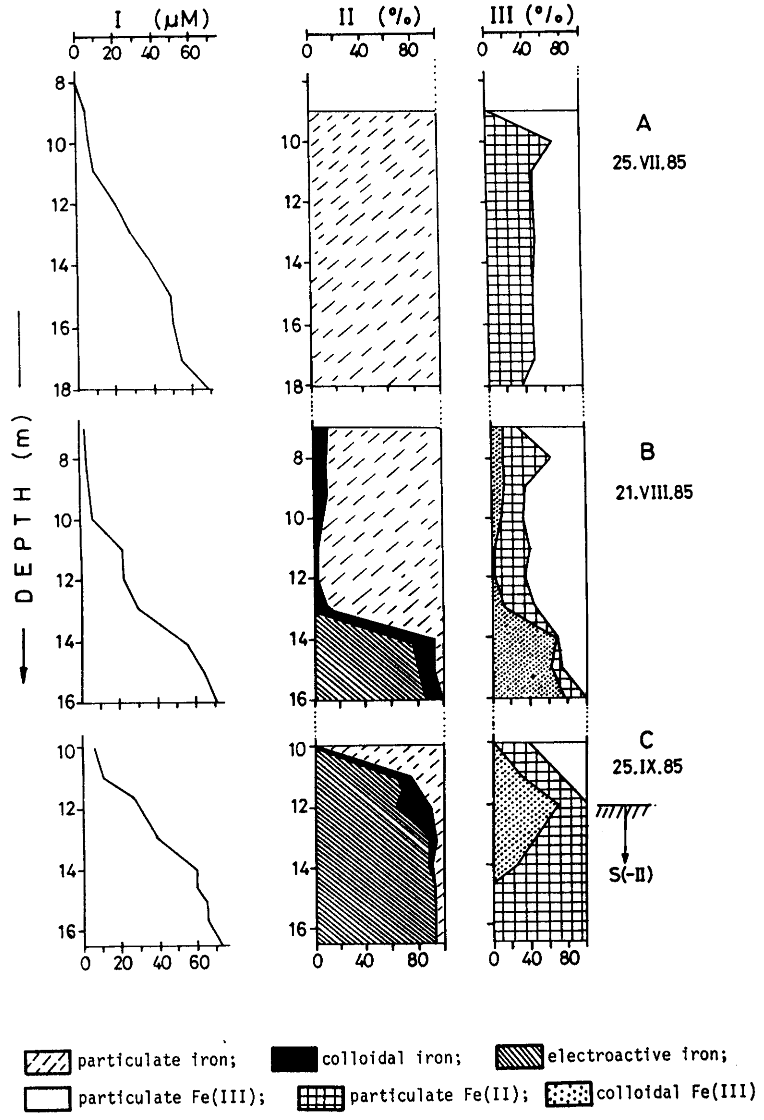

FIGURE 3. Depth Profiles for the Total Concentration (I), Size Fractions(II) and Redox State of Colloidal and Particulate Fractions (III) of Iron, for Three Dates During the Productivity Periods: 25 July (A), 21 August (B), and 25 September (C) (1985). The horizontal upper line in C gives the limit for the presence of S(-II).

Detailed distribution profiles of iron, sulfide and manganese and their seasonal evolution are given in [5,6]. Figure 3 shows examples of iron distribution profiles for 3 different dates. They can be compared with the total concentration profiles. In July there is no dissolved Fe(II). Interestingly about 50% of particulate Fe is Fe(II). In August the dissolved Fe(II) increases at the bottom of the lake, and in this layer, the proportion of colloidal iron increases with particulate concentration. The nature of particulate and colloidal iron in July and August is discussed below. The results (not shown in Figure 3) also indicate that there is very little S(-II) in the lake in August. In contrast in September, not only the Fe(II) layer increases compared to August concentrations, but high S(-II) concentrations (about 10-25 μM) appear in the water column below the indicated depth. In this case a peak of colloidal iron appears. The part of the profile corresponding to the decrease in concentration with depth presumably results from the reduction of sedimenting Fe(III) by S(-II). Interestingly colloidal Fe(III) persists well below the depth of appearance of S(-II), indicating that this colloidal Fe(III) is far from being instantaneously reactive. Particulate Fe(II) in the sulfide region is particulate FeS [10].

Obviously the distribution profiles give a much more comprehensive picture on the chemical reactivity of the element in the lake than the customary total or dissolved concentration profiles. These results also indicate the best depths for sampling in order to study a specific compound in detail. For example, in September (Figure 3 C) the mixed Fe(II)/Fe(III)/particles are best sampled at 10 m depth, whereas colloidal Fe(III) will be preferably sampled at 12 m, and particulate FeS below 14 m. It must be emphasized, however, that for establishing distribution profiles, two types of development are needed:

- techniques enabling one to record the profiles automatically and quickly. This is important since day to day variations may be observed and knowledge of these profiles is prerequisite for sampling and studying a given compound in the presence of the minimal amount of interfering ones.
- correct interpretation of the analytical response of each of the analytical methods used in relation to the physico-chemical characteristics of the test compound. In many cases little is known regarding this aspect. This is discussed below in more detail for the two critical steps in the scheme shown in Figure 2, namely polarography (Section 3) and filtration (Section 4).

