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COLLOIDAL IRON OXYHYDROXY-PHOSPHATE: THE SIZING AND MORPHOLOGY OF AN AMORPHOUS SPECIES IN RELATION TO PARTITIONING PHENOMENA

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ABSTRACT

An iron-rich fraction was isolated from a small eutrophic lake and studied on a particle-specific basis by (i) conventional transmission electron microscopy (TEM) and (ii) scanning transmission electron microscopy in conjunction with energy-dispersive spectroscopy (STEM/EDS). Additionally, the fraction per se was characterized by classical wet chemistry. A morphologically distinctive particulate was discovered to be rich in iron (in an X-ray amorphous form) and phosphorus (as phosphate), and it also contained some calcium. Particle sizes determined by TEM fell entirely into the colloidal range, with a great majority of those trapped by a cutoff filter of 0.45 μm showing a mean diameter $< 0.13\,\mu$m. Several morphotypes could be identified at 3 nm resolution in ultrathin sections of plastic-embedded colloid fractions. New technology transferred from the biomedical sciences was used to obtain ca. 1 nm resolution, and revealed (i) irregular arrays of subunits with a least diameter approaching the resolution limit and (ii) many varieties of the principal morphotypes. A range of 130 nm down towards the molecular range was found to be more representative of the true particle size distribution than one derived solely from filtration data based on filter pore sizes. Size distributions established by cascade filtration assessments are shown to be related to colloid aggregation phenomena, which may affect phosphate partitioning between the operationally-defined solid and soluble phases.

INTRODUCTION

Most of the colloidal phase (particles with a least dimension of approx. 0.001–1.0 μm) in lake water tends to be transported with the operationally-defined soluble fraction and analyzed as a soluble material, despite the fact that it has the physico-chemical properties of a solid. Models of contaminant partitioning in lakes cannot yield good predictions of solid–solution partitioning until they can take into account the colloids present (Morel and Gschwend, 1987). Considerable potential for characterizing lake colloids and quantifying the principal types is found in new non-intrusive optical techniques for counting and sizing. Coupled with energy-dispersive spectroscopy on a "per

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particle" basis (Leppard et al., 1988a,b), and minimally-perturbing techniques for sample handling (Buffle et al., 1988a,b), such new technology is now realizing its potential.

This paper presents an electron–optical analysis of the sizing and morphology of an amorphous species of colloidal iron oxyhydroxy-phosphate common to the oxic–anoxic interface of a small eutrophic lake. It differs from other studies of amorphous iron-rich colloids by combining three sets of complementary methodologies simultaneously: (i) minimizing sample perturbation at every step in sample collection and preparation; (ii) combining STEM/EDS analyses of individual particles and their aggregates with TEM morphological studies at ca. 0.001 μm resolution; (iii) to the greatest extent feasible, integrating data from wet chemistry performed in the field with new microscopical/spectroscopical information. Our technical advances allow for ultrastructural analyses of colloids which cover the full size range as defined operationally by Vold and Vold (1964), and as defined also by recent case studies (Leppard, 1983; Stumm, 1987). The resultant new observations are used to explain an apparently anomalous partitioning of phosphate in the standard water fractionation scheme for water quality analyses.

METHODS

The sample site was Lake Bret (in Vaud, near Lausanne), a well characterized Swiss lake currently under intensive limnological investigation with regard to its mechanisms of iron, manganese and phosphate cycling (Zali, 1983; De Vitre, 1986; De Vitre et al., 1988b). Colloidal material rich in iron and phosphorus and known to be concentrated in a well defined layer by natural lacustrine mechanisms (De Vitre et al., 1988b) was sampled directly from that layer at the oxic–anoxic interface. Oxygen effects, which would normally perturb such a sample, were minimized, and sample storage, prior to both the wet chemistry and the preparation for microscopy, was eliminated (De Vitre et al., 1988a; Buffle et al., 1988b). The advantages and limits of all these minimally-perturbing procedures have been reviewed recently (Buffle et al., 1988a; Perret et al., 1988).

