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# Large Lakes

## Ecological Structure and Function

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# Characterizing Autochthonous Iron Particles and Colloids—the Need for Better Particle Analysis Methods

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ABSTRACT The complexity involved in studying the biogeochemical cycles of particles and colloids in freshwaters is the result of several different factors: the great number of appearance and disappearance fluxes of these species, the difficulties associated with distinguishing and measuring those fluxes, and the difficulty of sampling colloidal particles without denaturation before their characterization. On the other hand, information obtained in laboratory studies of synthetic colloids are not always representative of the mechanisms encountered in heterogeneous natural environments. This article, taking autochthonous iron-rich colloids as a model, summarizes the state of the art concerning current knowledge of these colloids and emphasizes the need to develop new techniques specifically oriented towards analysis of natural colloids and their fluxes while taking care to minimize artifacts introduced by presently employed techniques. Finally, as a pertinent illustration, both the problems and the future uses of well-known filtration techniques are considered.

The transport of trace elements by colloids and particles plays a key role in controlling their biogeochemical cycling in lakes, estuaries, and oceans (Whitfield and Turner, 1987; Sigg, 1985; and refs. therein). The mechanistic details of these processes, however, are still poorly understood, largely because of a lack of appropriate techniques enabling determination of the nature, concentration, chemical reactivity, and physical fluxes of specific particulate species (Buffle et al., 1987). These processes are discussed below with particular emphasis being placed on those parameters which must be measured if we are to elucidate, in detail, the role played by particles (Section 12.2). In order to illustrate the specific types of problems that may be encountered, this discussion employs the case of iron oxyhydroxide particles which are formed at the redox boundary of eutrophic lakes and whose characteristics are described in Section 12.1. The possible artifacts which can be introduced by available techniques are illustrated using the specific but important case of filtration (Section 12.3).

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## 12.1 Nature of Autochthonous Iron Oxyhydroxide Produced at the Redox Boundary of Eutrophic Lakes

**Literature Survey** It is well established that iron hydroxide particles have strong adsorption properties (Crosby et al., 1983) for a large number of cations (Swallow et al., 1980; Balistieri and Murray, 1982) and anions, including phosphate (Lijklema, 1980) and humic substances (Tipping, 1981a). It can therefore be expected that the biogeochemical cycle of iron may play an important role in controlling the bioavailability of nutrients and, thereby, the trophic level of lakes (Mayer et al., 1982). This is likely to be particularly important for stratified lakes possessing an anoxic deep layer promoting the rapid cycling of iron at the redox boundary (see Figure 12.4). Assessment of the quantitative importance of this cycle, however, requires that each of the various fluxes in Figure 12.4 be determined. Davison in particular (Davison and Woof, 1984b; Davison, 1985; and refs. therein) has studied and discussed in detail the cycles of Fe (see Figures 12.1 and 12.4), Mn, and S at the redox boundary of eutrophic lakes. Fe particles have been studied by several authors (Tipping et al., 1981b; Crosby et al., 1983; Koenings, 1976), and they are often found to be a hydroxophosphate of iron (Mayer et al., 1982; Buffle et al., 1989) with a P/Fe ratio of up to 1:3. More recently, it has been shown (see below; Buffle et al., 1989) that they contain approximately equal amounts of Fe(II) and Fe(III). Particle sizes ranging from 1 to 10 $\mu$m have been reported (Laxen and Chandler, 1983), but these values were derived from filtration experiments, possibly leading to overestimation of sizes by a factor of 10 or more (see Section 12.3). Pore sizes of 20–200 Å and specific surface areas of 100 $m^2 \cdot g^{-1}$ have been measured by Crosby et al. (1983) for particles synthesized by oxidation of Fe(II) under conditions mimicking natural ones.

Although of great interest, the information reported in the literature is presently limited by the fact that the measurements were either made in the laboratory (where complete reproduction of natural conditions is not always easy) or were subject to possible important artifacts (e.g., using sediment traps for determination of fluxes or filtration for size distribution measurements; see Sections 12.2 and 12.3). The results presented below were obtained (i) from direct field measurements made (ii) on particles naturally formed within the water column while (iii) paying special attention to minimize artifacts from known sources. The study site was Lake Bret in the Canton of Vaud, Switzerland.