3. INTERPRETATION OF Fe(II) AND S(-II) POLAROGRAHIC SIGNALS

3.1 Nature of the Signals and Analytical Problems

Davison [7,8,9] was the first investigator to apply d.c. polarography, NPP and DPP systematically to the measurement of Fe(II), Mn(II) and S(-II) in fresh waters. The shape of the peaks obtained by DPP is shown in Figure 4, and the nature of their electrochemical reactions is given in the legend. In the limnological conditions used by Davison, "dissolved" S(-II), Fe(II) and Mn(II) (i.e. the species filterable through 0.45 µm) were only the protonated and aquated forms ($H_2S + HS^-$), Fe^{2+} and Mn^{2+}. In such a case, the corresponding peaks are just a measure of total S(-II), Fe(II) and Mn(II) concentrations.

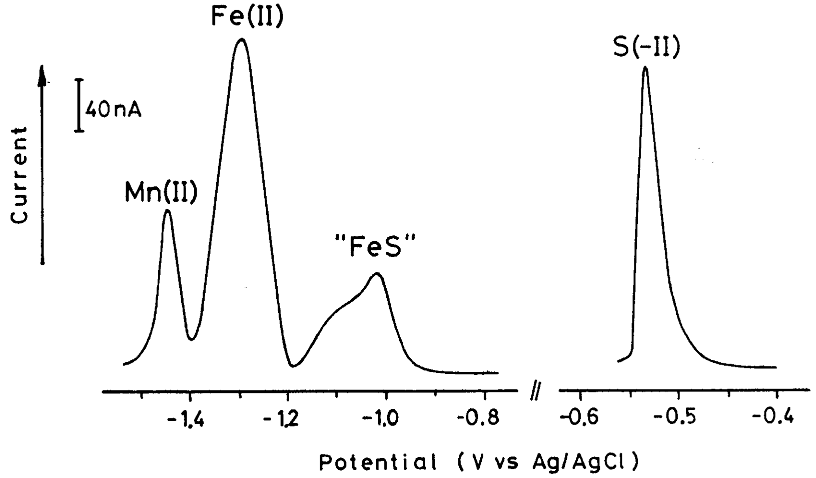

FIGURE 4. Example of a Field Differential Pulse Polarogram of Mn(II), Fe(II), "FeS" Complex and S(-II). The peaks labelled Fe(II) and Mn(II) correspond to the reduction of Fe^{2+} and Mn^{2+} or their labile complexes (after dissociation) to Fe^0 and Mn^0. The peak labelled "FeS" corresponds to the direct reduction of a "FeS" complex (see text) to Fe^0. The peak labelled S(-II) corresponds to the electrochemical oxidation of Hg^0 into adsorbed HgS.

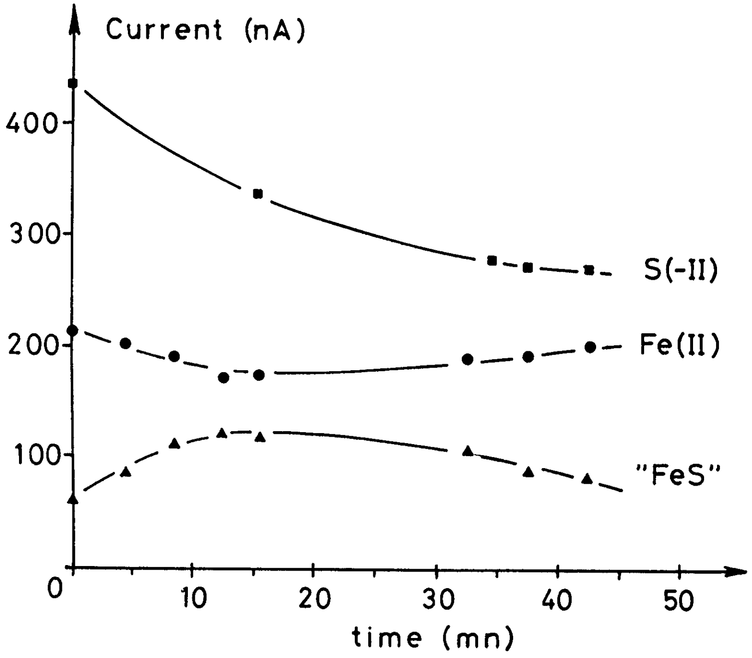

FIGURE 5. Changes in S(-II), Fe(II) and "FeS" DPP Peaks with Storage Time. Field measurements: the sample was collected directly into a polarographic cell completely filled and air tight.

