Analysis of environmental particles by atomic force microscopy, scanning and transmission electron microscopy

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Abstract Due to their large specific surface and their abundance, micro and nano particles play an important role in the transport of micropollutants in the environment. Natural particles are usually composed of a mixture of inorganic amorphous or crystalline material (mainly FeOOH, Fe₂O₃, Mn₂O₃ and clays) and organic material (humics and polysaccharides). They all tend to occur as very small particles (1–1,000 nm in diameter). Most natural amorphous particles are unstable and tend to transform with time towards more crystalline forms, either by aging or possibly, by dissolution and re-crystallization. Such transformations affect the fate of sorbed micropollutants and the scavenging properties are therefore changed. As these entities are sensitive to dehydration (aggregation, changes in the morphology), it is highly important to observe their morphology in their natural environment and understand their composition at the scale of the individual particles. Also for the understanding and optimization of water treatment technologies, the knowledge of the occurrence and behavior of nano-particles is of high importance.

Some of the possible particle analysis methods are presented: aggregation processes, biomineralization, bacterial adhesion, biofilms in freshwaters, ferrihydrite as heavy metals remover from storm water. These examples demonstrate the capabilities and focus of the microscopes. Atomic Force Microscopy (AFM) allows to analyze the particles in their own environment, meaning in air or in the water. As well, forces of interactions between particles or between particles and other surfaces such as membranes will be highly valuable data. Scanning Electron Microscopy (SEM) and for higher lateral resolution, Transmission Electron Microscopy (TEM) allow measurement of the morphology and composition. Especially, TEM coupled with Electron Energy Loss Spectroscopy (TEM-EELS) is a powerful technique for elemental analysis. Finally, general guidelines for the effective use of microscopic techniques are provided.

Keywords AFM; aggregation; bacteria; colloids; composition; dissolution; morphology; particle; SEM; TEM

Introduction

There is an increasing demand in environmental science for adequate analytical tools to identify submicrometre range particles and related issues such as aggregation processes, dissolution, micro-pollutant sorption and so forth. This need arises from considerations given below, and requires that conventional speciation schemes to be relayed by microscopic methods able to probe species at the level of the individual particles (Bancroft and Hyland, 1990; Hug et al., 1997; Xhoffer et al., 1992).

The present article shows examples and gives recommendations on the use of microscopic techniques to understand and analyze the various aspects and role of nano-particles in the environment and in water treatment. Since analytical tools have been developed for the study of nanometric objects and adapted to the highly heterogeneous suspensions of natural waters, micro and nano-particles can be analyzed in terms of their composition, crystallinity and morphology at their individual scale (Mavrocordatos et al., 1994). Amongst the microscopic techniques available, Scanning and Transmission Electron Microscopy (SEM and TEM) allow to observe particles from millimetre down to the sub-nanometre size. Due to the nature of the light in SEM and TEM, qualitative and quantitative
information on the composition can be obtained either by using fluorescence or absorption. The x-ray induced by the interaction of the electron and the specimen is observed by Energy Dispersive Spectroscopy (EDS) and permits a clear determination of the composition for elements heavier than oxygen (varies with detector). EDS gives a highly reliable signal for qualitative measurements but quantitative analysis generally leads to an error of approximately 20%. The complementary technique, which is only available in the TEM, is based on the absorption signal. Electron Energy Loss Spectroscopy (EELS) detects the loss of energy of the incident electron through the specimen. Thus, elements can be discriminated. Like EDS, EELS permits qualitative analysis even for light elements. Absolute quantification by EELS can be as accurate as 10% (Egerton, 1996). With respect to electron-specimen interactions, diffraction (Selected Area Electron Diffraction, SAED) is also measurable in the TEM and gives information on the crystalline properties (Mavrocordatos and Fortin, 2002). Thus, TEM and SEM as well as EDS, EELS and SAED are complementary techniques. However, all of these techniques require high vacuum and difficult specimen preparation.

Atomic Force Microscopy (AFM) is the ideal complementary tool for the morphological aspect. Indeed, AFM allows observation of particles in their natural environment with atomic resolution. Also, this technique gives true 3-dimensional information even in water. For instance, ionic force and acidity are controlled during imaging and the original morphology can be preserved. As opposed to electron microscopy, specimen preparation is less constringent.