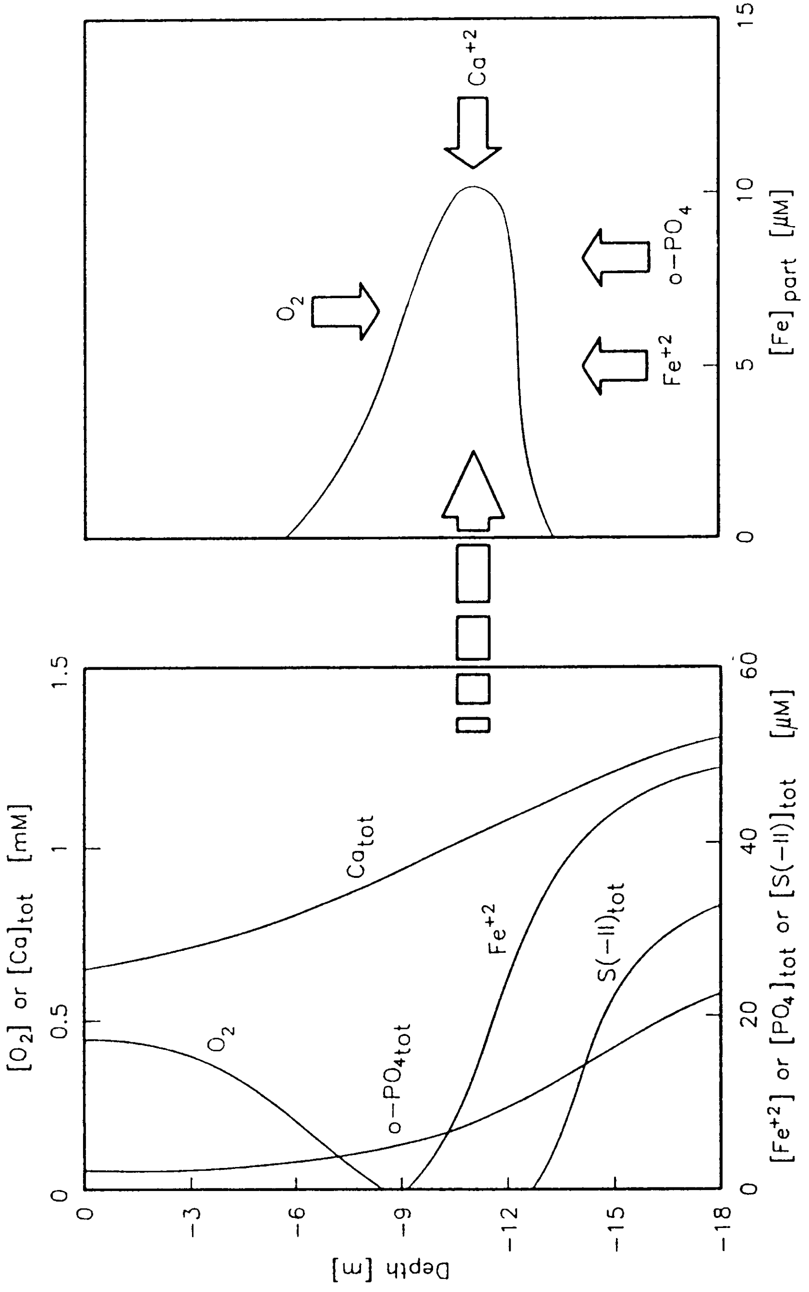

**Figure 12.1** Typical concentration profiles of species related to the iron cycle in Lake Bret during the summer; the transport of ferrous iron, oxygen, phosphate, and calcium into the transition zone leads to the formation of an autochthonous colloidal iron-rich species [Fe(II)/Fe(III)/OH/PO$_4$/Ca].

## Physico-chemical Characteristics of the Lacustrine Iron-rich Colloids

Morphological and size characterization of colloids, as well as their elemental composition on a particle-specific basis, were accomplished by transmission electron microscopy coupled to an energy dispersive spectrometer (TEM-EDS) (Leppard et al., 1988; Buffle et al., 1989). These results were corroborated and complemented by determination of the particle composition, on a wet chemical analysis basis, using a multi-method speciation approach (De Vitre et al., 1988). The chemical composition of the lake water (Figure 12.1) and reactivity of the iron colloids was assessed, *in situ*, using polarographic methods (Buffle et al., 1988b; De Vitre et al., 1988).

*Iron Particle Classes:* The TEM-EDS results show that most of the iron-rich particles contain three major elements (up to five in some cases) with other minor elements represented in very low proportions. They may be grouped into the following four classes (the proportions being based on a particle number basis): 49% contain Fe/P/Ca, 23% contain Fe/P/Ca/Si/Al, 15% contain Fe/P/Ca/Si, and 13% contain Fe and other elements. These various associations have been discussed elsewhere (Buffle et al., 1989) and it has been suggested that "pure iron particle units" are best represented by the class containing only Fe/P/Ca, the other classes containing inclusions of clay leaflets or diatom frustules. The characteristics of these "pure" particles are discussed below.

*Morphology:* Figure 12.2 shows that the structure of these iron particles is amorphous and porous, i.e., no discrete particle limit is observed, and that they are composed of spherical globules (which sometimes include dense globulets). Under high-resolution conditions it can be seen that the minimum grain size of these globules is 1–2 nm.

**Figure 12.2** Electron micrograph of iron-rich globules isolated from Lake Bret. Bar is 0.15 μm long.

*Size Distribution:*The spherical globules fall generally in the size range 45–310 nm (Figure 12.3), while the globulets are found between 7–16 nm. Aggregates of globules are also seen. It is important to note that the size of all globules is below the pore size of the membranes (0.45 $\mu$m) on which particles were retained during the sampling procedure. This particular point is discussed in Section 12.3, but, in any case, it is likely that the absence of particles smaller than 45 nm does not reflect the actual water column situation but rather the fact that these very small globules were not retained on the sampling membranes.

*Particle-Specific Elemental Composition:* EDS measurements have been done on 71 discrete particles previously isolated by means of the coupled TEM to ensure that they belonged to the Fe/P/Ca class. Values of $0.25 \pm 0.06$, $0.19 \pm 0.05$, and $1.34 \pm 0.32$ were obtained for the molar ratios P/Fe, Ca/Fe, and P/Ca, respectively.

*Wet Chemical Field Analysis:* The P/Fe ratio found above was confirmed by analysis of particulate $PO_4$ and Fe concentrations directly on the water sample. This, coupled with LAMMA (laser microprobe mass analyser) determinations, confirmed that the P in the globules observed by TEM is in the form of $PO_4$. Furthermore, wet analysis also showed that the Fe(II)/Fe(III) ratio in the iron particles is close to one. Both ratios were found to be statistically constant with season and depth.

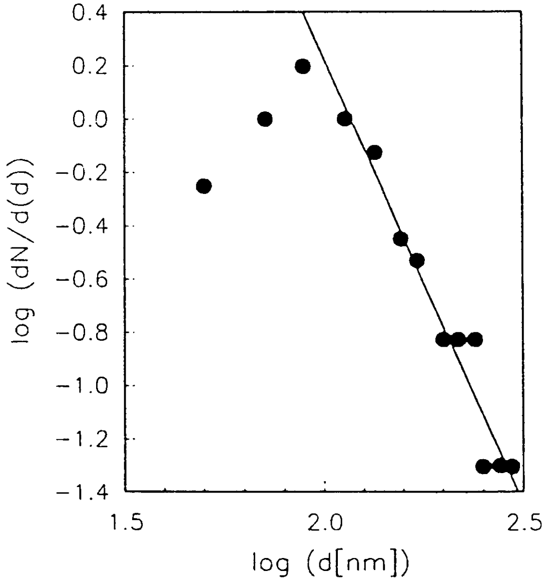

**Figure 12.3** Size distribution of 71 iron-rich globules, obtained by TEM examination and expressed according to the Pareto law, $\log[dN/d(d)] = \text{cte} - \beta \log(d)$, where N equals the number of globules with a size within a diameter interval d(d), and $\beta$ expresses the process of elimination of the particles in the water column.