The only (but important) advantage of voltammetry is that perturbations due to sampling and sample treatment are very much minimized compared to classical analysis (in particular no filtration is needed; furthermore voltammetry is well adapted to the study of anoxic water since these samples are naturally deoxygenated. It has been shown, however, [4,6,7,10,11] that another advantage of voltammetry is the ability to determine both the nature and concentration of colloidal and complexed species of iron sulfide, possibly formed in the water column when Fe(II) and S(-II) concentrations are high enough. This can be achieved by combining voltammetry, colorimetry and/or AAS methods as discussed in Section 3.2. The two above mentioned advantages of voltammetry, namely non perturbation of sample and species selectivity, are briefly examined below.

3.1.1 Non Perturbation of Sample

As mentioned above, a filtration step is not necessary in voltammetry, because the method, by its nature, is insensitive to "large" (> 50 nm) particles. As previously mentioned this avoids many artifacts. A further advantage of voltammetry is the simplicity and compactness of the apparatus which allows its use in the field. Thus it is possible to pump a water sample directly from a given depth into the polarographic cell, which minimizes the time lapse between sampling and analysis as well as the contact with intermediate vessels and tubing. The importance of this condition can be seen in Figure 5, which shows that the S(-II) peak decreases strongly and quickly after sampling in spite of the fact that the sample was stored directly inside the polarographic cell under carefully controlled air-tight conditions [5]. Since the S(-II) peak originates from the reaction of H_2S and HS^- with Hg (legend of Figure 4), its decrease corresponds to a natural degassing of H_2S caused by the difference in pressure between the sampling depth and the surface. Figure 5 also shows that this causes changes in concentrations of other species which are in equilibrium with H_2S. Note that this problem is general, irrespective of the analytical method used; polarography just enables one to point it out. It must then be concluded that, with such samples, storage must definitely be avoided and analyses have to be performed in the field. This was confirmed in our case by a systematic comparison of analyses [5] made in the field and in the laboratory after storage in carefully filled up air-tight Winkler bottles. Losses of H_2S up to 56% (and up to 85% with other vessels) and increases of the Fe(II) peak up to 21% were observed after 8 hours of storage.

3.1.2 Nature of the Polarographic Peaks

The electrochemical nature of Fe(II), Mn(II), "FeS" and S(-II) peaks has been reviewed in [7] and [11]. The main points can be summarized as follows:

- *S(-II)* peak corresponds to the electrochemical oxidation of Hg electrode. In the presence of S^{-2} produced by the dissociation of any labile S(-II) species (H_2S and HS^- being predominant in most cases), the oxidation of Hg is facilitated by the formation of adsorbed HgS:

$$\mathrm{S(-II)} \rightleftarrows \mathrm{S^{-2}} + \mathrm{Hg} \rightleftarrows \mathrm{HgS_{ads}} + 2e^-$$

- *Fe(II)* peak corresponds to the irreversible reduction of Fe^{2+} either present in this form in solution or after dissociation of the labile compounds (globally symbolized by Fe(II)) in which it is incorporated: $Fe(II) \rightleftarrows Fe^{2+} + 2e \rightleftarrows Fe^o$. The charge transfer of this reaction is very slow, and the corresponding peak (or half-wave) potential is much more negative (by about 600-700 mV) than the normal potential of the redox couple, Fe^{2+}/Fe^o. Therefore one may expect that any Fe(II) complex, capable of undergoing a direct faster charge transfer in its molecular orbitals (i.e. without an intermediate dissociation step), will give a separate peak at a more positive potential and will therefore not contribute to the Fe(II) peak. This is the case of the "FeS" peak.

- *"FeS"* peak. It has been shown [6,11) that this corresponds to the situation mentioned above. The exact nature of the complex has been studied by voltammetry in the field and is discussed below. It must be noted that this peak is also linked to a slight adsorption of the complex on the electrode. This, however, does not affect the measured current appreciably in the time scale of a dropping mercury electrode (seconds).

For Fe(II) the overall electrochemical process can be written as [2,4]:

$$Fe(II) \xrightarrow{D} Fe(II) \overset{k_d}{\rightleftarrows} Fe^{2+} \overset{2e^-}{\rightleftarrows} Fe^o$$

which shows that the corresponding current depends i) on the diffusion coefficient, D (therefore on the size) of all species contributing to the Fe(II) peak, and ii) on their dissociation rate constant, k_d. Obviously in complicated media like fresh waters which include a very large number of different species with wide ranges of k_d and D values, the measured current may be a complicated function of the medium composition. Much work remains to be done to allow a rigorous extraction of D and k_d distribution from the measured polarograms [2]. Nevertheless, the current properties can be used in an approximate manner to discriminate between different groups of chemical species by considering that even for species whose k_d is very large (fully labile species), the current will be negligible if D is small. Using the Stokes-Einstein relationship, it may be computed [2,4] that under this condition, the current produced by particles having radii greater than 50 nm is less than 8% of that produced by the same concentration of free Fe^{2+}. If k_d is not large (non-labile species), this proportion will be still lower. In view of other experimental errors, one may therefore take the value of 50 nm as an operational limit between electroactive and non electroactive compounds.

Exactly the same considerations apply to S(-II) species, and it would apply to the "FeS" complex as well, provided this electroactive compound is associated with larger particles. Field measurements have shown, however, [6,11] that the "FeS" complex has the same diffusion coefficient as Fe^{2+} which implies that such an association is not likely and hence the size of the "FeS" complex is small.