The wet chemistry for iron species, ortho-phosphate and relevant dissolved species (including calcium) was carried out according to Buffle et al. (1988b). The limnological context was characterized as previously (De Vitre et al., 1988b) using in situ probes in combination with analytical chemistry.

Microscopy preparation entailed adding a small volume (ca. 0.1 ml) of wet colloid fraction to a similar volume of a hydrated melamine resin (Nanoplast FB 101) developed by Bachhuber and Frosch (1983) to embed colloids for high-resolution electron microscopy analyses. This is to our knowledge the most minimally-perturbing approach to embedding currently applicable to non-cellular limnological specimens. There is no exposure to chemical fixatives, dehydrating solvents or heavy metal stains; additionally, the resin-hardening step occurs while the specimen is still hydrated. The embedding procedure, the ultrathin sectioning and the photomicrography were carried out according to Leppard et al. (1988a). Morphological and sizing analyses at ca. 1 nm resolution were performed with a Philips 300 TEM; these analyses were compared with those of a reference sample of the same colloid fraction prepared in a standard (perturbing) manner with an epoxy resin using fixatives, solvents and stains (see Leppard et al., 1988a).

Energy-dispersive spectroscopy on a "per particle" basis was performed on sectioned colloids according to Leppard et al. (1988a) with a dual-stage scanning electron microscope (I.S.I. DS-130), equipped with a STEM attachment and an energy-dispersive X-ray analyzer (γ-PGT/System 4). Section thickness was optimized with respect to colloid diameter and the diameter of the incident electron beam according to Leppard et al. (1988b). Quantification of EDS peak intensities was performed using a standardization procedure described elsewhere (Buffle et al., 1988b).

The following combination of techniques was used to ascertain the effect of filter-induced "coagulation" of iron oxyhydroxy-phosphate colloids on phosphate partitioning between solid and solution phases. Earlier studies (Buffle et al., 1988a; Perret et al., 1988) using classical filtration procedures had shown that many of the iron oxyhydroxy-phosphate aggregated particles retained by a given membrane had sizes smaller than the pore size. The retention mechanism for these particles was one of surface coagulation induced by high filtration flow rates. We extended these studies by TEM observations and found that flow rates $< 10 \text{cm h}^{-1}$ are necessary to minimize this effect. The conventional limnological approach to size analysis was in turn compared with a size analysis based on low-resolution micrographs of particles on the upper surface of the capture filter (micrographs produced by the SEM component of the STEM, which are representative of the low-resolution work carried out by many water scientists on aquatic particles). This SEM analysis of a chemically/operationally-defined fraction was then compared with high-resolution TEM images of the same particles in section view and the accompanying STEM/EDS measurements. This series of comparisons was performed at several flow rates to establish whether or not the particles operationally defined by SEM and filter pore sizes were in fact filtration-induced aggregates of smaller particles.

RESULTS AND DISCUSSION

Chemical characteristics of the colloidal material rich in both iron and phosphorus are summarized in Table 1. The limnological context is outlined in Table 2.

The minimization of perturbation of the sample prior to TEM analyses is related to a typical (perturbing) approach as follows:

(a) Our flow rate ($\leqslant$ 1 cm h^{-1}) to minimize surface coagulation vs typical flow rates (10^3 cm h^{-1}) routinely used with syringe filtration.

(b) Virtually zero storage time, since sample preparation is performed in the

TABLE 1

Chemical characteristics of the lacustrine globular colloids rich in iron and phosphate

	Wet chemical analyses of the fraction	Electron microscopical analyses of individual particles
[Fe(II)part]/[Fe(tot)part]	0.49 ± 0.03 (64 samplings over 12 dates in 1985–86–87)	Unavailable by this technique (no discrimination between oxidation states)
[o.PO_4 part]/[Fe part]	0.22 ± 0.01 (71 samplings over 12 dates in 1986–87)	0.25 ± 0.06 (71 individual particles)
[Ca part]/[Fe part]	Unavailable by this technique, due to low differences between [Ca] tot and [Ca] dissolved	0.19 ± 0.05 (71 individual particles)
Composition of major elements		49% containing Fe/P/Ca 23% containing Fe/P/Ca/Si/Al 15% containing Fe/P/Ca/Si 13% containing Fe + others (on a particle number basis)