*Chemical Reduction Rate:* Results consistent with zero-order kinetics were observed for the dissolution of at least 10% of Fe present in the colloids when ascorbic acid and S(−II) were used as reductants under lacustrine conditions. With S(−II) at lake water concentrations, dissolution rates of $3.4 \cdot 10^{-9}$ mole of Fe per hour were measured (De Vitre, unpublished results) which corresponds roughly to a reaction time of 8 hours for the reduction of 10% of the particulate Fe.

All these results are consistent with those of laboratory studies on the formation and reactivity of iron hydroxide particles and polymers (Pysik and Sommer, 1981; Schneider and Schwyn, 1987; Schneider, 1988) and are discussed elsewhere (Buffle et al., 1989). It can simply be pointed out that the remarkable constancy of the Fe/P/Ca and Fe(II)/Fe(III) ratios in these particles with time and depth, coupled with the EDS observation that $PO_4$ is not only adsorbed at the surface but belongs to the matrix of the particles, suggests that these particles are composed of a limited number of well-defined components.

## 12.2 The Nature of the Important Factors to be Measured in the Iron Cycle and the Available Methods

**Factors Characterizing the Iron Cycle** In order to assess the exact role of iron particles in the circulation of trace elements and nutrients, two types of information are needed:

1. Those related to the reactivity of trace elements and nutrients with the particles, including, in particular, the electrical charge of the particle, adsorption equilibria and rate constants as a function of pH, the specific surface area, porosity, and size distribution of the particle, and the diffusion coefficients of trace elements inside the particle.
2. Those related to the detailed characterization of the iron cycle and, in particular, the formation and elimination fluxes of the iron particles and their relative importance compared to those of other absorbing particles. These latter factors are, in fact, as important as adsorption strength, since the transport of adsorbable elements depends on the number and lifetime of settling particles.

For most of the *factors of type 1*, information is available in the literature (see Section 12.1), although it is most often based on laboratory studies. Comparison of our results with those of Crosby et al. (1983) suggests, however, that laboratory and field particles are similar if conditions close to natural systems are respected in the former case. Very little is presently known about the diffusion coefficient of elements inside such particles or about the relative scavenging role of the different size classes of particles, in particular the very small ones. Two remarks can, however, be made:

- Because of the high porosity of the particles, it is very likely that adsorption occurs at sites present *within* the particle structure, and not only at its surface. Furthermore, because of the small sizes of the particles, it may also be expected that diffusion times inside the particles will be short and will not appreciably affect adsorption equilibria.
- Determination of the relative scavenging role of the various size classes of particles can be assessed by transforming the particle size into a particle volume distribution (Lerman, 1979).

Very little is known regarding the *factors of type* 2 (iron cycle fluxes), largely because of experimental difficulties (see below). The results obtained with our data (see Section 12.1 and refs. therein) and cited in the literature, however, enable one to estimate the most important steps of the cycle as well as the orders of magnitude of the corresponding reaction times (Figure 12.4).

In Figure 12.4:

- Eddy diffusion times ($\tau_D$) are estimated from typical values of vertical eddy diffusion coefficients (0.03–10 $cm^2 \cdot s^{-1}$) (Lerman, 1979) and the concentration gradients of the species of interest in the lake (e.g., Figure 12.1).
- The oxidation time of Fe(II) ($\tau_{Ox}$) depends heavily on the exact nature of the oxidation reaction by $O_2$, noncatalyzed (Schneider and Schwyn, 1987; Davison and Seed, 1983; Sung and Morgan, 1980), catalyzed by Fe(III) hydroxide (Sung and Morgan, 1980) or influenced by other factors (e.g., $PO_4$, ionic strength and alkalinity (Sung and Morgan, 1980; Sarikaya, 1979)). Fe(II) may also be oxidized by $MnO_x$ formed by oxidation of Mn(II) by $O_2$ in the same water strata. The oxidation time indicated in Figure 12.4 is estimated from the literature values corresponding to aquatic conditions similar to those prevailing in Lake Bret.
- Particle formation times ($\tau_P$) are estimated from Schneider (1988) for globules containing Fe(III), ($\tau_{P,III}$), and from experimental observations for FeS precipitation ($\tau_{P,II}$).
- The time needed for coagulation of Fe/P/Ca globules with other settling particles of the water column is presently unknown. The occurrence of this process is, however, likely, due to the relatively small electrical charge of iron hydroxide particles at the lacustrine pH (7.5) and is strongly suggested by the fact that the size distribution follows a Pareto law with $\beta = 3.2$ (Figure 12.3); such values of $\beta$ are indicative of elimination of particles by perikinetic coagulation (Kavanaugh et al., 1980).
- The time indicated for reduction of globules by S(−II) ($\tau_R$) results from experimental observations (Section 12.1).
- The sedimentation times of Fe/P/Ca globules ($\tau_{S,G}$) are estimated from