Thus on the basis of polarographic results and their combination with colorimetric data (which give total Fe(II) and S(-II) concentrations), one can define the following fractions:

- colloidal + particulate Fe(II) (size > 50 nm) obtained from the difference between Fe(II) colorimetry and Fe(II) DPP peak;
- colloidal + particulate S(-II) (size > 50 nm) obtained from S(-II) colorimetry minus S(-II) DPP peak;
- Fe(II) in "FeS" complex from the "FeS" DPP peak;
- concentrations of Fe^{2+} and its known labile complexes (size < 50 nm), obtained from the Fe(II) DPP peak and the distribution calculations based on equilibrium constants of complexes;
- concentrations of S^{-2} and its known labile complexes, obtained from the S(-II) DPP peak, as described above for Fe(II).

Note that colloidal and particulate fractions of Fe(II) and S(-II) can still be discriminated by comparing polarographic and filtration results. Although all these fractions are more or less operationally defined, they yield a much better description of the system composition than total concentration measurements. Their determinations make use of only two techniques (colorimetry and polarography) along with very little sample handling. Since the physico-chemical principles of polarography are well known, it is also possible to get a quantitative estimation of the errors due to the approximations made in defining the fractions.

3.2 Examples of Limnological Results

Three examples of the application of the above scheme to lake composition characterization are discussed as follows:

- The FeS ionic product, IP = $[Fe^{2+}][S^{-2}]$ (or equivalently [4], IP' = $[Fe^{2+}][HS^{-}]/[H^{+}]$), is often determined to find out the degree of saturation of the water column with respect to FeS, by comparing the IP value to that of the corresponding solubility product. Most of the IP' values reported in the literature have been computed using the "dissolved" concentration of Fe(II) and S(-II) instead of the actual aquated ion concentrations, [Fe^{2+}] and [HS^-]. However the former may be much higher than the later in the case where Fe(II) and/or S(-II) colloids are formed (Figure 8 in [4]), and this in turn may lead to a significant over-estimation of IP'. From this standpoint the use of the above scheme enables one to get more realistic values for [Fe^{2+}] and [HS^-] and hence IP' profiles [2,4,6,7].

- Our studies [2,4,5,6,10] of the Fe(II)/S(-II) system in Lake Bret since 1981 have shown in particular that i) appreciable amounts of particulate FeS were never formed despite the fact that IP' values frequently exceeded the solubility product by a factor of 1-2 log unit, and ii) storage in air-tight sampling bottles for about 8 hours or more increases the proportion of colloidal and/or particulate FeS species. These observations suggest that IP' values are not very useful indicators as to whether a solid (particulate or colloidal) FeS compound can be formed in the water column, because thermodynamic equilibrium is not reached. On the other hand, the multimethod approach gives a more detailed and a more realistic insight into the actual chemical composition of the medium.

- The analytical approach described above also enables one to determine the exact nature of the "FeS" complex. By recording the seasonal trend in the "FeS" peak depth profiles, the results indicate [6] that "FeS" peak appears only when IP' > 10^{-3} M, i.e. for sufficiently large concentrations of Fe^{2+} and S^{-2} (although for our conditions this corresponds to undersaturation conditions, since the value of the solubility product written as $K'_{sp} = [Fe^{2+}][HS^-]/[H^+]$ is $10^{-2.35}$ [6]). Furthermore the fact that Fe(II) and "FeS" curves in Figure 5 are symmetrical suggests that a labile equilibrium between these two species exists in the water column. Finally the voltammetric properties of "FeS" [6,11] suggest that this complex is small sized (identical diffusion coefficient for "FeS" and Fe^{2+}) and possesses no electric charge (a likely property [e.g. 12] since this species is adsorbable on a negatively charged electrode). By combining these characteristics, the following equilibrium may be expected:

$$n\,Fe^{2+} \quad + \quad n\,HS^- \quad \rightleftarrows \quad Fe_nS_n^o \quad + \quad n\,H^+$$

where n should be a small integer, but greater than one. From the mass action law, one can write:

$$[Fe_nS_n] = K\,[Fe^{2+}]^n\,[HS^-]^n\,/\,[H^+]^n = K\,(IP')^n$$

where $[\mathrm{Fe}_n\mathrm{S}_n]$ is the concentration of the species to which the "FeS" peak current, i_p is proportional, and K is the equilibrium constant. Figure 6 shows that i_p varies directly with $(\mathrm{IP}')^2$, suggesting that n = 2 and that "FeS" is a dimer. The existence of $\mathrm{Fe}_2\mathrm{S}_2$ in the water column seems likely, since $\mathrm{Fe}_2\mathrm{S}_2$ and $\mathrm{Fe}_3\mathrm{S}_3$ are known to exist in biological media (often stabilized by other ligands).

