Natural Microparticles-Macromolecules Interactions: an EFTEM Approach

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1. INTRODUCTION

Natural waters contain a broad diversity of mineral and organic particles (minerals : clays, iron and manganese (oxy)hydroxides, silica, carbonates; organics : humic and fulvic substances, polysaccharides, peptides). Submicron mineral particles may play an important role in the transport processes of toxics and nutrients by adsorption, due to their large specific surface area and their high number concentration (Perret et al 1994). These particles are frequently associated to natural organic macromolecules (Fig. 1; Leppard 1992). Physico-chemical characterization of these entities, at the scale of the individual particles, will help understanding the fluxes of heterotrophic species within aquatic compartments.

In our study, we have used well defined synthetic hematite particles (α-Fe_2O_3; 60-80nm diameter) and exocellular polysaccharides (xanthan produced by *Xanthomonas Campestris*) as models. The use of an Energy Filtred Transmission Electron Microscopic (EFTEM) approach (Reimer et al 1992) allowed their morphological and chemical characterization.

2. MINERAL PARTICLES/ORGANIC MACROMOLECULES INTERACTIONS

Conventional TEM (CTEM) of mixtures of hematite and xanthan at concentrations representative of natural levels (respectively 0.1 and 0.2ppm) shows strong interactions between the two species (Fig. 2a). These associations are fully comparable to those observed in natural systems (Fig.1) and validate the choice of our model species.

However, polysaccharide fibrils are only weakly contrasted in both cases : natural ones are visualized owing to the submicron mineral particles they bear, while synthetic ones are only observed after staining (uranyl acetate 0.1%; en bloc).

We propose the following scheme for an accurate characterization of these associations : (i) Contrast-Tuning (CT) of organic macromolecules, and (ii) Electron Spectroscopic Imaging/Electron Energy Loss Spectrometry (ESI/EELS) of mineral particles.

2.1 Observation of organic macromolecules by EFTEM-CT

EFTEM-CT allows a high contrasting of poorly electron-dense structures when filtered images are obtained at 250eV, just below the carbon K edge. This is illustrated on Fig. 2b by the fact that very small fibrils (5-7nm thickness) are revealed in contrast to Fig. 2a.

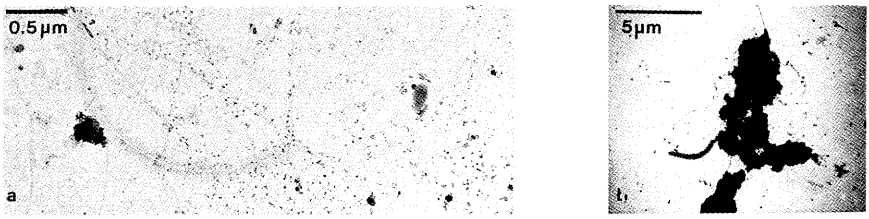

Figure 1 : CTEM of (a) aquatic and (b) sediment samples, showing biotic/mineral interactions.

This spectroscopic procedure allows a better understanding of the network of macromolecules trapping mineral particles, with nodes between fibrils and coalescence of others, which are not observed by CTEM.

Furthermore, the non-perturbing CT method is opposed to classical contrasting techniques, which require a fairly high concentration of electron-dense stains capable of introducing coagulation and structural artefacts in natural samples containing fragile organic structures.

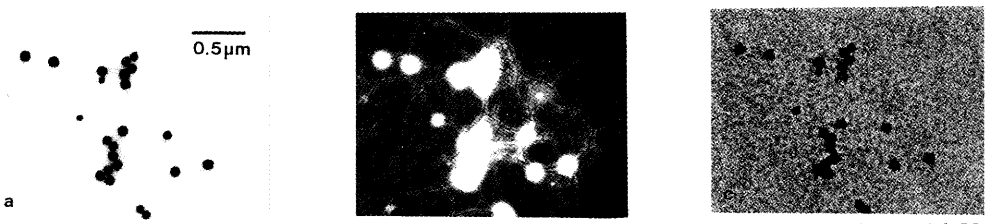

Figure 2 : α-Fe_2O_3/xanthan : (a) CTEM; (b) EFTEM-CT at 250eV; (c) EFTEM-ESI at 716eV.

2.2 Elemental characterization of mineral particles by EFTEM-ESI/EFTEM-EELS

EFTEM-ESI allows the identification of individual elements present in the particles. In contrast to CTEM (Fig. 2a), Fig. 2c shows the unique contribution of Fe present as iron oxide on the filtered image. This visualization procedure represents the very first step in the analysis of natural specimens and is actually extended to sort the chemical composition of the different particles in a natural heterogeneous sample, through the electronic images produced.

EFTEM-EELS gives the complete spectroscopic signal of the analyzed particle. As an example, Fig. 3 shows a spectrum obtained on one of the hematite particles present in the sample. This spectrometric procedure could be used for natural submicrometric particles (ca. <100nm) in order to ascertain their composition and quantitatively determine their elemental ratios.

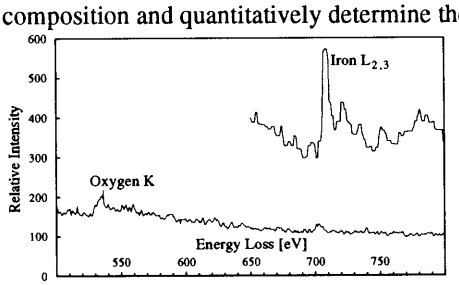

Figure 3 : EFTEM-EELS spectrum of a α-Fe_2O_3 particle present on Fig. 2.

Leppard G.G. (1992) ch 6 in Environmental Particles, vol 1 (Lewis, Chelsea) pp 231-289.
Perret D. et al (1994) Wat. Res. **28**, 91-106.
Reimer L. et al (1992) Ultramicroscopy **46**, 335-47.