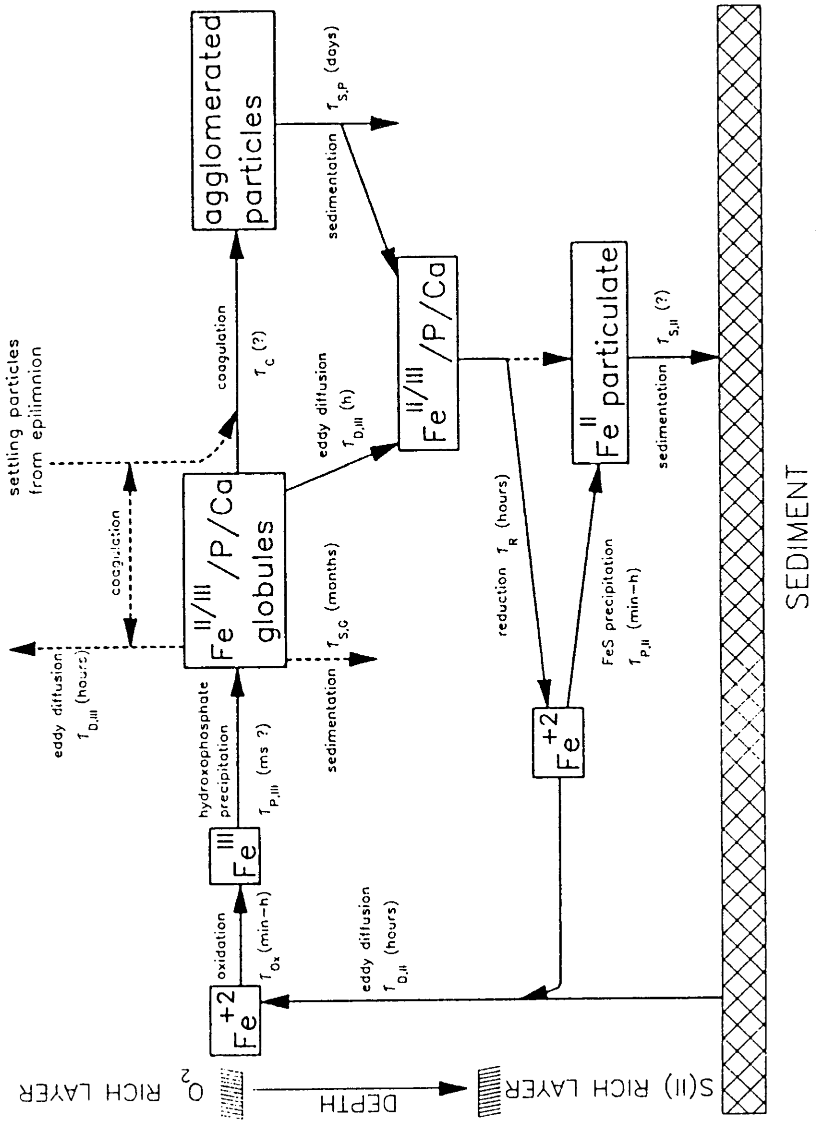

**Figure 12.4** Schematic overview of the different physical and chemical fluxes involving the iron species at the oxic-anoxic boundary. The subscripts of the reaction time $\tau$ refer to: *first subscript*—eddy diffusion (D), coagulation (C), sedimentation (S), precipitation (P), oxidation (Ox), or reduction (R); *second subscript*—$Fe^{II}$ particles (II), $Fe^{III}$-containing particles (III), coagulated particles (P), or Fe/P/Ca globules (G).

typical values of sedimentation coefficients (Lerman, 1979) and the size range observed for globules (Figures 12.3 and 12.8). The sedimentation rate of FeS particles is as yet unknown.

Although the above reaction times are only rough estimates, Figure 12.4 allows an important fact to be pointed out. Experimental evidence suggests that, during the three months of summer, the concentration of iron particles (Figure 12.1) can be considered as the result of a quasi-stationary state in the iron cycle. If this is true, the rate of the elimination process must be of the same order of magnitude as that of the slowest step in the chain of the particle production mechanisms (eddy diffusion and/or oxidation), i.e., hours). Free globule sedimentation ($\tau_{S,G}$, i.e., months) can therefore be ruled out, and even sedimentation of agglomerated particles ($\tau_{S,P}$, i.e., days—weeks) is not very likely since these processes are probably much too slow. The most probable processes, therefore, are either reductive dissolution of setting agglomerates or downward eddy diffusion of globules due to the concentration gradient produced by reductive dissolution at depth. The existence of a high concentration gradient of iron particles at the limit of the sulfidic layer (Figure 12.1) strongly supports the important role of this reductive dissolution.

**Limitations of Available Methods for Particle Studies** Figure 12.4 also emphasizes the intricacy of the iron cycle at the redox boundary, which is sharply at odds with the very poor resolution of presently available methods for particle sampling, characterization, and determination of fluxes.

Of the six types of flux-producing processes (see Fig. 12.4 and its discussion) only *eddy diffusion* (the $\tau_D$'s) can be measured unambiguously *in situ* (Lerman, 1979). As regards the four "*chemical fluxes,*" (i.e., oxidation, $\tau_{Ox}$ (Sung and Morgan, 1980; Davison and Seed, 1983); reduction, $\tau_R$ (Waite and Morel, 1984; Zinder et al., 1986; Stone and Morgan, 1987; Pysik and Sommer, 1981); precipitation, $\tau_P$'s; and coagulation, $\tau_C$ (Weilenmann, 1986)), reported data were largely obtained from laboratory studies (information for the latter two being especially scarce). These four processes involve surface (heterogeneous) reactions and are often highly dependent on particle concentration, the presence of "foreign" particles in solution (precipitation by heterogeneous nucleation, coagulation of particle mixtures, catalytic oxidation), microbial action (oxidation, reduction), and the presence of dissolved adsorbing and complexing agents (catalytic reductive dissolution). All of these effects are difficult to control individually and their exact environmental roles are difficult to estimate by laboratory simulations. *In situ* chemical flux determinations are, therefore, extremely necessary. Finally, *sedimentation fluxes* are most often determined by means of sedimentation traps (Simpson, 1982; Bloesch and Burns, 1980), which, as is discussed in these latter papers, have several limitations including, in particular, an often poorly controlled trapping efficiency (dependent on trap shape, turbulency, sediment resuspension, etc.). Of particular importance for studies of the iron cycle are that:

1. Traps must generally be left *in situ* for long periods (days) implying that they do not give real-time information but, rather, are a time-integrating collection method. They are also a depth-integrating method since they collect particles from all depths above the trap.
2. Traps are also nonselective. This is a very important limitation when studying the iron cycle since autochthonous iron oxyhydroxides are minor particulate components when compared to others such as calcite, clays, and allochthonous iron oxides. Since the latter two contain relatively large amounts of Fe, the sedimentation fluxes specifically due to the iron cycle of Figure 12.4 are determined by the difference of the Fe fluxes above and below the redox boundary layer (Davison et al., 1982). This leads to high uncertainties because of the much larger amount of Fe in clays compared to Fe/P/Ca particles.
3. Because traps must be left *in situ* for days, important biological and chemical transformations may occur during the sampling period. This is particularly important for Fe(III) particles which may easily be reduced to soluble Fe(II), thereby allowing its diffusion back into the water column resulting in severe underestimation of the fluxes.

As a result of these very important limitations, the development of flux determination methods which are selective for particular chemical species is certainly necessary if we are to gain a more detailed insight into the iron redox cycle.