To sum up, all of the results obtained by the above methods [6,11] suggest that in the bottom layer of the lake, where both Fe(II) and S(-II) are present, the following sequence of reactions may occur:

$$\begin{array}{r}\mathrm{Fe}^{2+} + 2\,\mathrm{HS}^- \underset{\text{fast}}{\rightleftarrows} \overset{\text{labile}}{\mathrm{Fe}_2\mathrm{S}_2} \xrightarrow[\text{slow}]{} \overset{\text{colloidal}}{\mathrm{FeS}_s} \xrightarrow[\text{coagulation}]{} \text{particles} \\ \downarrow\quad \\ \text{sedimentation}\end{array}$$

where FeS_s is solid FeS. In order to determine the possible scavenging role of FeS_s, the rate of colloid and particle formation and sedimentation should be determined precisely, but the present results suggest that these processes are slow (at least hours to days).

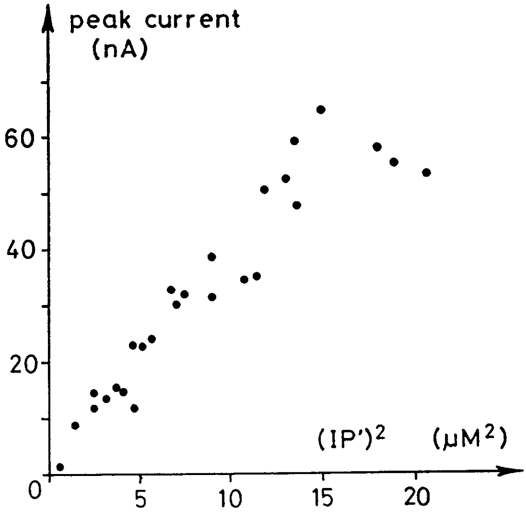

FIGURE 6. Correlation Between the DPP Peak Current of the "FeS" Peak and the Ionic Product, IP', Computed as Indicated in the Text from $[\mathrm{Fe}^{2+}]$ and $[\mathrm{S}^{-2}]$ Values also Measured by DPP. The graph reflects the data obtained at all depths and all dates during the period August - October, 1985.

4. NATURE AND SIZE DISTRIBUTION OF IRON PARTICLES AT THE OXIC/ANOXIC INTERFACE (Non-Sulfidic Layer)

4.1 Size Distribution Problems

Filtration and ultrafiltration have been extensively used for size fractionation of compounds and particles in natural waters despite the numerous claims regarding the problems encountered in this technique. These can be classified into two categories: i) contamination of the water sample by trace metals or organic compounds of the membrane or adsorption of trace compounds of the test medium by the membrane [13,14], and ii) fouling of the membrane by the colloids and macromolecules to be filtered, possibly resulting in gel formation which acts as an additional less permeable filter.

The first type of problem is most important when dealing with trace compounds studies (typically $< 10^{-7}$ M). The second one is particularly relevant when filtration is used to characterize the properties (in particular size distribution) of the whole spectrum of colloids and macromolecules. Since fractionation methods not based on membrane separation are not numerous, it would be particularly important to study in detail the factors causing the above problems. However, although membrane fouling is an important industrial research field (e.g. [15,16]), very few investigations have been carried out with respect to natural water applications [17], since the earlier papers of Sheldon [18,19] who systematically compared the cut-off limits of various membranes. Laxen *et al* [20] have also compared the results of laboratory versus field cascade filtration for iron and manganese oxide and conclude that the latter is more reliable.

To test the fouling effect in more detail, we carried out a systematic study of the effects of filtration conditions on the Fe(II)/Fe(III) particles formed above the sulfide layer in Lake Bret (Figure 3). Figure 7 shows three size distributions for the same water sample obtained by three different methods.

Method A is the most commonly used. The sample is filtered in the field immediately after sampling through a set of filters having different pore sizes; great care is taken to avoid air contamination by using a classical syringe assembly. A new sample is used for each filter (i.e. no cascade filtration), and the distribution is computed from all the filtration results. Laxen [20] has obtained the same range of size distributions for iron particles as that shown in Figure 7A.

Method B is an *in situ* (i.e. at depth) cascade filtration performed by means of a home-made filtration system [21]. In use it was very carefully purged to avoid air contamination. The main differences from method A are: i) a slower flow-rate through the membranes (particularly for the less porous membranes), ii) better oxygen contamination control, iii) strong magnetic stirring of the solution being filtered just above the membrane, and iv) filters used in cascade. The last two points are particularly important.

Method C consists of size distribution using transmission electron microscopy (TEM) and visual enumeration of the particles (total number = 122). The TEM analysis was done on a sample *retained* on 0.45 μm membranes. However, all iron particles were found to be *smaller* than 0.3 μm. Because TEM sample preparation may introduce important artifacts, several preparation modes were compared [22], and one of them consisted of imbedding the wet sample into a resin without prior dessication. Results from all of these techniques confirm that the size

