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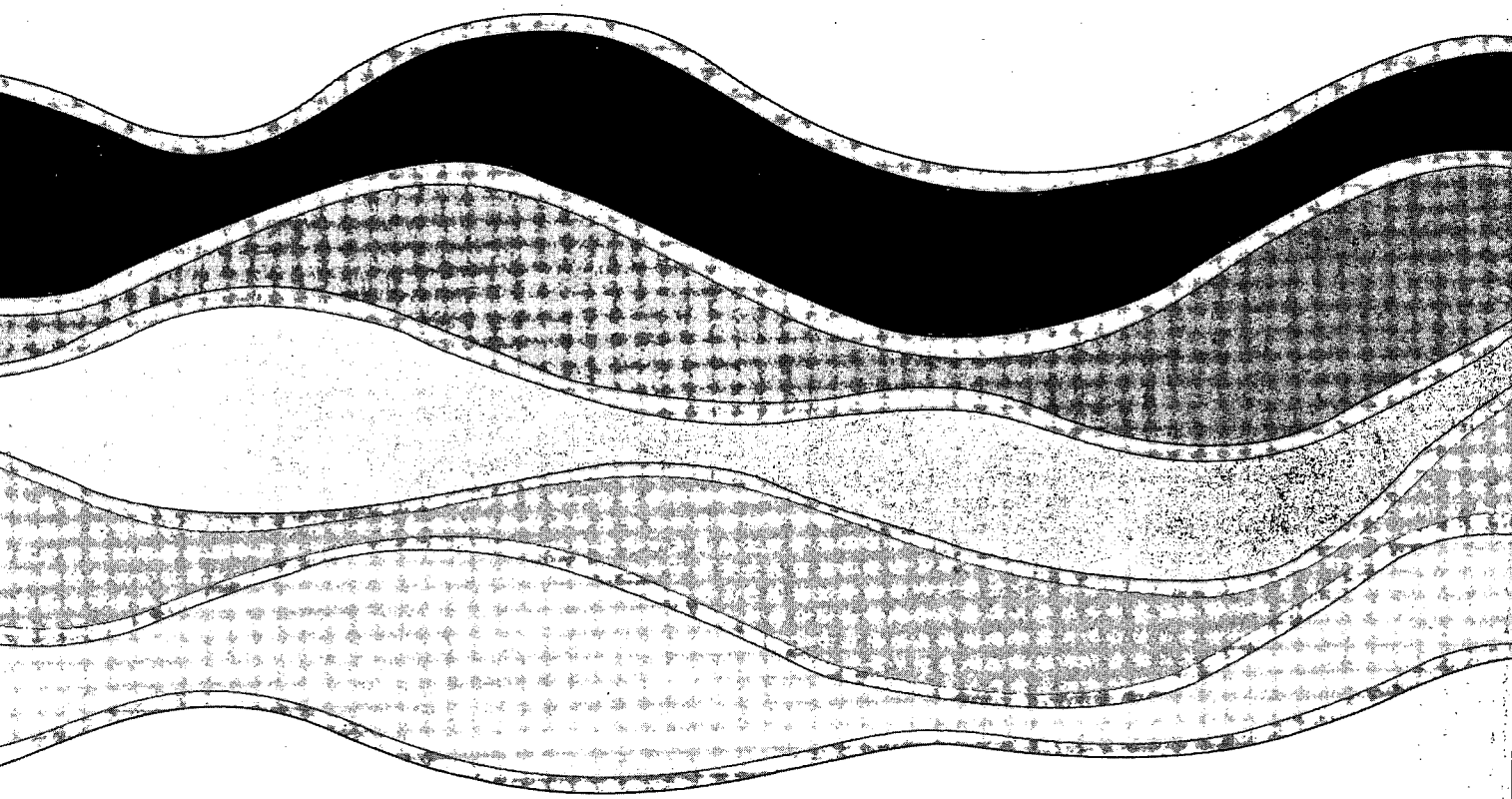

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D. Perret[1], G.G. Leppard[2], M. Müller[3], N. Belzile[1], R. De Vitre[1] and J. Buffle[1]*

[1] Department of Inorganic, Analytical, and Applied Chemistry
University of Geneva
Geneva, Switzerland

[2] Lakes Research Branch
National Water Research Institute
Burlington, Ontario, Canada L7R 4A6

[3] Laboratory for Electron Microscopy
Swiss Federal Institute of Technology
Zürich, Switzerland

* corresponding author

NWRI Contribution No. 91-27

MANAGEMENT PERSPECTIVE

This paper describes a simple and powerful procedure for the specimen preparation of aquatic colloidal particles for direct observation by Transmission Electron Microscopy (TEM) at a resolution of 0.001 μm. It is applicable to aquatic colloidal systems rich in humics, fulvics, iron oxyhydroxides, manganese oxides, polysaccharides, clay minerals, and silicates. These are the quantitatively significant colloids implicated as mediators in the biogeochemical circulation processes for contaminants, both organic and inorganic. Their characterization as natural aquatic materials is essential for an understanding of how to model contaminant dispersion and bioavailability changes. The procedure is simple and fast and applicable in the field. It can be applied so as to minimize the risk of physico-chemical transformations of fragile and unstable particles. It can also be used to record changes in colloid systems and flocs with respect to space and time, an important application for understanding colloid-mediated environmental processes.

PERSPECTIVES DE LA DIRECTION

Nous décrivons dans le présent document une méthode simple et puissante pour la préparation de spécimens de particules colloïdales aquatiques en vue de l'observation directe au microscope électronique à transmission (MET), avec une résolution de 0,001 um. Cette méthode s'applique à des systèmes colloïdaux riches en acides humiques, en acides fulviques, en oxyhydroxydes de fer, en oxydes de manganèse, en polysaccharides, en minéraux argileux et en silicates. Ce sont les colloïdes les plus nombreux qui servent de médiateurs dans le processus de circulation biogéochimique des contaminants, tant organiques qu'inorganiques. Leur caractérisation comme matériaux aquatiques naturels est essentielle pour comprendre comment modéliser les changements au niveau de la dispersion des contaminants et de la biodisponibilité. La technique est simple, rapide, et applicable sur le terrain. Elle peut être appliquée de manière à réduire le risque de transformations physico-chimiques des particules fragiles et instables. Elle peut aussi servir à enregistrer les modifications des systèmes colloïdaux et des flocs en fonction du temps et de l'espace, une application importante pour comprendre des processus environnementaux à médiation colloïdale.

ABSTRACT

This paper describes a simple and powerful procedure for the specimen preparation of aquatic colloidal particles prior to direct observation by Transmission Electron Microscopy (TEM). Four preparation schemes are described. For waters containing sufficiently large concentrations of colloids, aqueous solutions of particles are mixed with Nanoplast, a water-soluble embedding resin, and centrifuged over specimen grids placed on a horizontal disk; after curing, the resulting film has optimum quality for studies of particles at ultra-high resolution (≥ 1 nm). For waters with low concentrations of colloids, or when size fractionation is desired, particles are collected directly on a TEM grid placed at the bottom of a centrifugation tube; after deposition and curing of a film of Nanoplast over the specimen, the grid can be used directly for TEM observation. The advantages of these methods, and of two other applications of Nanoplast for aquatic and sediment particles, are compared to those of classical specimen preparation schemes. A large number of specimens may be quickly prepared in the field, using the above procedures; at the same time, most of the preparation artefacts linked to classical procedures are avoided. The new procedures should help to make TEM a semi-routine analysis method for studying the nature and behaviour of aquatic colloids.

KEY WORDS: Aquatic colloids, Transmission Electron Microscopy, Nanoplast water-soluble embedding resin, lake, river, sediment.