Due to their large specific surface and their abundance, micro and nano particles play an important role in the transport of micropollutants in the environment. Natural particles in freshwaters are usually composed of a mixture of inorganic amorphous or crystalline material (mainly FeOOH, Fe₂O₃, Mn₃O₄ and clays) and organic material (humics and polysaccharides). They all tend to occur as very small particles (1–1,000 nm in diameter). Additionally, particles formed by combustion processes and spread out in the atmosphere or produced by erosion of natural and synthetic material can be found in surface waters as well as in road run-off.

Synthetic materials can undergo aging pathways such as dissolution of aggregation with other suspended particles. Most natural amorphous particles are unstable and tend to transform with time towards more crystalline forms, either by aging or possibly, by dissolution and re-crystallization. Such transformations affect their fate and the fate of the associated pollutants.

The concentration, residence time, bioavailability and fate of vital and toxic trace elements in ecosystems are mainly governed by their interactions with solid surfaces, such as suspended material under a wide range of environmental conditions (Buffle and van Leeuwen, 1992). Trapping of trace elements leads to their preconcentration onto/into solid phases as compared to the solution, and to a strong coupling of the cycle of trace elements and particles. To assess the behavior of vital and toxic species and predict their distribution in ecosystems and their effects on human health, it is therefore compulsory to understand the detailed pathways and interactions between trace elements and suspended material. These processes depend on the properties of the trace element, the interacting particle and the surrounding medium (Salbu and Steinnes, 1992). Sorbed micropollutants will then be affected by these aging processes and the scavenging properties are therefore changed.

A detailed knowledge about the character and fate of particles is also essential for the understanding of water treatment techniques for drinking water or wastewater treatment: particles can influence the performance of these techniques strongly and the removal of pollutants will be affected if they are associated with particles.
Material and methods

Specimen preparation

The techniques of specimen preparation for particles in suspension demand to preserve as much as possible their native aspect (Lienemann et al., 1998). For instance, structural information can easily be lost during preparation and misinterpretations will arise. The scheme in Figure 1 proposes a general pathway showing the various techniques available for particle analysis. Plain lines are the most adapted specimen preparations and allow full exploitation of the potential of the microscope for all parameters. On the other hand, dotted lines are possible specimen preparation methods that can lead to some artifacts. For instance, direct deposition does not allow control of the thickness of the specimen and thus EELS signal can be biased for thick specimens (Mavrocordatos and Perret, 1998). On the other hand, sectioning particles either by ion beam or by ultramicrotomy will not preserve the morphology to a full extent and difficult interpretations of the structure will arise.

Scanning electron microscopy

In the present studies, specimens for SEM were prepared by depositing the dry particles onto an aluminium stub coated with a carbon sticker. The preparation was then coated with a Pt thin film (approximately 20 nm). Measurements were conducted with a Philips XL30 equipped with a LaB₆ source. An accelerating voltage of 20keV with coated material, was used.

Transmission electron microscopy

Depending on the information needed, we used ultracentrifugation, direct deposition of the suspension onto the TEM grids and Focused Ion Beam (FIB, described in the next paragraph). The ultracentrifugation allows deposition of the suspended material directly onto the TEM grids. These grids were previously covered with a formvar film and coated with a C° film. Thus, a representative image of the sample is observed. The direct deposition onto a lacy carbon supporting film (grid of copper 200 mesh) is not representative of the sample but allows us to observe particles that are lying over a hole and analyze them without interference of the supporting film.

An Energy Filtered – TEM (EFTEM, Leo 912, LaB6 cathode, 120keV) and a TEM (Philips CM30 LaB6 cathode, 300keV) were both used for the TEM analysis. The EFTEM

![Figure 1](https://iwaponline.com/wst/article-pdf/50/12/9/419514/9.pdf)
gives the access to the EELS whereas the Philips CM30 is mounted with an EDS detector (EDAX). SAED can be obtained in both microscopes.

**Focused ion beam**

FIB allows us to section the material of interest with a beam of cations. The focused beam sublimates the material and thus allows the user to decide the region of interest. A FEI microscope working with a dual beam (electron: Field Emission Gun and Cations: liquid Ga source) was used for the experiments. Prior to the sectioning, a Pt coating was deposited onto the material. The specimen was then sectioned to prepare the lamella. The lift-out technique with a micromanipulator (Nashige) allowed us to deposit the section onto the TEM grid.

We tried to obtain the thinnest section with the FIB. Nevertheless, the ion beam produces an amorphous layer on the surface of the section that limits the thickness to approximately 100 nm.