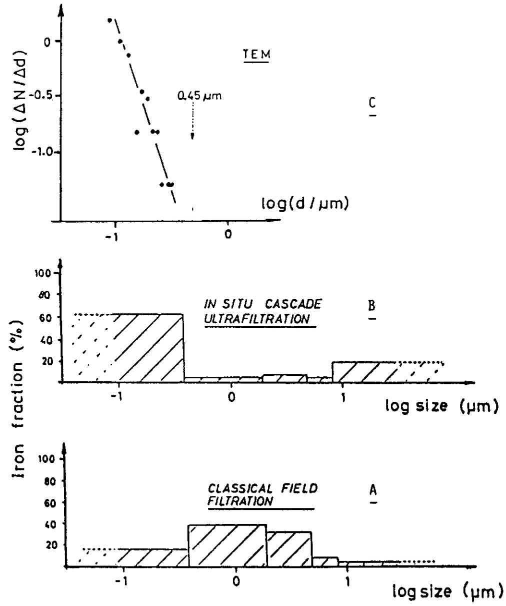

FIGURE 7. Size Distribution Diagrams of Iron Determined by Three Different Methods, Using the Same Water Sample Located at the Oxic/Anoxic Interface Above the Sulfidic Layer (see Figure 3C). A: Classical field syringe filtration. B: in situ (at depth) cascade ultra-filtration; C: Particle counting by TEM; observation of the particles retained on 0.45 μm filter (N and d = particle number and diameter).

distribution in Figure 7C is likely to be representative of that in the water column.

By comparing Figures 7A, 7B and 7C, it is obvious that the filtration process gives an apparent size distribution with particle sizes which are too high. The results, however, depend on filtration conditions. The classical syringe filtration data differ from the TEM distributions by about an order of magnitude whereas the agreement with TEM data is much better with *in situ* cascade filtration. The agreement is attributed mostly to points iii) and iv) mentioned above.

Figure 8 shows examples of the type of results obtained in an extended study to test systematically the effect of i) the nature of the membrane, ii) the solution flow-rate through the membrane, iii) the stirring rate of solution above the membrane, and iv) the storage duration. The water samples were filtered (in the field for short storage times) in a home-made filtration unit which excluded air contamination and permitted control of flow-rate and stirring-rate. For each flow-rate and stirring mode, filtration was performed successively through a set of 3 µm, 0.2 µm and 0.02 µm pore-size membranes. These measurements were repeated with 5 sets of different types of commercial membranes (Nuclepore, Schleicher-Schuell, Rhone-Poulenc, Gelman, DDS).

In all of the cases, the proportion of particulate iron adsorbed on the membranes was determined as a function of the conditions used. Figure 8 shows an example of the results obtained for the adsorption on 0.2 µm membranes, in stirred solutions, at two very different flow rates (0.05 and 1 mL/min). Figure 8 points out that very low flow-rates must be used to avoid adsorption. Even a flow-rate of 1 mL/min which is low compared to that normally used with syringe filtration (>10 mL/min), results in strong adsorption. This considerable effect of the flow rate is attributed to its influence on the ***polarization concentration*** at the membrane surface [2]. During filtration, the concentration $[X]_o$ of the retained particles inside the diffusion layer (thickness = $\delta \approx 10 - 30$ µm) adjacent to the membrane surface, is larger than that in the bulk solution ($[X]_b$), and $[X]_o/[X]_b$ increases drastically with flow-rate, according to Equation 1. Therefore even for a very low flow-rate (see below), $[X]_o$ may become much larger than $[X]_b$ by several orders of magnitude, resulting in coagulation and flocculation at the membrane surface.

This effect may be estimated quantitatively from Equation 1:

$$[X]_o/[X]_b = \exp(J\,\sigma\,\delta/D) \tag{1}$$

where J = flow-rate, σ = retention coefficient of the membrane ($0<\sigma<1$), and D is the diffusion coefficient of the retained particle. For particle size of 0.5 µm, one can estimate that the ratio, $[X]_0/[X]_b$, is equal to 3 for J = 0.01 mL/min, to 20000 for J = 0.1 mL/min, and almost infinite for J = 1 mL/min. Even if these figures are only approximate, they confirm qualitatively the great importance of polarization concentration.