RÉSUMÉ

Nous décrivons dans le présent document une méthode simple et puissante pour la préparation de spécimens de particules colloïdales aquatiques avant de les observer directement au microscope électronique à transmission (MET). Quatre méthodes de préparation sont décrites. Dans le cas d'eaux contenant des concentrations assez importantes de colloïdes, des solutions aqueuses de particules sont mélangées à du Nanoplast, résine d'inclusion hydrosoluble, et centrifugées au-dessus de grilles de spécimens placés sur un disque horizontal; après séchage, le film obtenu est d'une qualité maximale pour des études de particules à ultra-haute résolution (≥ 1 nm). Dans le cas d'eaux à faibles concentrations de colloïdes, ou lorsque l'on veut un fractionnement selon la taille, les particules sont recueillies directement sur une grille de MET placée au fond du tube de centrifugation; après le dépôt et le séchage d'un film Nanoplast sur le spécimen, la grille peut être utilisée directement pour observation au MET. Les avantages de ces méthodes, et de deux autres applications du Nanoplast pour des particules aquatiques et des particules de sédiments, sont comparées à ceux des méthodes classiques de préparation des spécimens. Les méthodes décrites ci-dessus permettent de préparer rapidement un grand nombre de spécimens sur le terrain; on évite en même temps la plupart des artefacts de préparation liés aux méthodes classiques. Avec ces nouvelles techniques, le MET devrait devenir une méthode d'analyse semi-systématique pour l'étude de la nature et du comportement des colloïdes aquatiques.

MOTS-CLÉS : Colloïdes aquatiques, microscopie électronique à transmission, résine d'inclusion hydrosolubles Nanoplast, lac, rivière, sédiment.

INTRODUCTION

It is well established that organic and inorganic particles in aquatic systems, such as humics, polysaccharides, iron oxyhydroxides, manganese oxides, or clays, act as mediators in the circulation processes of vital and toxic minor organic and inorganic species (Kavanaugh and Leckie, 1980; Anderson and Rubin, 1981; Whitfield, 1981; Baccini, 1984; Salomons and Förstner, 1984; Buffle, 1988); therefore, the physico-chemical characterization of these aquatic colloids is of a primary importance to ocean, lake, sediment, and soil scientists.

Among available techniques, Electron Microscopy (EM), coupled to Energy Dispersive Spectroscopy (EDS) is a powerful tool for the determination of characteristics such as size, morphology, porosity, degree of aggregation, crystallinity, and elemental composition of single particles (Leppard et al., 1988; Buffle et al., 1989). The application of the full potential of EM necessitates the use of Transmission Electron Microscopy (TEM), since Scanning Electron Microscopy (SEM), of the conventional kind used by aquatic scientists, has a too poor resolution limit to allow the study of very fine substructures of particles down to the molecular level (Leppard et al., 1990; Perret et al., 1990). However, the classical procedures of specimen preparation for TEM observation of aquatic particles are time-consuming and, for some kinds of particles (e.g. those subject to collapse by dehydration or alteration by oxidation), may be subject to the production of artefacts.

Two major types of problems can arise when particles are isolated for characterization by TEM (Leppard et al., 1990):

- artefacts linked to sampling and sample handling, which may induce physico-chemical transformations of particles (adsorption, coagulation or redox modifications of the particles) caused by changes in pH, oxygenation and aquatic compound concentrations;

- physical artefacts (shrinkage, aggregation/disaggregation of particles) produced by the steps required specifically to prepare sections of specimen for TEM observations.

Problems related to collection and concentration of colloidal particles by filtration are discussed in detail in Buffle et al. (1988), Perret (1990), and Perret et al. (1990). They can be avoided by minimizing sample handling, performing it in the field and preparing embedded specimens on grids for TEM in the field, without any storage step. Four procedures are reported here, where sample handling is completely avoided (for solutions with high enough concentrations of particles), or highly minimized (when a preconcentration step is unavoidable).

The second type of problem can only be avoided by drastically changing the classical preparation procedures for specimen preparation. The present paper will focus on this latter aspect. Advantages and drawbacks of the existing procedures are first discussed with respect to their possible artefacts. New procedures are then proposed and exemplified in the second part of the paper.

I DISCUSSION OF THE CLASSICAL PROCEDURES

I.1 Sample Handling

Discussing this step in depth is outside the scope of this paper. It is however worthwhile to briefly illustrate the importance of the corresponding possible artefacts. Sample handling steps which are the most currently used for sample collection are as follows:

- storage for some time;
- filtration (for fractionation and/or preconcentration);

- centrifugation (for fractionation and/or preconcentration);
- freeze-drying.

Each of these steps may or may not produce artefacts, depending on the nature of the colloids present and the physical and chemical heterogeneity of these colloids. Any generalization should therefore be taken with caution. However, the little information presently available in this field suggests that: preconcentration and storage for more than a day both can produce important coagulation changes (up to at least 50%) for both inorganic (Laxen and Chandler, 1982, 1983; Perret, 1990) and organic colloids (Figure 1, and Leppard et al., 1990);

- filtration produces considerable artefacts because of coagulation at the membrane surface, unless very low flow rates (<1 cm/h) are used (Buffle et al., 1988; Perret, 1990; Perret et al., 1990);

- centrifugation is sometimes reported as producing aggregates (Salim and Cooksey, 1981); no systematic study is presently available for aquatic colloids, although the biomedical literature treats such artefacts in depth for colloidal parts of living cells;

- freeze-drying may induce drastic, irreversible aggregation of colloids (Figure 1), although it does not necessarily affect the morphology of some individual inorganic particles (e.g. iron hydroxides formed in lakes : Leppard et al., 1988), or some individual organic particles (e.g. polysaccharide-rich fibrils secreted by many lacustrine organisms: Leppard et al., 1990).

All these results suggest that sample handling must be minimized as much as possible for aquatic colloids whose literature is just now being created.

I.2 Classical Preparation Modes of Specimen Grids for TEM Observations

Figure 2 sums up some of the most commonly used preparation schemes for preparation of TEM grids of aquatic colloids. Procedures 1 and 2 originate from technology transfer from methods used for studying biological structures. Procedure 3 has recently been proposed (Nomizu and Mizuike, 1986; Nomizu et al., 1987, 1988) for the specific study of aquatic colloids. Procedures 4 to 7, summarized in Figure 3, make use of a new hydrophilic resin (Nanoplast), and are proposed and discussed in detail in this paper.

I.2.1 Embedding of particles in hydrophobic resins (Figure 2; routes 1 and 2)

Biological techniques of sample preparation were developed originally in response to the need to:

- stabilize cells for examination of specific features under conditions of high vacuum required in the TEM;
- reduce the thickness of specimen to permit an optimal transmission of the electron beam.

These techniques led to the use of chemical fixatives, and to the embedding of the fixed material in hard resins, permitting ultrathin sectioning. The drawback of the embedding step is that, until recently, the best resins were hydrophobic, thus requiring lengthy and perturbing extra preparation steps to dehydrate the studied material. Major artefacts are the redistribution of some mobile structural subunits, the shrinkage, the rupture and the distortion of some

delicate structures, and the misleading aggregation of subunits (see Causton (1985) and Leppard et al. (1990) for more details).

Biological dehydration procedure has been applied to aquatic colloids, either after sequential chemical fixation (Massalski and Leppard, 1979; Burnison and Leppard, 1983), or directly without fixation (Leppard et al., 1986). The dehydration artefacts, however, are potentially more difficult to minimize and interpret when investigating highly hydrated aquatic colloidal gels than with rigid and/or previously well-characterized biological components (Lima-de-Faria, 1969), and several additional drawbacks exist:

- the method is time consuming and not applicable in situ as required by considerations of section I.1;

- the use of a large number of reagents and handling steps makes them a source of potential contamination of the specimen by foreign particles;

- some of the organic part of the colloids may be sensitive to the solvents used for dehydration and to the resin used for embedding (Causton, 1985).