TABLE 2

General morphological and hydrological characteristics of Lake Bret (Vaud, Switzerland)

Maximum length	1600 m
Maximum width	380 m
Maximum depth	20 m
Maximum volume	4.6×10^6 m^3
Lake watershed	2 km^2
Tributary watershed	21.3 km^2
Mean water outflow	150 m^3 s^{-1}
Mean water residence time	1 year

field vs typical range of storage times for water quality analyses (hours to days).

(c) Minimizing oxidation effects by performing filtration and sample preparation under nitrogen. This problem is important when dealing with samples from anoxic or oxygen-depleted waters, which may contain ferrous iron.

(d) Reduction of concentration polarization effects by stirring the solution during filtration, use of low flow rates and maintaining a low ($\leqslant 10$) solution concentration factor (initial volume/final volume) in the filtration cell.

(e) Using a minimally-perturbing dehydration procedure for TEM vs the standard TEM procedure for viewing limnological samples.

The TEM sizing analysis of globular iron phosphate colloids (or globules, our only abundant type of amorphous iron phosphate colloid) produced a curve similar to that published earlier (Leppard et al., 1988a). The sizing analysis revealed a maximum particle size near 0.3 μm (least dimension), a mean size near 0.1 μm and an abrupt lower limit cutoff near 0.05 μm. As noted earlier (Leppard et al., 1988a), perturbations (such as permitting severe sample dehydration conditions to occur so as to conform to standard TEM preparatory technology) had little effect on individual globule shape or size. In contrast, the principal effect of sample perturbation was revealed as a coagulation of globules with each other and with other colloid types to form aggregates whose size extended into the accepted size range for true particles. This coagulation effect is being dissected into its major components, but only its relationship to phosphate partitioning will be reported here. It is discussed in more detail below (Figs 3 and 4).

Previous high-resolution TEM analysis (Leppard et al., 1988a) found an indistinct granular ultrastructure within some of the electron-opaque material which constitutes the matrix of a globule, thus provoking a search for a matrix unit structure. This finding was re-investigated here with the practical resolution limit reduced to ca. 0.001 μm through the use of a minimal grain-size medium for embedding (Nanoplast FB 101 in its medium-hard formulation) and through the use of extreme-ultrathin sections. Thousands of images were examined for the presence of discrete, electron-opaque granules whose diameter was below the practical resolution limit of earlier TEM studies. Figure 1 shows examples of such a granule having a diameter in the range of 0.001–0.003 μm. For globule matrices shown by STEM/EDS to be rich in iron,

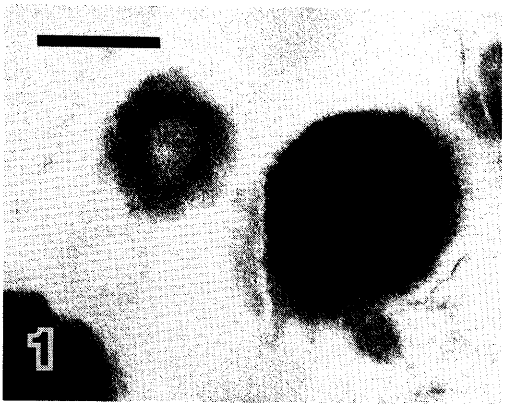

Fig. 1. The granular ultrastructure of globules of iron oxyhydroxy-phosphate. The large globule shows granules at its surface and the small one shows granules at both its surface and its core. The bar represents 100 nm.