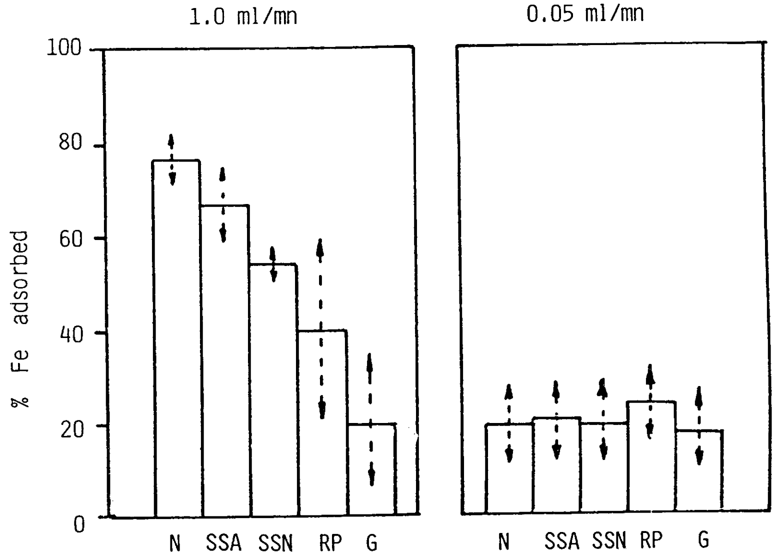

FIGURE 8. ***Influence of the Membrane Filter Type and Flow-Rate on the Proportion of Iron Particles Adsorbed on the 0.2 µm Pore-Size Filter. In all of the cases cascade filtration of the same initial water sample was performed using 3 µm, 0.2 µm and 0.02 µm filters. N = Nuclepore (polycarbonate); SSA = Schleicher-Schuell (cellulose acetate); SSN = Schleicher-Schuell (cellulose nitrate); RP = Rhone-Poulenc (polyvinylfluoride); G = Gelman (acrylic copolymer).***

Although flow-rate has been found to be the most important factor, three other factors influence the results:

- *Stirring* decreases adsorption although not drastically (about 10% decrease in stirred compared to unstirred solutions). Stirring decreases δ, and therefore the ratio of $[X]_0/[X]_b$, but facilitates coagulation processes at the membrane surface. These two effects are opposite in nature.

- ***The nature of the membrane,*** although not negligible, is less important than that suggested by Figure 8. Indeed an inverse correlation was observed between the proportion of iron adsorbed on the 0.2 μm membrane and that adsorbed on the 3 μm membrane of the same cascade. As a result, the cumulative proportions of iron adsorbed on 3 μm and 0.2 μm membranes are not very dependent on the membrane type, including Nuclepore.
- ***Storage duration*** was found to be important, confirming the recommendations of Laxen and Chandler [20] on the necessity of performing filtration in the field. Under the best conditions (low flow-rates), the proportion of iron adsorbed on the membrane increased from 0 to 40% for storage periods increasing from 0 to 75h. This imposes a limitation on the minimum usable flow-rate since 16h are needed to filter a 10 mL sample at 0.01 mL/min.

4.2 Particle Characterization

The above results clearly indicate that great precautions must be taken when using filtration for particle size characterization. In particular, no *a priori* assumption can be made on the relationship between pore-size of the membrane and the actual size of the retained and unretained particles. There may be some agreement if correct filtration conditions are used, but they must be checked in each case by independent methods.

Even if this type of calibration is not possible, however, filtration can be fruitfully used, provided it is considered merely as an operational means to obtain fractions; actual size is not necessarily the major discriminating factor. Filtration is then only used to get fractions "enriched" in the test compound so that it may be studied more readily. The only requirement for this type of assessment is that filtration be done under conditions where coagulation on the membrane surface is minimum. (The above results may be useful in that respect).

Figure 7C shows an example of this kind of application. Particles were "enriched" by successively filtering through 100 μm and 0.45 μm filters. The particles retained on 0.45 μm were then studied by TEM coupled to an energy dispersive X-ray fluorescence probe (EDS) [22,23]. The iron particles could be classified morphologically into four groups. The fundamental group was a spongy type, amorphous, nearly spherical globules of 40 - 300 nm. These globules may include small denser globulets (5-15 nm). They may also form tightly packed (0.1 - 1 μm) and loosely packed aggregates (0.5 - > 10 μm). Figure 7C shows that the size distribution of individual globules (determined by TEM observation of a large number of globules) seem to obey the Pareto law [2,24], where log (ΔN/Δd) is proportional to log Δd (N and d are particle number and diameter respectively). The slope of the line of 3.2 suggests that one of the main factors giving rise to this particular size distribution is the elimination of particles by perikinetic coagulation followed by sedimentation [2].

With EDS coupled to TEM, it is possible to make elemental determinations on each globule. Measurements done on 127 globules showed that their major elemental components are Fe, Ca and P (and probably unmeasurable OH) in the respective atomic proportions of 4.2/1.3/1 [22]. P was shown to be PO_4 (-III) by use of LAMMA (Laser Microprobe Mass Analyzer), and this has been confirmed by classical chemical analysis of particles. Finally note that the multimethod scheme discussed previously (Figure 3) showed that in those particles retained on 0.45 µm filter, 50% of the iron is in the Fe(II) form and 50% in the Fe(III) form, regardless of the depth (Figure 3) and particle size [5,6].

5. APPLICATION TO INTERPRETATION OF LIMNOLOGICAL PROCESSES

By applying the multimethod approach described above to follow the spatial and seasonal trends in lake composition, it was possible to get a more precise description i) of the detailed chemical stratification of the anoxic zone and ii) of the oxic/anoxic interface redox components. This is summarized in Figure 9 which shows the chemical stratification at a given date. The figure includes the profiles of Mn(II) and MnO_x whose determinations are not discussed here in detail. In summary, a multi-method approach similar to that used for iron (Figure 3) was employed for Mn, except that, in addition, three determinations of MnO_x were done; two of them were based on reduction reactions, complementary in terms of reducing power of the reagent [5,6]. Consequently these three methods allow one to get an insight on the redox reactivity of MnO_x [5].