Another drawback of the classical embedding approach is that particles are embedded into molds or capsules, to derive hard blocks from which ultrathin sections (ca. 50 nm thickness) have to be prepared by ultramicrotomy before they can be examined by TEM. This slicing step, usually done with a diamond knife, is very much time consuming since days or weeks may be necessary before obtaining representative sections. In addition, the sectioning procedure can produce local physical artefacts on the surrounding matrix for inorganic particles, caused by differences of hardness between resin and particles; these artefacts, in which

sectioning becomes scraping, can be of importance when dealing with ultra-fine substructures close to the nanometer level.

I.2.2 Particle collection by centrifugation, and observation without resin (Figure 2; route 3)

This procedure has been proposed recently by Nomizu and Mizuike (1986) and Nomizu et al. (1987, 1988) as an alternative route to the embedding of non-living aquatic particles into resins. Aqueous samples are centrifuged in conventional tubes on the bottom of which specimen grids have been positioned; depending on the force and time of centrifugation, the method allows a quantitative recovery of submicron particles onto the grids. Although it is an important improvement compared to routes 1 and 2, there are three limitations to the use of this procedure for fragile particles collected in the field:

- ultracentrifugation, necessary to collect the smallest particles (< 10 nm), cannot be performed readily in the field;

- the method of Nomizu and Mizuike (1986) uses grids covered simply by a carbon film; this film is delicate and can be strongly damaged during centrifugation;

- after centrifugation, grids are recovered and air-dried; this step can be a source of important structural modifications, similar to or even much more important than those occurring during the dehydration steps performed before the embedding of particles in hydrophobic resins.

II PREPARATION OF GRIDS BASED ON THE HYDROPHILIC NANOPLAST RESIN

Nanoplast FB101 is a melamine resin (Bachhuber and Frosch, 1983; Frösch and Westphal, 1989), which has hydrophilic properties. During the polymerization/desiccation step, water is produced, which evaporates slowly, together with the bulk water of the sample; thus the polymer slowly replaces the bulk water, and perhaps later the water of hydration, to keep the studied structure unaltered with respect to dehydration-induced shrinkage. Thanks to these properties, aquatic particles can be embedded in Nanoplast without a prior dehydration step. Furthermore, the simplicity of this embedding step makes it usable in the field, allowing one to prepare easily a large number of directly observable grids. Four procedures are presented below and summarized in Figure 3.

II.1 Direct Embedding of the Water Sample on a Grid of Nanoplast Preparation (Figure 3; route 7)

When the particle concentration of the water sample is large enough (e.g. $>10^8$part/mL for particle diameter = 100 nm, or $>10^{11}$ part/mL for particle diameter = 10 nm) and no preliminary fractionation is desired, the water sample may be directly mixed with Nanoplast, and the mixture deposited on a TEM grid as described below. The whole procedure includes three steps:

II.1.1 Preparation of grids

This step is the classical preliminary preparation of grids, prior to further deposition of the Nanoplast film. It must be done in the laboratory, but a large

number of grids may be prepared in advance and stored for months. Metallic grids (Cu, Ni, Au) are covered with a thin film of collodion (10-50 nm thickness), followed by a thin carbon film (5-10 nm thickness for a good transparency). The latter allows a good thermal and electrical conduction of electrons under the beam of the microscope, while the collodion film serves as a very planar support for the carbon film. This classical preparation procedure (Meek, 1976; Möldner, 1980) is briefly described below:

- A clean glass slide is dipped into a solution of 0.5% collodion (cellulose nitrate; Elmis) in amylacetate (isopentylacetate; Merck) and then allowed to dry vertically in a dust free atmosphere; slide sides are grazed with a blade and the slide is carefully placed with tweezers at the surface of clean bidistilled water contained in a large dish. When contact between the slide and water is established, the slide is gently dipped so that the thin collodion film peels off and floats at the water surface; 200-mesh copper grids (Plano; washed in chloroform) are then gently placed at the surface of the film; finally, film and grids are recovered with a cardboard (grids being taken in sandwich between cardboard and adhering film), and allowed to dry. The cardboard-grid-collodion set is then placed in a vacuum coater (Edwards E306) equipped with carbon rods which are evaporated and sprayed over the grids under a vacuum of 5.10^{-5} Torr at least. These grids may be stored for months.

II.1.2 Preparation of specimen grids in the field

- Melamine resin (Nanoplast FB101; Rolf Bachhuber) is freshly prepared by mixing a catalyst into the monomer solution, since polymerization starts slowly but immediately after mixing. 0.025 g of

p-toluolsulfonic acid (catalyst B52) is mixed with 1.0 g of hexamethylol-melamine-methyl-ether (monomer MME7002); Nanoplast:sample mixtures are prepared by gently mixing 1 part of resin with 10 parts (v:v) of aqueous sample containing the particles for study;

- less than 5 μL of the mixture are then pipetted onto the surface of a specimen grid placed on the horizontal disk of the microcentrifuge shown in Figure 4 (the cardboard-grid-collodion-carbon set sticks to the horizontal disk with double-sided tape); after a waiting time of 30 s, the grid is centrifuged for 10 s at 7000 rpm and then removed from the disk (Yaffee, 1988; Perret, 1990).

II.1.3 Polymerization and hardening of the resin

Prior to TEM examination, the film of resin containing particles has to polymerize evenly in order to avoid tearing; the curing steps are achieved the following way:

- the grids are placed in a desiccator without vacuum for 12 h at 40°C, then in an oven for 12 h at 60°C and finally for 12 h at 80°C;

- collodion film can be dissolved by placing the grids in a Petri dish containing amylacetate for 24 to 48 h. However, this dissolution step is only necessary for high resolution observations; for studying particles > 10 nm, collodion does not need to be removed.

II.2 Combining Fractionation/Preconcentration by Conventional Centrifugation, to Direct Preparation of Grids (Figure 3; route 6)

When particle concentration in the water is too low, the method described by Nomizu and Mizuike (1986) can be used to concentrate these particles directly on grids positioned at the bottom of centrifugation tubes. Centrifugation may also be used for a solution sufficiently concentrated, but to which it is desirable to apply a fractionation based on size and density. In these cases, field preparation of grids is possible only for studying particles which can be collected with transportable centrifuges (roughly for particle size >100 nm, assuming density = 2 g/cm^3, and maximum relative centrifugal field = 4000 g).

The use of Nanoplast in these cases is recommended for three reasons:

- grids may be coated with a Nanoplast film before centrifugation. The hard resin then protects the carbon film, which otherwise is easily broken;

- after centrifugation, grids are further coated with a Nanoplast film, which avoids losing particles during the transport of grids;

- finally and most importantly, the last protective Nanoplast film allows dehydration of the collected particles to occur smoothly in a stabilizing matrix, without artefact, as in the case of section II.1.

The two protective Nanoplast films are prepared according to the same procedure as the one described in section II.1.

II.3 Embedding of Aquatic Particles in Molds (Figure 3; route 4)

Embedding of lacustrine samples containing iron oxyhydroxy-phosphate colloids formed in situ (Buffle et al., 1989), or of pieces of membranes used for studying the behaviour of iron colloids during filtration (Perret, 1990), have been done in molds of Nanoplast. After curing, the molds of solid Nanoplast must then be sliced by ultramicrotomy. As mentioned above, this is much more time consuming than the direct preparation of grids (section II.1), and requires exceptional skill by the technician in the use of the ultramicrotome. However it may be a useful approach for studying solid material. In these cases, the procedure is as follows (Leppard et al., 1988, 1989): the sample (50 μL of water or a piece of solid material of interest) is mixed with 150 μL of a fresh Nanoplast solution (1.0 g monomer MME7002 + 0.02 g catalyst B52) in a resin mold; the resin is then allowed to polymerize and harden (2 days in a desiccator without vacuum at 40°C, then 2 days outside the desiccator at 60°) prior to ultramicrotomy.