In *particle characterization*, the most important problems are linked to sampling and fractionation. In most cases, filtration or centrifugation is used and the particulate material thus obtained is examined by characterization techniques, such as chemical analysis (Laxen and Chandler, 1983), X-ray fluorescence, electrophoresis (Tipping, 1981a), spectroscopy (IR, EPR, Mössbauer: Davison and Dickson, 1984a), TEM-EDS (see Section 12.1) or light scattering (Rees, 1987; see also Simpson, 1982, for a good review). In most cases, the application of such techniques to the real situation existing in the water column is compromized by particle denaturation during the initial sampling (and possibly the pre-concentration) step. Centrifugation problems are due to the absence of size selectivity for particles larger than 0.1 $\mu$m and the difficulty or impossibility of controlling denaturations due to coagulation during centrifugation (Kavanaugh et al., 1977; Salim and Cooksey, 1981), chemical reactions caused by differences in chemical conditions in the centrifugate with respect to the bulk solution, and, finally, the difficulty in maintaining, during centrifugation, the partial pressures of $O_2$, $CO_2$ and $H_2S$ prevailing *in situ*. Problems associated with filtration are discussed in Section 12.3. In any case, the development of characterization methods applicable *in situ* without, or with a minimum of sample handling (De Vitre et al., 1988; Buffle et al., 1988b), is highly desirable.

## 12.3 Filtration of Lacustrine Colloids: Can It Reflect Particle Size and in What Way?

As was mentioned above, filtration is one of the most ubiquitous operations in any study of aquatic samples, despite its important limitations and possible artifacts. Appropriate fractionation strategies require knowledge of the exact nature and relative importance of these limitations which can be grouped into three categories:

1. Contamination of samples by trace components present in the membrane or losses of trace compounds by adsorption at the membrane surface (Truitt and Weber, 1979).
2. Denaturation of water sample colloids between field collection and their subsequent study if storage and filtration periods are too long (Laxen and Chandler, 1982).
3. Denaturation of colloids at the membrane surface due to possible coagulation, retention by the membrane, and, ultimately, gel formation (clogging) (Suki et al., 1984).

When filtration is used for studying the properties of aquatic colloids, such as those of the iron cycle, the first type of problem is not limiting since it concerns only components present at the sub-micromolar level. The second type of problem can also be made negligible if filtration is done directly in the field or, even better, at depth (De Vitre et al., 1987). Consequently, we shall focus here on the artifacts produced at the membrane surface.

**Problems and Potential Remedies** Studies of membrane filtration processes focusing on their application to water analysis are very scarce, but much information can be gleaned from the extensive studies performed for industrial applications. Numerous theoretical or empirical models have been used to explain the fact that solution flux through a membrane is generally observed to decrease with time. They are based on changes in osmotic pressure (Michaels, 1968; Kozinski and Lightfoot, 1972; Trettin and Doshi, 1979), occurrence of interactions between different solutes or between the solute and the membrane (Fane et al., 1983; Matthiasson, 1983), variation of hydraulic resistances (Suki et al., 1984), or particle collisions which lead to coagulation within the membrane (Green and Belfort, 1980). These industrial-condition-oriented models are generally not directly applicable to limnological studies since particle concentrations are much lower and their nature is much more diverse (large heterogeneity, as shown in Figure 12.5). Coagulation rate (and consequently gel formation) is decreased by the first condition but increased by the second.

Nevertheless, drawing upon these models, it may be schematically stated that two basic processes may occur at the membrane surface (Figure 12.6) leading to the retention of colloids, which, if only their size were to be considered, should pass through the membrane pores. These basic processes are:

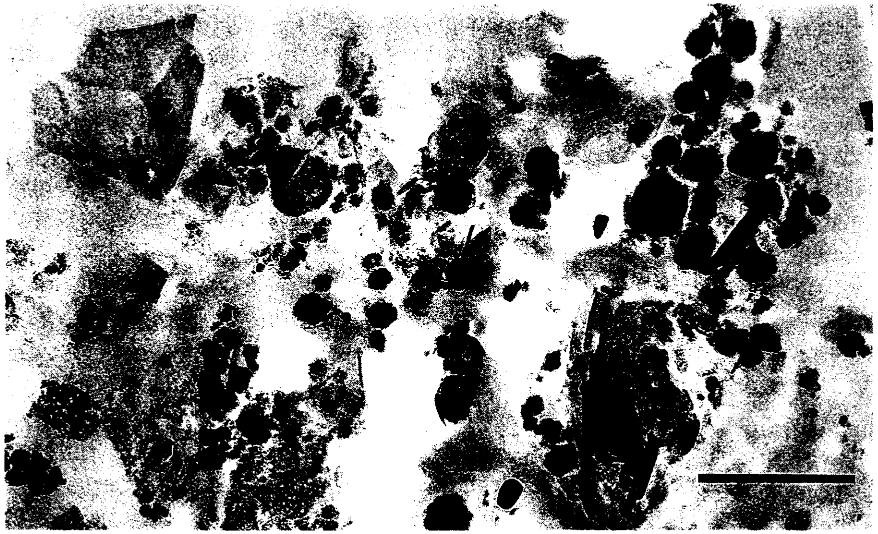

**Figure 12.5** Electron micrograph showing the large heterogeneity of a natural sample containing iron-rich colloids in a matrix of calcite crystals, clays, and organic fragments. Bar is 0.45 $\mu$m long.

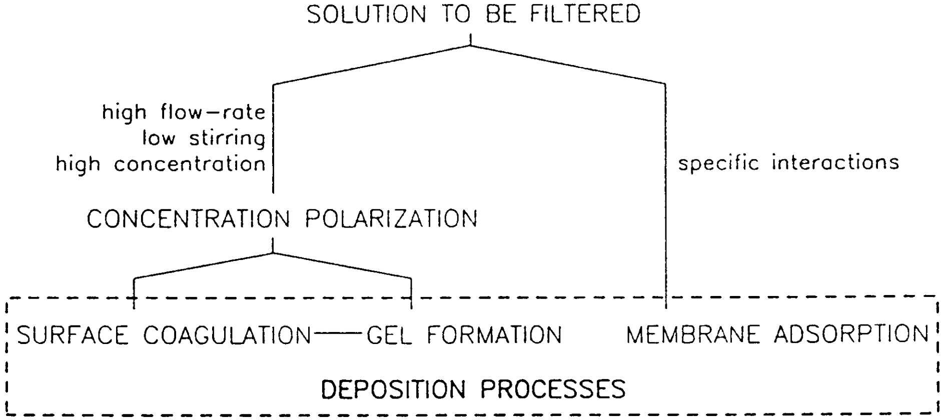

**Figure 12.6** Main causes of decrease in flow rate during filtration. "Deposition processes" is a global term for retention on a membrane.