**Atomic force microscopy**

As mentioned earlier, the AFM demands less preparation. One way of preparing specimen is to deposit a drop of the suspension directly into the specimen holder, a 1 cm² piece of freshly cleaved mica (muscovite, KAl₂[AlSi₃]O₁₀(OH)₂). Once the water has evaporated, the hydrated specimen is placed in the AFM and a controlled solution (in term of pH, ionic force) is injected in the microscope. This preparation enables the particles to stick to the mica and be analyzed.

The microscope used for all the experiment is a multimode AFM with a low noise head, a Nanoscope IIIa controller and the Nanoscope III v3.2 software, (Digital Instrument, Veeco). For all experiments, the contact mode SiN₄ cantilevers (V-shaped), 100 µm in length with a square pyramidal shape tip (half angle of 35°, measured by Scanning Electron Microscopy) were used. Images were recorded with a 125 µm scanner (J type, Digital Instrument). The Set Point was between 1.5–2 volts with a scan speed of 1–2 Hz and an integral and proportional gain between 1 and 4. These settings were applied to all the recorded images. Finally, a real-time plane fit image filter was applied.

**Results and discussion**

**Mechanism analysis**

Natural particles are sensitive to their physico chemical environment. Inorganic material such as oxides (i.e. Fe₂O₃, Mn₃O₄) are affected by pH or redox conditions. On the other hand, clay particles are less or not sensitive and remain the same in term of their composition and morphology. Organic materials tend to decompose and form highly refractory material such as fulvic or humic substances. Such dissolution phenomenon can be measured by bulk techniques, but at the scale of the individual particle, only AFM can reveal it.

Figure 2 illustrates the dissolution of a Sb₂O₃ particle. There is nowadays a concern about the fate of antimony in the environment. Indeed, these oxides are used as fire-retardant in plastics and since now very little is known about the behavior of these particles after waste treatment. Figure 2a shows an AFM image of the particle at pH = 7. The pyramid-like shape is deposited onto a carbon film for a better adhesion during measurements. From Figure 2b it appears that the Sb₂O₃ particle has changed its shape after it was exposed for 2 hours to a buffer solution of pH = 4. The dissolution takes place and the morphology is dramatically affected.

Another similar effect is observed with wood combustion particles. Indeed, Figure 3a shows the particles and aggregates in the air while Figure 3b presents the same specimen during exposure to a neutral solution. In this case, the morphology change can be associated
to a loss of structure. As these entities are composed of organic matter and inorganic salts, one can speculate that the salt dissolves while the organic part is the one observed in Figure 3b.

On the other hand, formation of amorphous or crystalline materials can take place in natural or industrial waters. As an illustration, fine CaCO3 precipitates can be formed directly from water containing sufficient calcium (Figure 4a). In such a case, the grains which are formed are amorphous and very small (ca 50–100 nm). In order to analyze these entities, both high lateral resolution and elemental analyses are needed. TEM allows us to achieve both requirements.

Synthetic or natural crystalline calcite can also be observed in natural waters (Figure 4b). Such large crystals are easily observed by SEM. In this case, qualitative morphology analysis shows crystallinity. TEM-SAED mode applied on thin specimens allows determination with precision the crystal form observed (Figure 4c). Each bright spot represents an oriented different diffraction plan of the crystal. When measured accurately, the corresponding crystal is then retrieved.

Entities such as oxides, combustion products, organic matter and so forth tend to aggregate in the aqueous environment. In the TEM micrograph of wood combustion sample (Figure 5), the particles in the aggregate are all from the same source and of the same composition. As shown in this example, TEM can provide a high resolution which is necessary for physicochemical investigations. The analysis and results that can be obtained from the TEM image are highly valuable, such specimen represents the ideal case for a fractal analysis.
Indeed, Fractal dimension ($D_f$) can in theory only be applied for one type of particle. $D_f$ will inform us on the aggregation regime (Lin et al., 1989; Jullien, 1992; Mavrocordatos et al., 2002).

Aggregation can also take place amongst different types of particles. In Figure 6a, an AFM image of an aggregate in natural water shows the heterogeneity of this aggregate. One can distinguish angular particles (inorganic crystalline material), smooth surfaces (organic residues) and fibrillar material either sorbed on the surface of mica or still attached to the cell. Figure 6b illustrate the aggregation of the same material (calcium sulfoaluminate hydrate:monosulfate form $3\text{CaO}\cdot\text{Al}_2\text{O}_3\cdot\text{CaSO}_4\cdot12\text{H}_2\text{O}$) appearing to form crystals of different shapes.