II.4 TEM Observations of Sediment Particles Deposited on Teflon Plates (Figure 3; route 5)

Iron and manganese oxyhydroxide particles formed in lacustrine sediments have been separated and collected using Teflon plates (Belzile et al., 1989) inserted directly into the sediment. The oxyhydroxide particles are deposited on the surface of the Teflon as two distinct layers, the Mn layer above the Fe layer in agreement with thermodynamic considerations. Preparation for TEM is done by depositing 100-500 μL of Nanoplast (10 g monomer MME7002 + 0.2 g catalyst B52) using a micropipette on the surface of the Teflon covered with either Mn or Fe particles, in order to form a film of ca. 1 mm thickness. This was done directly in the field some 5-10 min after removing the Teflon plates from the sediments. Curing of the Nanoplast was initiated within 4 h using a portable oven set at 40°C; further

hardening of the resin was done as above in the laboratory. Once the curing of the Nanoplast resin is complete, the films, along with the particles, may be readily peeled off the Teflon surface by gently bending the Teflon; the films are then reembedded in a conventional epoxy resin in molds, in order to allow ultrathin (ca. 50 nm) sections to be prepared by ultramicrotomy for observation at 1 nm resolution.

III RESULTS AND DISCUSSION

III.1 Examples of Applications

This section gives a few examples of pictures of aquatic particles obtained with the various procedures described above. Their purpose is only to illustrate the possible application of Nanoplast in this field. Detailed results have been reported elsewhere.

Figure 5 gives the pictures of material collected on Teflon plates inserted at the sediment-water interface of two different lakes, and processed according to the procedure of section II.4. Figure 5a shows iron and manganese oxyhydroxides from an oxic lake (Brady Lake, Ontario, Canada; pH=6.9; Belzile et al., 1989; De Vitre et al., 1989). Figure 5b shows iron oxyhydroxide material collected in the anoxic layer of a eutrophic lake (Lac de Bret, Vaud, Switzerland; pH=7.5); spherical iron oxyhydroxide globules present on this picture are very similar to those collected in the water column (see Figure 6).

Figure 6 shows particles of iron oxyhydroxy-phosphate formed in a eutrophic lake (Lac de Bret, Vaud, Switzerland). Figure 6a was obtained according to the procedure of section II.3 (embedding of particles in molds), and shows large size globules. Figures 6b and 6c were obtained by directly embedding water samples in Nanoplast on grids (procedure of section II.1); they show small size granules (dark

particles) associated to organic matter (light gray small particles), probably mostly pedogenic fulvic compounds.

Figure 7 shows natural river particles (River Rhine, Switzerland) collected directly on a grid by centrifugation according to the procedure of section II.2. Figure 7a was obtained from a raw, untreated sample, and shows a wide range of particles and aggregates. After a 2.5 h centrifugation of the raw sample at 4000 g to eliminate large and dense particles, the supernatant was centrifuged 5 h at 140000 g over a grid, in order to obtain Figure 7b, which shows uniformly dispersed small particles.

III.2 Discussion

During polymerization, the water soluble hexamethylol-melamine-methyl ether monomers penetrate the porous substructures of particles present in the samples, and polycondensate with each other, resulting in a macromolecular matrix which is less and less soluble in water as polymerization proceeds. The initial polymerization step is crucial in obtaining a finely and homogeneously dispersed resin which completely embeds particles; this is achieved thanks to the optimum Nanoplast:water sample ratio, and to the slow elimination process of the water by evaporation. It must be emphasized that polycondensation produces up to 6 molecules of water per monomer, which is an additional favorable factor for avoiding denaturation of hydrophilic structures.

For the preparation of specimen by films centrifuged on grids (section II.1), local inhomogeneities in the resulting film create folds and fractures. Such damaged surfaces must be avoided as much as possible, as inorganic particles tend to accumulate at fracture interfaces, thereby leading to artefactual coagulation processes. Several Nanoplast:water sample ratios have been investigated, in the

range 1:3 to 1:15; it has been found that 1:10 (v:v) is an optimum ratio for the preparation of ultrathin films showing few defects (Figure 8a). Most films produced with a higher proportion of resin tend to reticulate, fold and fracture (Figure 8b). Lower Nanoplast:water sample ratios produce very fragile films in which particles are poorly embedded.

After polymerization of the film, observation of a large number of grids under a classical light microscope shows that the ones prepared with the optimum ratio have more than 70% of their surface free of physical damage. It must be emphasized that a ratio of 1 Nanoplast:10 water sample results in an effective preconcentration of particles by a factor of 10, which is a much favorable factor in most natural waters where particle concentration is often low.

Figures 5a to 8a show that the grain of the Nanoplast resin is extremely fine, less than one nanometer, and that no physical interaction appears at the interface between inorganic objects and resin. Furthermore, iron granules present in Figures 6b and 6c are associated to weakly electron opaque clouds of natural organic material. Such loose associations are often perturbed by conventional dehydration steps. A number of other pictures with physical structures of fulvics, polysaccharides, fibrils, and iron hydroxide particles, as well as the aggregation of the latter with themselves, bacteria, clays, amorphous silica, and organic debris, have also been obtained by the procedures described above, showing that physical and aggregate structures are well preserved.

In addition, the comparison of the above procedures with several classical preparation schemes have shown that the former do not produce extra coagulation artefacts with natural systems (Leppard et al., 1988). Although a systematic study of preservation of physical structure with model systems still needs to be done, a large number of specimens have been analysed until now in our

laboratory with many different types of aquatic samples, which all suggest that the above techniques are non perturbing.

The actual thickness of films prepared by horizontal centrifugation (sections II.1 and II.2) has not yet been measured; however, numerous TEM observations of Nanoplast sections and films indicate that the latter have thicknesses comparable to the ones obtained for ultrathin sections (less than 100nm; see Figures 6a, 6b, and 6c). Horizontal centrifugation speed can be adapted to modify the thickness of films. With the speed used here (ca. 7000 rpm), particles on the grid are subjected to less than 200 g; however, it has been observed that lower speeds may produce inhomogeneous films, whereas much higher speeds would produce fragile films.

IV CONCLUSION

The use of Nanoplast in conjunction with mild in situ procedures for the minimally perturbing preparation of non-living TEM specimens results in films of colloids with no or much fewer artefacts than when these colloid specimens are prepared by classical methods using hydrophobic resins and ultramicrotomy. The high quality of Nanoplast films, together with the absence of interaction between inorganic or organic colloids and the melamine resin, makes these procedures a promising route to ultrastructural analysis of aquatic samples. Although they have been largely applied to fresh water systems (water and sediments), the proposed procedures could probably require some modification before being applied to marine samples, because micro-crystallization of salts may occur during polymerization of the Nanoplast resin, due to the high ionic strength.

Nanoplast allows preparation of sections thinner (down to 10 nm) than classical hydrophobic resins (Frösch and Westphal, 1985), and presents very smooth

surfaces after sectioning, together with a very fine grain (<1 nm) (Frösch et al., 1985). This permits studies of ultrasmall particles; moreover, the methods proposed here are simple and fast, and are directly applicable in the field. All these conditions minimize the risk of physico-chemical transformations of fragile and sensitive particles, and allow semi-routine analysis in order to record in detail possible changes of colloid nature and morphology with respect to space and time. This last aspect is extremely important for understanding environmental processes in details.