1. Retention by adsorption due to specific interaction between the colloid and the membrane surface. One may expect this process to depend on the chemical nature of the membrane.

2. Retention due to coagulation of the small colloids into agglomerates larger than the pore size. This process may result from the *"concentration polarization"* effect, which always occurs at the solution-membrane interface on the retentate side of the membrane (Figure 12.7). Those particles which cannot pass through the pores accumulate in a layer close to the membrane and their concentration becomes larger than that of the same particles in the bulk solution. As a result of this concentration gradient, these particles will diffuse back towards the bulk solution but, the larger these particles, the smaller their diffusion rate will be.

The concentration polarization effect can be represented by the following approximate equation (Meares, 1976; Buffle, 1988a), which gives the ratio of the particle concentrations at the membrane surface ($C_0$) to that in the bulk solution (C):

$$C_0/C = \exp(J\cdot\sigma\cdot\delta/D)$$

where J ($cm\cdot s^{-1}$) is the flow rate of solution through the membrane per unit surface area, $\sigma$ is the retention coefficient ($\sigma = 1$ for fully retained particles; $\sigma = 0$ for particles not retained at all), $\delta$ is the diffusion layer thickness (cm), and D ($cm^2\cdot s^{-1}$) is the diffusion coefficient of the particle. This equation enables one to estimate that $C_0$ may take on values many orders of magnitude larger than C if the calculation is made for particle sizes of 0.04–0.3 $\mu$m (Figure 12.3) and with solution flow rates typical of those used in syringe filtration (100-300 $ml\cdot min^{-1}$) (Buffle et al., 1988b). With such a high particle concentration, coagulation becomes very rapid and produces agglomerates, which are deposited on the membrane and may finally form a clogging gel (Figures 12.6 and 12.7). Note that, in contrast to the specific adsorption effect, the coagulation effect is expected to be rather independent of the chemical nature of the membrane. Specific adsorption effects are more difficult to minimize than coagulation ones, but our results suggest that the latter are often predominant when filtration conditions are not chosen with care.

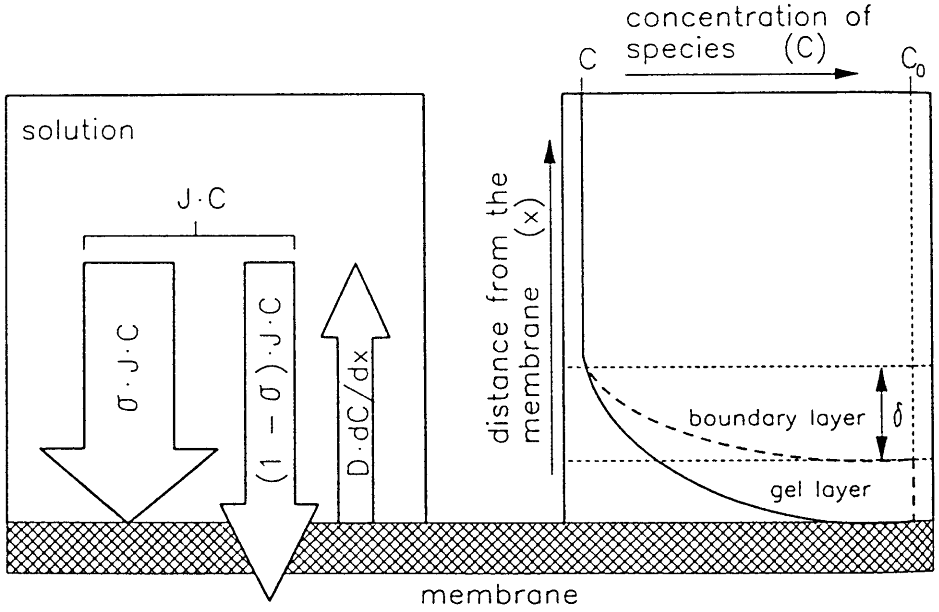

**Figure 12.7** Polarization concentration model. *Left:* the three driving forces leading to increased concentration at the membrane surface, transport through the membrane, and back-transport to the solution. *Right:* schematic concentration gradient showing the existence of a boundary layer, and, in extreme cases, a gel layer (dashed curve) at the membrane surface; C, concentration in the bulk solution; $C_0$, concentration at the membrane surface.

To minimize coagulation effects, all parameters must be selected so as to decrease the $C_0/C$ ratio. The above equation shows that this can be achieved by:

- Using very low solution flow-rates, J, through the membrane. For filtrations involving large volumes this necessarily implies that the filtering surface area be increased (e.g., membranes with large surfaces, hollow fibers) if the filtration time is to remain acceptable; this, however, also increases the risks of specific surface adsorption.
- Increasing the diffusion flux of particles back into solution by decreasing $\delta$ through induced turbulency (e.g., by efficient stirring at the membrane surface). Note, however, that turbulency has the simultaneous adverse effect of increasing the rate of orthokinetic coagulation of colloids in solution.
- Limiting the concentration factor, $V_0/V_f$, where $V_0$ and $V_f$ are, respectively, the volumes in the filtration cell initially and at the end of filtration. This prevents the bulk concentration from increasing excessively.

It should be noted that, at present, none of these conditions are normally applied in the case of routine use of syringe filtration.