These 3 different situations illustrate aggregation possibilities. Also, in all cases, slight
differences can only be seen thanks to the microscopic approach. The aggregation process can be determined for homogeneous particles by the calculus of $D_f$ with TEM or AFM. SEM on the other hand does not measure the height and thus only 2-dimensional analyses of objects can be performed.

Similar techniques as described above can be used to observe dispersion processes or stable (non-aggregating) systems. An example shows oil combustion particles arising from a furnace as analyzed by AFM (Figure 7). Figure 7b shows a zoom of Figure 7a displayed in 3-D. Thanks to image analysis, the size distribution can be accurately measured. The number of observed particles will determine the accuracy of the size distribution.

Sorption processes can take place in various forms. Cells attaching to surfaces can develop large networks of fibril and colonize the surface (biofilm formation). In Figure 8a, an example is shown of cell attachment to a Mica surface exposed to river water. Clearly the branches of extracellular polymers can be distinguished which attach to the surface. In other cases, bacteria can simply sorb on a surface and form a biofilm (Figure 8b) or if the surface is a membrane, such sorption leads to fouling (Figure 8c).

Other techniques as shown above are required to investigate adsorption processes of dissolved compounds. For example, studies were performed on the adsorption of copper onto amorphous iron oxide particles using TEM-EELS technique. Elemental maps of Fe and Cu are shown in Figure 9a and 9b, respectively. These elemental maps where obtained by TEM-EELS in image mode. Further interpretation allows to quantify the amount of Cu sorbed.

Biomineralization is the least defined mechanism. Nowadays, only hypotheses are proposed for this mechanism. Nevertheless, evidences show that in presence of cells, precipitates of iron oxides or calcite appear on their surface whereas no particles can be found beside the cells. As an illustration, calcite crystals formed in a supersaturated solution in presence of bacteria are shown here (Figure 10 a, b). The AFM image Figure 10a displays the 3D image

![Figure 7](image7.png) AFM images (a, b), size distribution (c) of oil combustion particles (scale in nm)

![Figure 8](image8.png) Biofilm growth determined by AFM (a, scale in µm) and SEM (a). Membrane fouling by bacteria determined by AFM (c)
showing fine CaCO₃ particles precipitated on the surface. Figure 10b shows a FIB cross-section of the cell with the embedded crystal. Such cross-section should lead to the understanding of the mechanism of biomineralization. Indeed, if the interface can be resolved at the sub-nanometric scale, one can define the degree of intimacy between crystal and cell walls.

**Conclusions**

Microscopic techniques can be powerful tools to analyze the behavior of particles and associated pollutants in the environment. Dissolution and transformation processes can be monitored in the aqueous phase using AFM techniques as shown in Figures 2 and 3. As presented in Figure 4, precipitation processes can effectively be determined using AFM, TEM and TEM-SAED techniques. Aggregation processes of homogeneous and heterogeneous systems can be envisaged using TEM, SEM, and AFM. TEM provides very precise information, as required for example for fractal analysis, but only provides 2-dimensional information. SEM on the other hand provides 3-dimensional information. AFM allows us to observe aggregation processes *in situ* without sample preparation or drying (see Figures 5 and 6). Similar techniques can be used to observe dispersion processes or stable (non-aggregating) systems (Figure 7). Different types of sorption processes can take place on surfaces. Biofilm growth can be determined effectively by AFM and SEM, as illustrated in Figure 8. The adsorption of elements can be observed by TEM-EELS techniques (Figure 9). Also complex environmental mechanisms like biomineralization can be analyzed by microscopic techniques such as AFM and FIB preparation (Figure 10).

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**Figure 9** Elemental maps obtained by EF-TEM of (a) Cu sorbed on FeOOH and (b) Fe (magnification: 4000×)

**Figure 10** Calcite crystals formed on cells. (a) Image recorded by AFM in water (scan size 10 × 10 µm) and (b) FIB cross-section of the cell previously deposited onto a filter of 0.2 µm pore size and coated with Pt (thick layer above the cells).
It is expected that microscopic characterization techniques will develop further and will enable even more sophisticated analyses in the future. Based on the present state-of-the-art, as illustrated by the examples in this article, general guidelines for the analysis of environmental mechanisms can be established, as presented in Figure 11. This figure can serve as an illustration of the versatility of microscopic techniques, but also as a map to give directions how to investigate particle-related environmental problems.

This scheme proposes only the essential instruments for the designated mechanism. Some techniques can be applied on the specimen for more detailed information.

References