Thanks to the numerous advantages of the techniques proposed here, it may be expected that important progress will be possible in the field of aquatic colloids, in the near future, in particular for:

- the development of a well documented ultrastructural literature on the nature and morphology of aquatic organic and inorganic colloids;
- detailed studies of natural aggregate morphologies, and formation processes, with discrimination between reality and artefacts;
- high resolution analysis of individual very small particles with a better definition of the limit between "amorphous" and "crystalline" particles;
- a better understanding of the nature of associations between inorganic and organic colloids.

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FIGURE CAPTIONS

Figure 1: Three different views of ultrathin sections of organic fibrils embedded in epoxy resin, showing degree of aggregation in relation to the preparation of specimen. (a) Fibrils gently centrifuged from water. (b) Fibrils gently centrifuged from water, freeze-dried, stored, rehydrated and then gently centrifuged again, to test the effect of the freeze-drying step. (c) Natural fibril gel organized into a biofilm by the organisms which secreted the fibrils into water; this natural aggregate presents an intermediate state of fibril aggregation.

Figure 2: Overview of methods for specimen preparation for TEM of natural aquatic particles. Schemes 1 and 2 are discussed in detail in Burnison and Leppard (1983), Leppard et al. (1986), and Leppard et al. (1988); scheme 3 is discussed in Nomizu and Mizuike, (1986) and Nomizu et al. (1987, 1988); schemes 4 to 7 make use of the hydrophilic Nanoplast resin and are described in this paper (see also Figure 3).

Figure 3: Four different applications of Nanoplast embedding of natural aquatic particles. Scheme 4 is discussed in detail in Leppard et al. (1988) and Buffle et al. (1989); scheme 5 has been used in Belzile et al. (1989) and De Vitre et al. (1989), and scheme 7 in Perret (1990). Detailed procedures of schemes 5, 6, and 7 are described in this paper.

Figure 4: Home-made microcentrifuge for horizontal centrifugation of Nanoplast:aqueous sample mixtures; (1) horizontal centrifugation disk, (2) removable aluminium protection ring, (3) battery operated motor with adjustable speed, (4) on/off switch, (5) 12V(DC) power supply.

Figure 5: TEM of material collected on Teflon plates vertically inserted in lacustrine sediments, then embedded into Nanoplast after recovery; before ultramicrotomy, the layer of Nanoplast was reembedded in epoxy resin. T he upper part of each picture represents the water side, and the lower part represents the Teflon side. (a) The dark area reveals fibril-like crystals and amorphous granular material of the overlap zone between iron and manganese oxyhydroxide bands in the sediment of an oxic lake. (b) Iron oxyhydroxides (some of which are spherical) collected in the anoxic sediment of a eutrophic lake.

Figure 6: TEM of natural particles of iron oxyhydroxy-phosphate formed in a eutrophic lake (see Leppard et al. (1988), Buffle et al. (1989), and Leppard et al. (1989) for more details). (a) Ultrathin section of Nanoplast showing large size globules (ca. 100 nm) of iron oxyhydroxy-phosphate. (b) and (c) Very tiny iron-rich granules homogeneously dispersed in the Nanoplast film obtained by direct embedding of the water sample on the grid; the granules are surrounded by natural organic material (pedogenic fulvics), probably acting as a binder for the granules. A globule (arrow) is also present in (c), indicating that the procedure described in section II.1 allows the recovery of small to large size iron oxyhydroxy-phosphate particles.

Figure 7: TEM of Nanoplast films containing river particles. (a) Raw sample directly centrifuged over a grid placed at the bottom of a conventional centrifuge tube. (b) The sample, after centrifugation to eliminate large and dense particles, was centrifuged over a grid placed at the bottom of a conventional centrifuge tube. After centrifugation, both grids were coated with a protective Nanoplast film.

Figure 8: (a) TEM of horse spleen ferritin (Sigma Chemicals) obtained by horizontal centrifugation of Nanoplast:sample mixture under optimum conditions (see text); arrows point to negligible defects in the film. (b) TEM of a Nanoplast film obtained by centrifugation of a 1:3 Nanoplast:sample mixture; under these conditions, the film is reticulated and presents fractures.