## Tests of Filtration Factors for Iron Particles

*Results* The above factors have been systematically tested by studying the results of filtration of the iron particles formed at the redox boundary (see Figures 12.1 and 12.4). Figure 12.8 shows the (apparent) size distributions of these colloids obtained by two different filtration modes. In syringe filtration (Figure 12.8*a*), the untreated water sample is filtered on membranes with different pore sizes, without solution stirring and with uncontrolled but "normal" flow rates (200 $ml\cdot min^{-1}$ = 1 $cm\cdot s^{-1}$ for a filter area of 3.3 $cm^2$; note that these

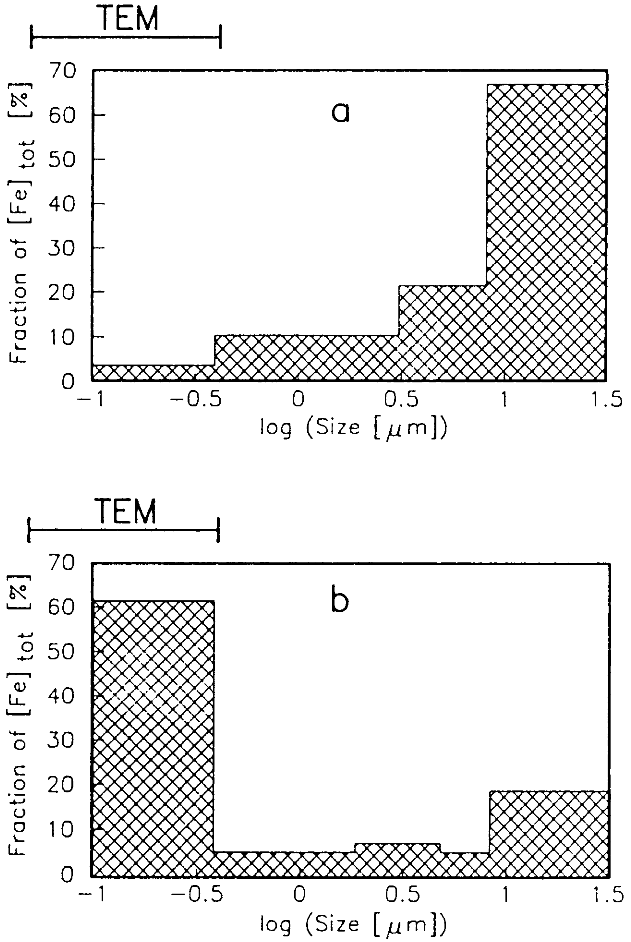

**Figure 12.8** Size distributions of iron-rich colloids in Lake Bret obtained by (*a*) syringe filtration (cellulose nitrate membranes, fast flow rate, no cascade, no stirring, field filtration), and (*b*) cascade filtration (polycarbonate membranes, low flow rate, cascade mode, stirring, in situ filtration).

values are high compared to those discussed below). In this case, size fractions are computed by differences between the various filtrates. At depth cascade filtration (Figure 12.8*b*; De Vitre et al., 1987) was performed with a custom-made submersible apparatus. In this case, the same water sample is passed successively through a series of membranes with decreasing porosities under conditions of slower flow rates for successive membranes, stirring at the membrane surface, and under an $N_2$ atmosphere. Clearly the use of these conditions, which all favor minimization of artifacts, yields particle sizes which are about one order of magnitude smaller than the ones given by syringe filtration.

The fact that the above difference is mainly due to surface coagulation has been shown by TEM observations of membranes used at different flow rates; an example can be seen in Figure 12.9 which shows the nature of the "particles" retained on filters. By using scanning electron microscopy (SEM; low resolution) to get a top view of the surface membrane after filtration, particles of a few μm seem to be retained (Figure 12.9*a*) while, when TEM (high resolution) is used to get profile micrographs of thin sections through the membrane (Figure 12.9*b*), it is clear that these "particles" are, in fact, agglomerates of much smaller ones.

Figure 12.10 shows the separate effects of flow rate (J), concentration factor ($V_0/V_f$), and nature of the membrane, these being discussed below. It should simply be noted that Figure 12.10*b* shows the fraction of iron retained on the membrane, even though for the membrane pore size used (3 μm), none of the iron particles (see Figure 12.3) should be retained based on their size. Any retention is therefore due to secondary effects such as those given above (retention by adsorption, coagulation).

It must also be stressed that, due to the large heterogeneity of water samples (see Figure 12.5), the reproducibility of the results (over more than 150 filtration operations) was found to be about 10%. This implies that any filtration operation must necessarily be repeated several times for statistically

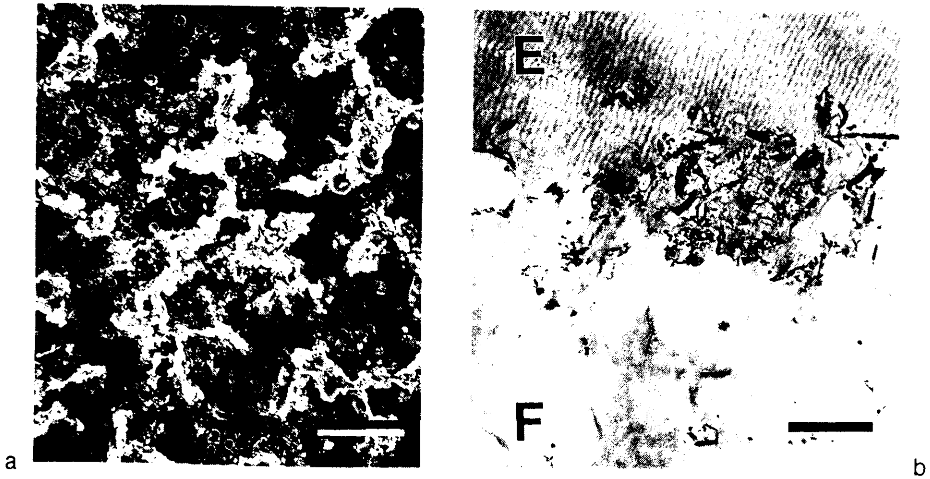

**Figure 12.9** (*a*) Scanning electron micrograph and (*b*) transmission electron micrograph of particles retained on filtration membranes, showing the aggregation and coagulation problems produced during filtration, and the possible overestimation of particle size when using SEM (low resolution) instead of TEM. SEM picture shows a membrane surface, while TEM picture corresponds to a perpendicular view of a membrane surface (F = filter material; E = external solution on the top part of the membrane); clearly, the "pack" retained by the membrane is an aggregate of very small particles which should have been able to pass through the pores. Bar in a) is 30 μm long. Bar in b) is 3 μm long.