The results shown in Figure 9 are discussed in detail in [5]; only a few salient points are emphasized here. An important feature of Figure 9 is that Mn(II) profiles are peak shaped. The conditions for the occurrence of a peaked profile for a given compound have been discussed by Davison [25]. A necessary condition is that both formation and elimination sources must exist in the water column, the former source being located at a shallower depth than the latter. It is also noteworthy that the depth scale also corresponds qualitatively to a decreasing oxidation power scale {O_2>MnO_x> Fe(III)} and an increasing reduction power scale {Mn(II)> Fe(II)> S(-II)}.

By taking into account the detailed results of the multimethod approach [6,5], the above lake stratification can be interpreted as being the result of a series of reactions:

- Mn(II) (mostly Mn^{2+}) undergoes a microbiologically catalyzed oxidation with O_2 to give *MnO_x*, which sediments. During the course of sedimentation it is reduced (although not always completely) by Fe(II) and S(-II).
- Fe(II) (mostly Fe^{2+} in the layer where S(-II) = 0) is oxidized by oxygen and possibly MnO_x to *Fe(II)/Fe(III) mixed particles* (see 4.2). These particles, although small (40 - 300 nm) can undergo coagulation followed by sedimentation. They are completely reduced in the sulfidic zone. These particles contain a large proportion of phosphate. In fact the Fe(II)/Fe(III) particle peak corresponds to the upper limit of the concentration profile of dissolved PO_4(-III). Therefore it is likely that the iron cycle hinders the upward diffusion of dissolved phosphate.

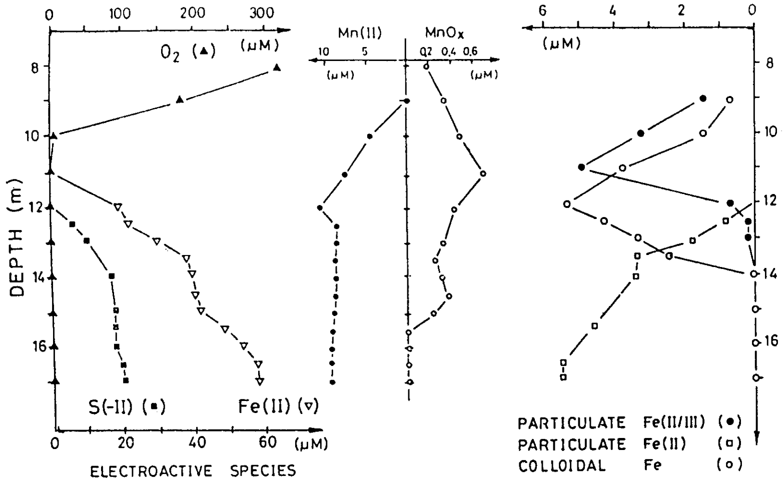

FIGURE 9. Typical Example of Chemical Stratification of Lake Bret (18 Sept, 1987) Based on Depth Profiles of Particulate and Dissolved Mn, Fe and S(-II) Species.

- A peaked profile for *colloidal Fe(III)* appears at a slightly lower depth than that of the Fe(II)/Fe(III) particle peak. The different compositions, sizes and locations of Fe(II)/Fe(III) particles and Fe(III) colloids, suggest that they are produced by different mechanisms (see [5]).
- Finally, at lower depths, both colloidal and particulate Fe(III) disappear probably because of their reduction by S(-II). As mentioned earlier, *Fe_2S_2 and colloidal FeS* may be formed, but precipitation of FeS is a slow process and its exact importance is yet to be established under lake conditions. It is also interesting to note that although MnO_x is a stronger oxidant than iron, colloidal MnO_x exists in layers deeper than iron particles. This suggests that MnO_x exhibits a relatively inert behavior compared to iron particles, either due to its intrinsic nature, or due to the formation of a protective layer at its surface.

6. CONCLUSIONS

This overall picture needs to be confirmed by further studies. In any case, the establishment of a dynamic description of the lake composition is certainly an essential step for understanding the exact mechanisms of trace element cycles in the water column. The successive transformation of one solid phase to another (as discussed above) implies that trace elements will also follow a series of adsorption/dissolution steps. The net result for their cycling will depend on the relative rates of each of these chemical steps and of the particle sedimentation rates. This is probably not a simple problem, but in any case it is apparent that in such a system predictions about metal cycling can not be made just from thermodynamic calculations based on the competition of a mixture of the above mentioned solid phases for the metal of interest. Kinetic factors probably are at least as important.

In general, the multimethod approach should open up a wide range of applications, since it can make use of the very large pool of instrumental techniques developed in the last two decades. It also follows the trend discussed in the introduction, that an analytical chemistry approach based more and more on the chemical reactivities of the compounds will evolve. This, however, does not imply that instrumental development is no longer required. At least two improvements are desirable in the field of limnologic speciation: i) the development of new techniques with newer physico-chemical selectivities or whose selectivity is more easily defined in terms of rigorous physico-chemical parameters, and ii) the development of techniques readily applicable in the field and even preferably *in situ*, which implies improvement in automation and miniaturization.

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