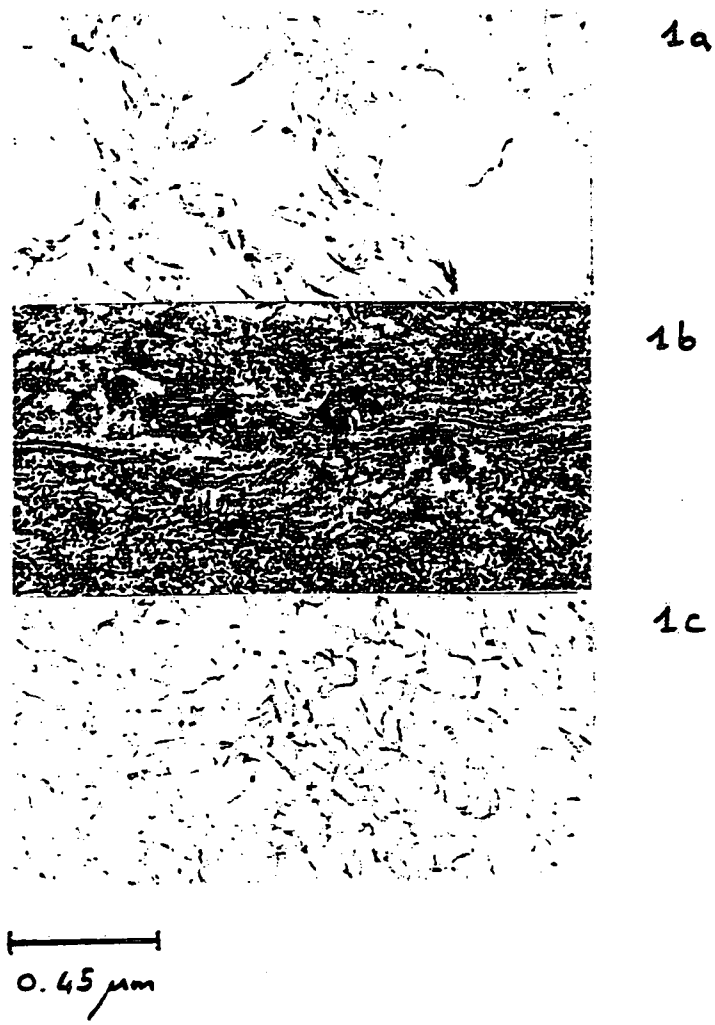


FIGURE 1

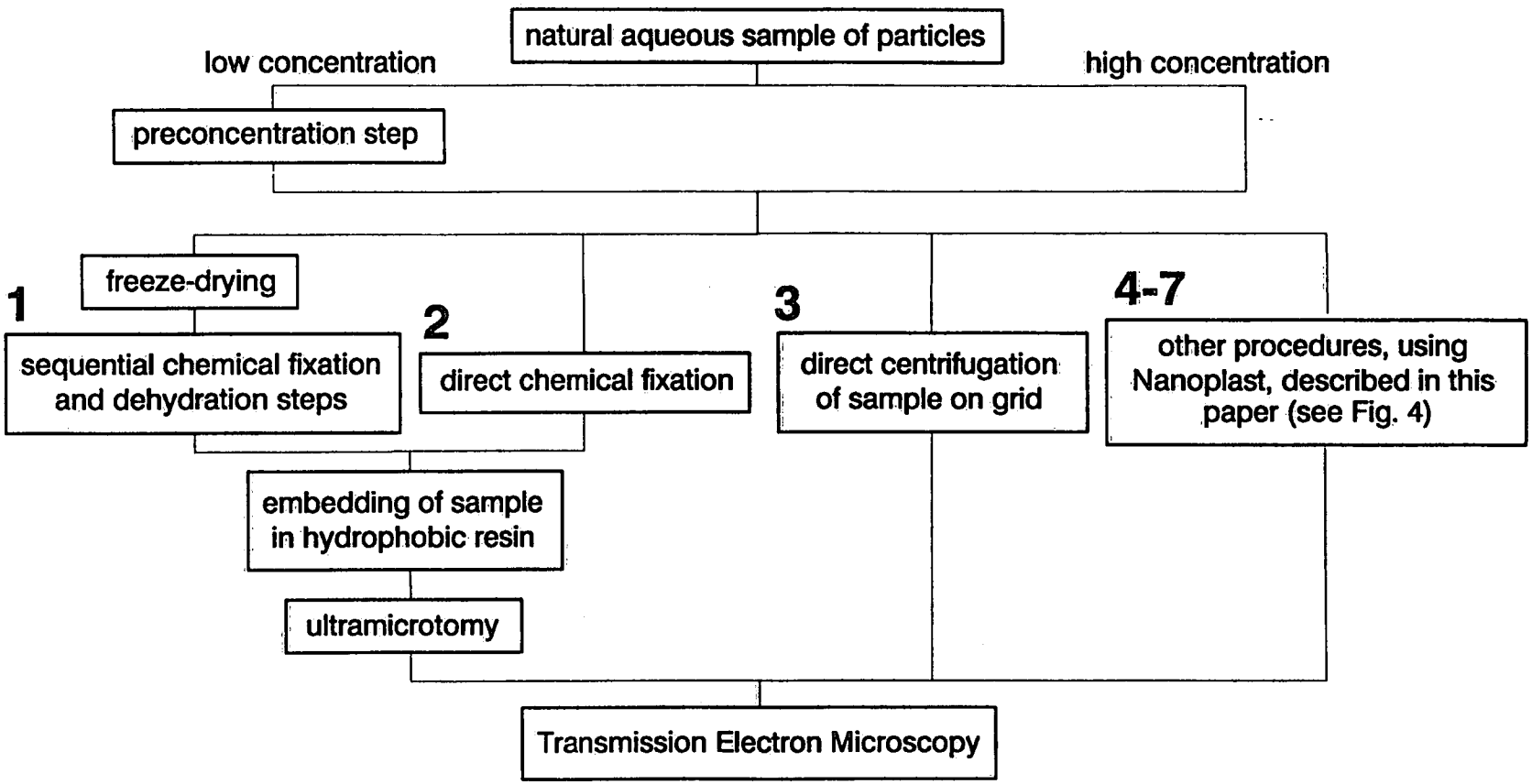

natural aqueous sample of particles
low concentration
high concentration
preconcentration step
freeze-drying
1
sequential chemical fixation and dehydration steps
2
direct chemical fixation
3
direct centrifugation of sample on grid
4-7
other procedures, using Nanoplast, described in this paper (see Fig. 4)
embedding of sample in hydrophobic resin
ultramicrotomy
Transmission Electron Microscopy

FIGURE 2

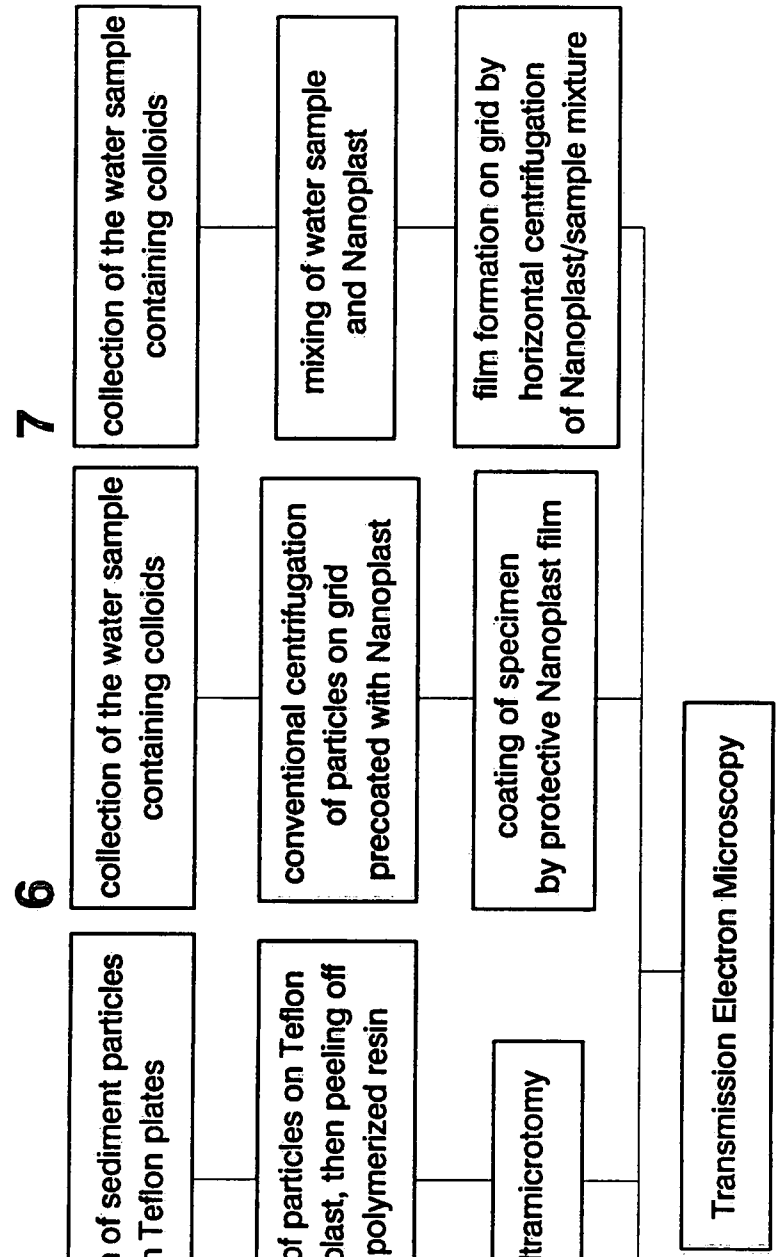

4
conventional sampling of aqueous samples
conventional embedding of aqueous samples in Nanoplast capsules
ultramicrotomy
5
collection of sediment particles on Teflon plates
coating of particles on Teflon by Nanoplast, then peeling off of the polymerized resin
ultramicrotomy
6
collection of the water sample containing colloids
conventional centrifugation of particles on grid precoated with Nanoplast
coating of specimen by protective Nanoplast film
7
collection of the water sample containing colloids
mixing of water sample and Nanoplast
film formation on grid by horizontal centrifugation of Nanoplast/sample mixture
Transmission Electron Microscopy