Fig. 2. A cloud of granules bridging the individual globules of an aggregate. The bar represents 100 nm.

phosphorus and calcium, these granules could be observed at two locations. These were: (a) within the core portion of the matrix some of the time; (b) at the diffuse interface between globule and its external milieu. These tiny granules and those which sometimes appear to bridge individual globules within an aggregate (Fig. 2) do not necessarily represent a lower size limit for matrix granules, but they certainly suggest that the ultimate unit of ultrastructure is in the range of molecular dimensions as previously suggested by the work of Schneider and Schwyn (1987) on synthetic particles. No evidence for crystalline arrays of granules was seen. Representative spectra for globules are found in Leppard et al. (1988a,b) and in Buffle et al. (1988b).

Sections which provide such improved views of globule matrices can also serve to extend further the categorization of globule morphotypes, an electron–optical attempt at speciation begun earlier (Leppard et al., 1988a). Indeed, the details of the structural integration of globules with other colloids in an aggregate and the details of globule inclusions are better visualized now than was possible at 3 nm resolution. However, the interpretation of such detail must await future development of a data base on the structure of common aquatic mineral colloids as viewed in TEM sections. Thus, while it is currently possible to establish morphological sub-categories of the earlier major morphotypes, current research on globule speciation seems best advised to follow a combined chemical/spectroscopical approach such as that being established by Buffle et al. (1988b) for analyses on a "per particle" basis.

Improved structural resolution focussed attention on an earlier finding relating to the shape of the sizing curve, namely a severe dropoff in globule numbers between the mean and 0.048 μm, the earlier lower limit (Leppard et al., 1988b). Under the slightly-perturbing isolation conditions in use at the time, globules in the size range 0.048–0.307 μm could be captured as a multi-component coagulum (with clays, fibrils, etc.) on the surface of a standard 0.45 μm filter. This defined them operationally as true particles when in fact their measured diameters defined them as solubles. With the new information given here that a globule matrix contains subunits which may be 40 times smaller again, we suspect that our earlier best sizing curves (Leppard et al., 1988a) may have been incomplete. A straightforward attempt to identify solitary globules in the size range 0.001–0.048 μm in films of coagulum at capture filter surfaces failed for two simple reasons. Firstly, spherical grains of any electron-opaque material in that size range tend to have a similar appearance in ultrathin sections, with the problem of discrimination becoming more acute with decreasing size: this undermines the morphological basis for selecting iron-rich globules for EDS analyses. Secondly, confirmation by STEM/EDS of significant iron, phosphorus and calcium peaks in globules < 0.04 μm is made difficult by instrumental limitations, although these limitations appear subject to improvement. A quantitative isolation of the smallest globules on ultrafine filters followed by a STEM/EDS confirmation based on a grouping technique (Leppard et al., 1986) would permit us to estimate the importance of the smallest globules as iron-containing structural units; such an experiment is currently in progress. Nevertheless, it is clear that: (a) the range of 0.13 μm down to molecular dimensions accounts for most of the globules, and (b) "large" particles (0.45 to several μm), seen in classical syringe filtration experiments using various membranes with different porosities and in some low-resolution SEM images, are actually aggregates of globules which are usually heterogeneous.

What does this new information on colloid sizing mean in terms of water quality measures? Since small "particles" of phosphate (a species referred to here as an amorphous iron oxyhydroxy-phosphate globule) can be trapped by a filter having a relatively large pore size (0.05 μm particles trapped over 0.45 μm pores), one is forced to question the meaning of universally-employed water quality tests based on distinguishing between particulate and dissolved phosphate through the use of a filtration operation. A reasonable question is, "what is the effect of filtration flow rate on phosphate partitioning between the operationally-defined particulate and soluble phases?" Since the filter capture of particulate phosphate in sizes < 0.45 μm is dependent, at least in part, on the formation of a phosphate-rich coagulum at the upper surface of the capture filter, then flow rates which minimize or delay coagulum formation should maximize the ratio of soluble to particulate phosphate.