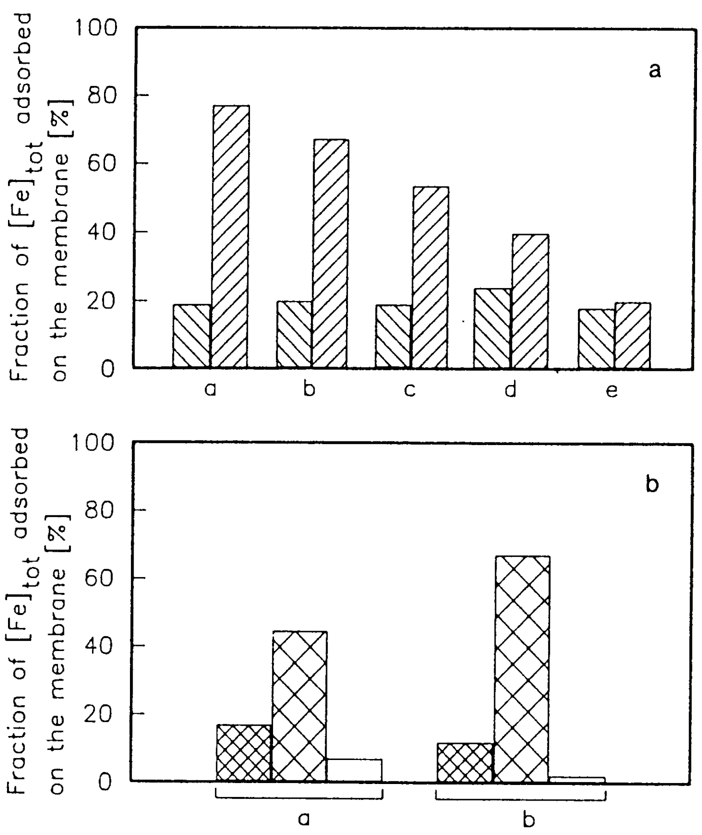

**Figure 12.10** (*a*) Effect of the flow rate on the fraction of iron retained on the membrane. Membrane types: a, polycarbonate; b, cellulose nitrate; c, cellulose acetate; d, polyvinylidene fluoride; e, acrylic copolymer. Nominal pore size is 0.2 μm; ▧ = 1 cm·h⁻¹, ▨ = 20 cm·h⁻¹. (*b*) Effect of the ratio of $V_0/V_f$ (= initial volume/final volume in the cell) on the fraction of iron retained on the membrane. Membrane types: a, polycarbonate; b, cellulose nitrate. Nominal pore size is 3 μm; ▩ = 1.1 (20 ml/18 ml), ⊠ = 5.0 (20 ml/4 ml), □ = 1.7 (200 ml/120 ml); in the third case, stirring was more efficient, leading to a lower value of iron retained on the membrane.

valid results to be obtained. All results presented here are averages of triplicates.

*Discussion* The results of cascade filtration are much closer to those of TEM than those of syringe filtration (see Figures 12.3 and 12.8). As suggested before, this is due to the fact that, in the former case, all conditions favor the minimization of artifacts: oxygen contamination—and therefore possible oxidation of Fe(II)—is avoided by at depth operation; rather low solution flow rate is imposed by the less porous membrane; efficient stirring decreases $\delta$; and $V_0/V_f$ never exceeds five. In addition, cascade filtration progressively eliminates the large particles from the successive filtrates, thus decreasing the probability of coagulation.

Figure 12.10*a* shows how the flow rate, J, is a predominant factor in producing artifacts by surface coagulation irrespective of the nature of the membrane. SEM observations suggest that with the smallest flow rate (1 cm·h⁻¹) the surface coagulation process is negligible. It must be noted, however, that, by using such very low flow rates for filtering large volumes, the time necessary to perform the filtration becomes so long that coagulation may occur in the filtration cell itself (Perret, unpublished results).

These results have also shown (Buffle et al., 1988b) that stirring somewhat decreases the proportion of Fe retained on the membrane (i.e., the coagulation effect), but only by 10% (with the classical magnetic stirring used). More efficient turbulency might be created at the membrane surface (Matthiasson and Sivik, 1980), but, as mentioned above, this might also favor coagulation in solution. Figure 12.10*b* shows that, as expected from the artifacts interpretation based on coagulation, an increase in the concentration factor, $V_0/V_f$, leads to an increase in iron retention.

Finally, the effect of the chemical nature of the membrane must be considered. The data in Figure 12.10*a* indeed show that retention significantly depends on the membrane nature and seem to suggest that polycarbonate membranes (Nuclepore) are the worst offenders of the membranes tested. Taken by themselves, the results shown in Figure 12.10*a* are, however, misleading in that respect. Indeed other data (Perret, unpublished results) show that an inverse effect of the membrane nature is observed when membranes of higher porosity (3 μm) are used. Although the exact reason of this inverse behavior is not yet fully understood, the important point to stress here is that the overall retention in cascade filtration is always high at high flow rates, irrespective of the chemical nature of the membrane.

**Conclusions** All of the above results show that important precautions must be taken if iron colloids are to be filtered without artifacts. Specifically, filtration must be performed at low and controlled flow rates ($\leq$10 cm·h⁻¹), preferably in cascade, with small concentration factors, in stirred solutions, and under an $N_2$ atmosphere. Although iron colloids might be particularly sensitive to coagulation due to their low electrical charge at the lake pH (7.5), it is quite likely that these precautions are applicable to most aquatic colloids and, indeed, these results are in agreement with previous observations (Danielson, 1982).

These results have important implications concerning the use of filtration for size distribution measurements, whether using cascade filtration followed by chemical analysis of the filtrates or by microscopic observations of particles retained on filters. In the latter case, it has already been discussed that the retained "particles" observed by SEM (see Figure 12.9) are in fact agglomerates of much smaller colloids. Great caution must therefore be used when determining size distributions from SEM observation of particles on filters, as is common practice. The use of cascade filtration for size distribution measurements must also be accompanied by important precautions. Tests of the influence of filtration conditions, in particular flow rate, is highly recommended. In any case, TEM is certainly by far the best method presently available and a few TEM observations, at least, should complement any other method. On the other hand, filtration remains a useful tool for operational fractionation, provided that the above filtration conditions are well controlled to minimize artifacts and that particle size is not inferred from the pore size of the membrane used. It must be stressed that well controlled filtration conditions imply, in particular, *avoiding* syringe filtration, because of its high and ill-controlled flow rate.

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