FIGURE 3

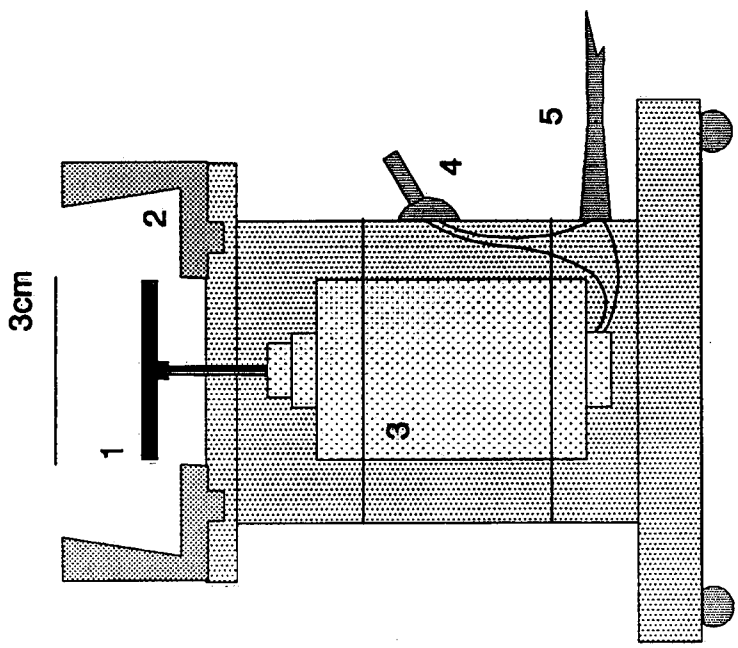


FIGURE 4

FIGURE 5

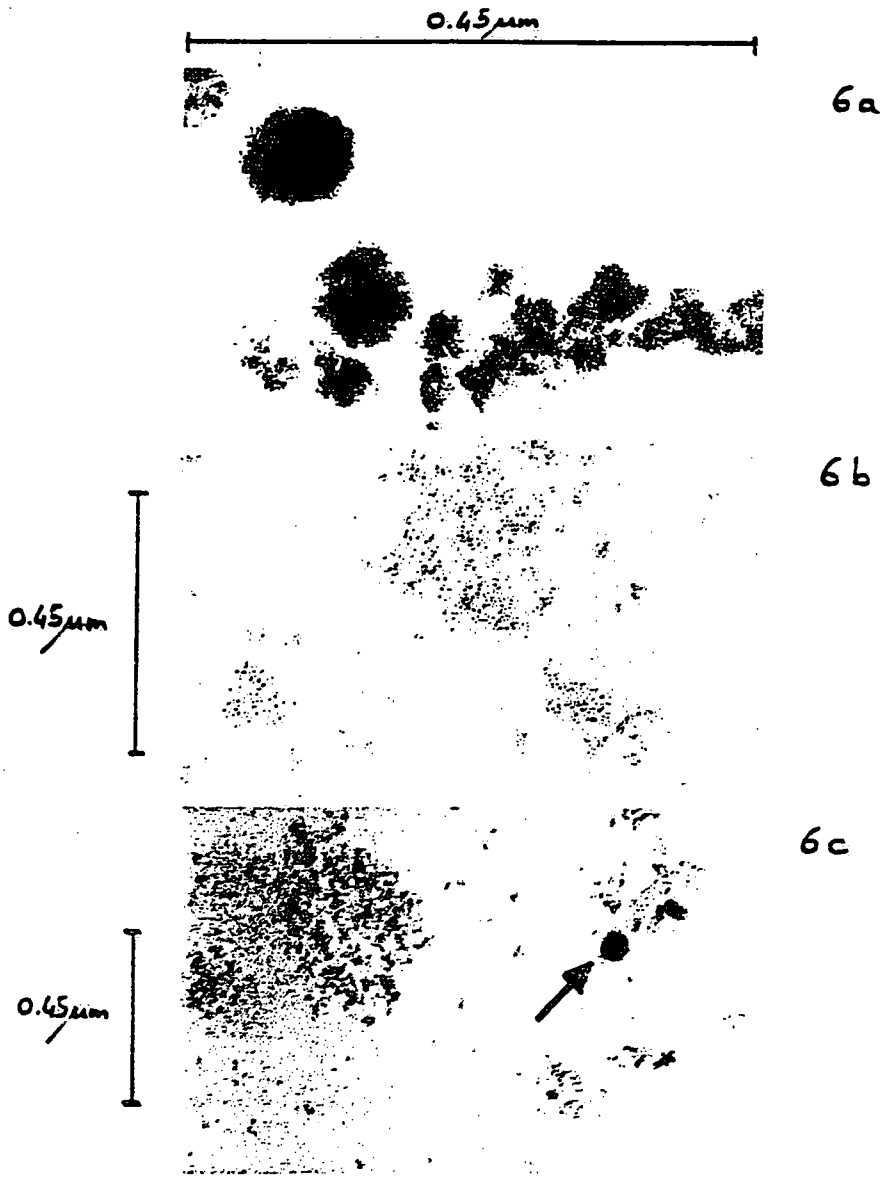


FIGURE 6

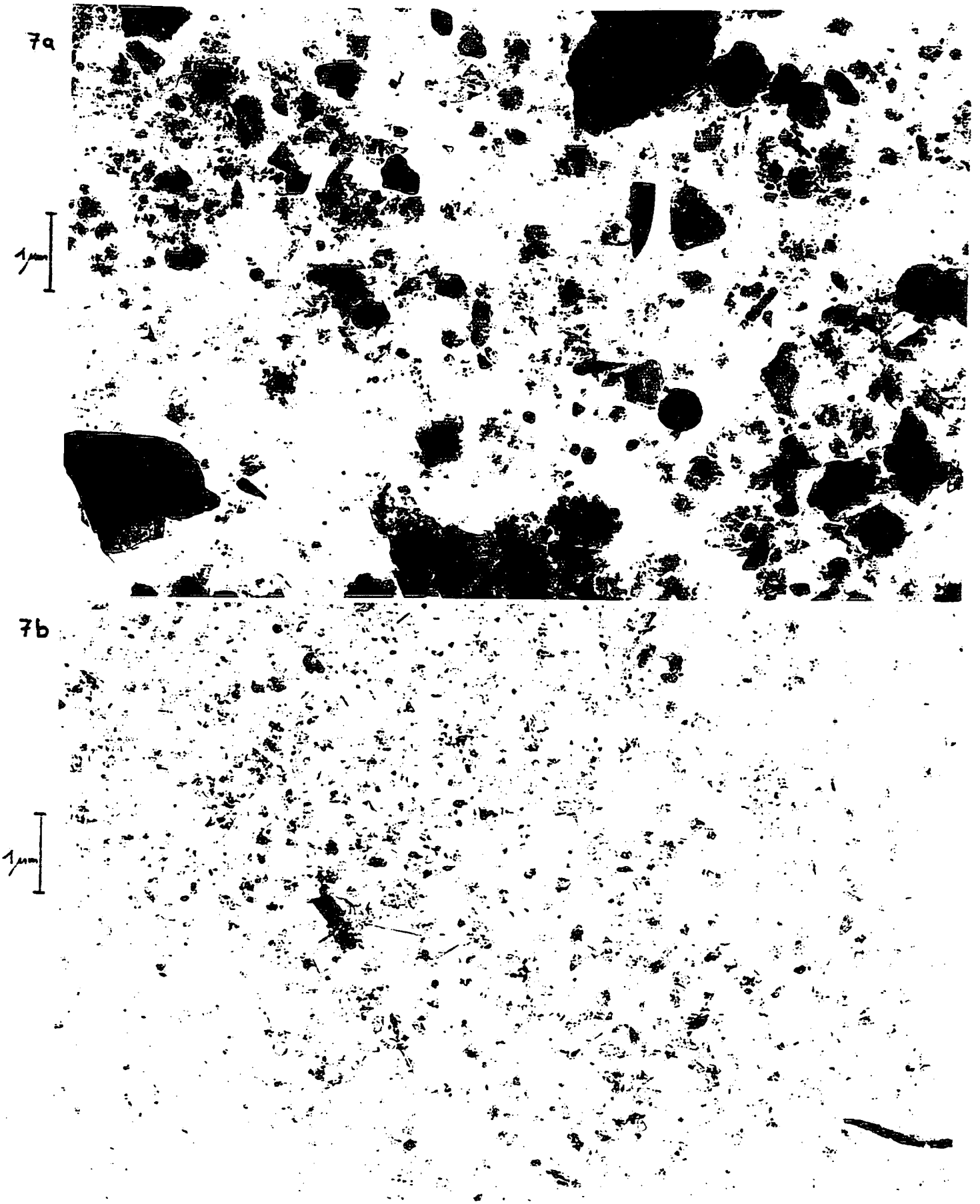


FIGURE 7

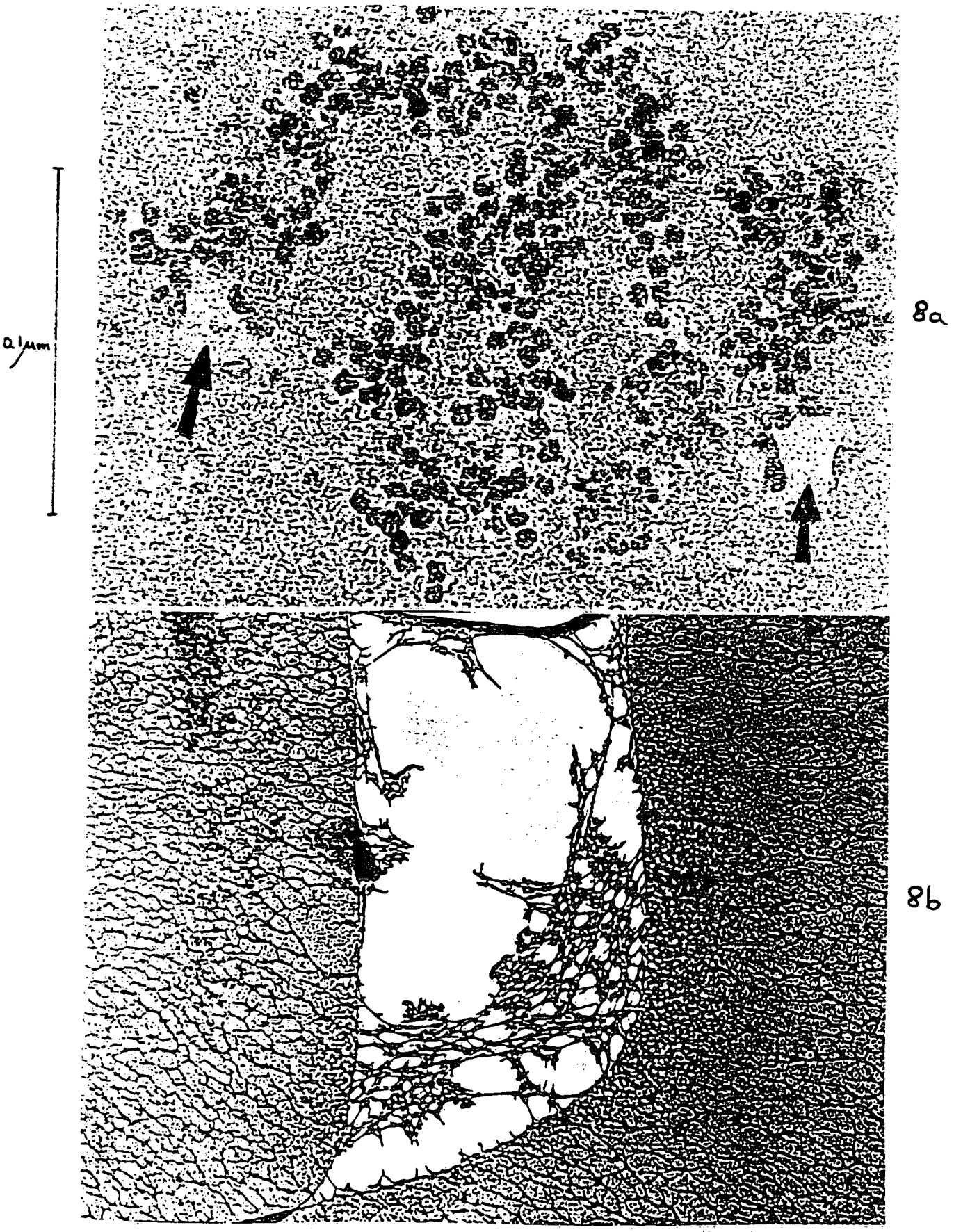
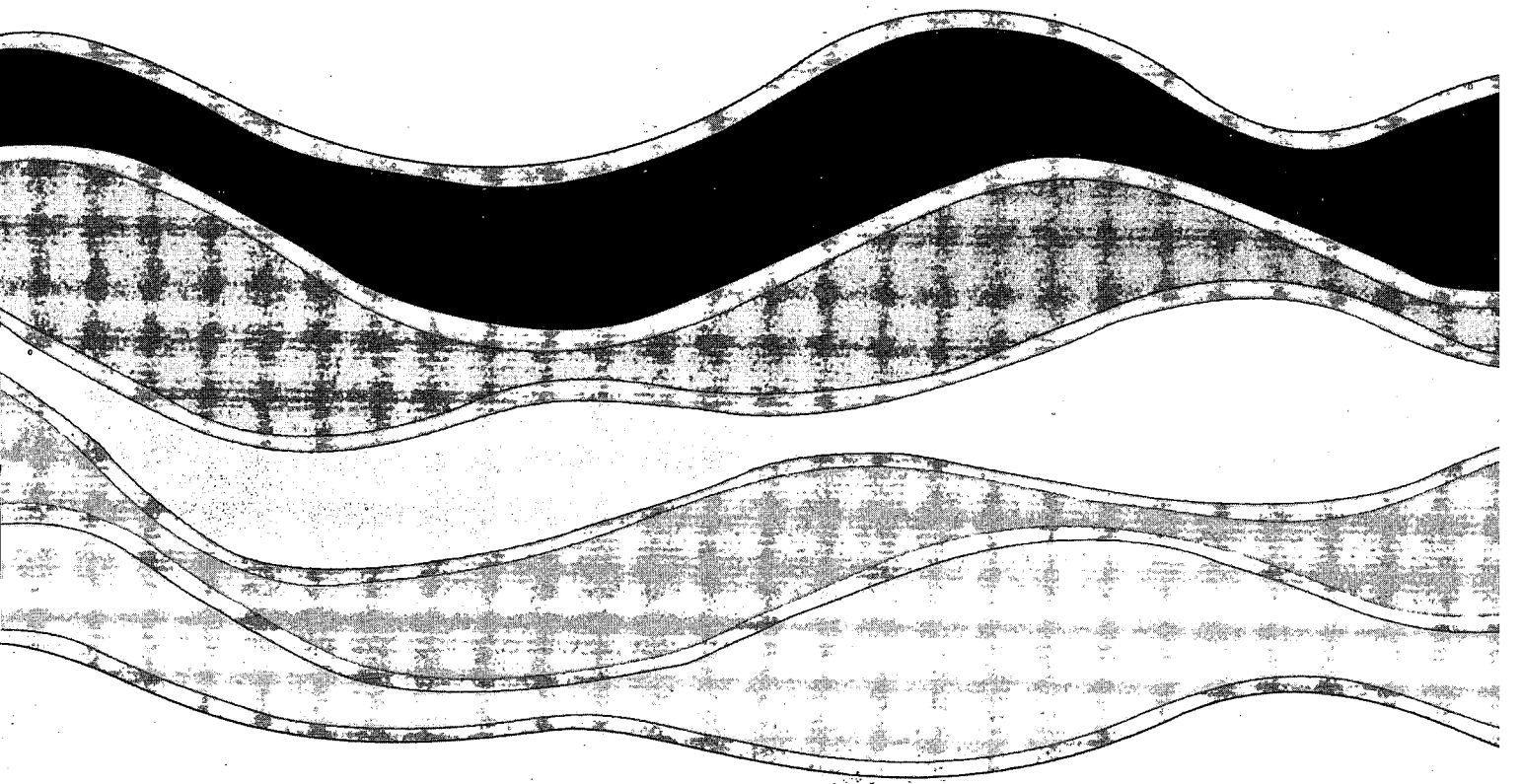


FIGURE 8



NATIONAL WATER RESEARCH INSTITUTE
P.O. BOX 5050, BURLINGTON, ONTARIO L7R 4A6

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INSTITUT NATIONAL DE RECHERCHE SUR LES EAUX
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