The quantitative chemical analyses presented in Table 3 show the effect of flow rate on the fraction of particulate iron retained on the filter's surface, using two different Nuclepore filter porosities of 3 and 0.2 μm. Regardless of

TABLE 3

Fraction (%) of iron retained/adsorbed on the surface of polycarbonate (Nuclepore) filters as a function of flow rate and pore size. Data given is from three replicate experiments

Flow rate (cm h^{-1})	% iron retained on membrane's surface
Pore size 3 μm	
0.2	0
10.0	20
20.0	45–60
Pore size 0.2 μm	
1.0	15–20
20.0	80

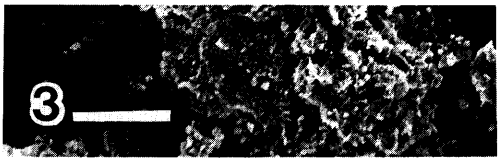

Fig. 3. A scanning electron micrograph of a "particle" of growing coagulum at the upper surface of a filter. The bar represents 3 μm.

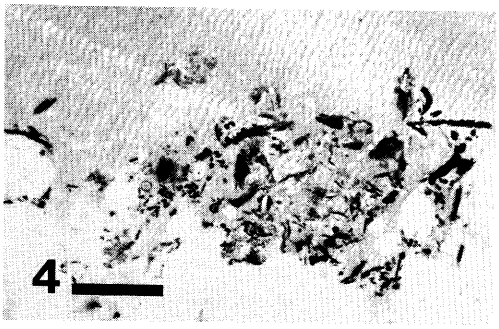

Fig. 4. A transmission electron micrograph of a section taken through a "particle" like the one shown in Fig. 3. Note that the increased resolution of Fig. 4 reveals the "particle" to be a heterogeneous aggregate of colloids, one which includes globules. The bar represents 3 μm.

filter porosity, the amount of particulate iron retained is highly dependent on the flow rate used, sharply increasing at high flow rates (20 cm h^{-1}). This factor will consequently have a pronounced effect on the analytical determination of "soluble" and particulate phosphate concentrations. It should be stressed that other filtration parameters, such as the concentration factor, stirring rate and membrane type, have also been shown to affect the amount of iron retained (Perret et al., 1988); however, the most important factor is flow rate.

Low-resolution SEM micrographs (Fig. 3) of "particles of growing coagulum" can graphically reveal the changing nature of the upper filter surface in response to flow rate. A growing expanse of coagulum can quickly and effectively create an extra filter between the standard filter and the incoming lake water. High-resolution TEM micrographs (Fig. 4) of sections taken through a "particle", like the one shown in Fig. 3, reveal that an individual particle of coagulum is, in reality, an aggregate of colloids. The heterogeneity of this aggregate accounts for the great chemical complexity of a coagulum with respect to the relatively simpler chemistry of an individual colloid type within that aggregate.

CONCLUSIONS

The results presented above clearly demonstrate that precautions must be taken to interpret filtration data in terms of size fractionation. In order to minimize artefacts, filtration should be performed using low, well controlled flow rates (ca. 10 cm h^{-1}), a cascade filtration mode, small concentration factors ($\leqslant 10$), a stirred solution and filtration under nitrogen for anoxic samples. Although iron oxyhydroxy-phosphate colloids might be particularly sensitive to coagulation due to their low electrical charge at pH values close to 7, it is likely that the above precautions are applicable to most aquatic colloids.

Our results have important implications related to the use of filtration in size distribution measurements. If the latter are measured by SEM observation of particles retained by a given filter, with no accompanying TEM analyses, there is a great risk that agglomerates formed during the filtration step be interpreted as true large particles. For the same reasons, size distributions artefactually shifted towards too large size classes are also observed when cascade filtration at high flow rates (followed by chemical analysis of the filtrate) is used to determine a size distribution.

Nevertheless, filtration may remain a useful tool for operational fractionation provided that: (i) the above-mentioned variables are well controlled in order to minimize artefacts (in particular, tests on the influence of the flow rate are recommended); and that (ii) particle size is not inferred from the pore size of the membrane used. The use of electron microscopical observations (both TEM and EM/EDS) is advised whenever an investigator suspects that a filter fractionation scheme does not deliver what was intended.

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