Project Report SG88: observing colloidal matter in coastal seawater samples with the help of a new SEM-EDXA protocol

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DESCRIPTION/RATIONALE OF THE PROJECT

Terrestrial dissolved organic matter may be fundamental for transporting iron (and other elements) to marine waters, and most of the iron-organic associations present in river water as well as the receiving coastal waters seem to occur in the colloidal size range, here defined as 2–400 nm. Until now, these colloidal forms have been characterised essentially by their elemental contents or their UV-vis molecular absorption properties. The present study was conducted to determine if it was possible to visualise individual peat-derived colloids in natural waters by scanning electron microscopy (SEM) using a novel technology developed to visualise bacteria, blood cells and viruses in medical and pharmaceutical applications. If yes, then it would be of interest to perform elemental analysis on individual colloids by energy dispersive X-ray analysis (EDXA) which is a technique used in tandem with the SEM instrument. Elemental composition would provide clues as to whether inorganic solid colloids were present, presumably as iron oxides or aluminosilicates, or whether iron was entirely bound to organic substances such as humic substances. This has important implications for the reactivity and fate of terrestrially derived iron in the coastal ocean.

METHODOLOGY

Sampling was conducted in Thurso River and Thurso Bay from a sea kayak (Fig. 1) on 1 February and 3 November 2013. Four-litre water samples were collected from the surface at two locations after salinity had been checked to be S = 0 and S = 30.0, respectively. The samples were filtered through 0.4 μm polycarbonate membranes to remove particulate material prior to the isolation of the colloids. Filtered samples were ultra-filtered using a Sartoflow Slice 200 (Sartorius) cross-flow filtration system (Fig. 2) equipped with a peristaltic pump, PTFE tubing, a polysulfone feed reservoir (500 ml) coupled to a larger feed reservoir and a 5-kDa Hydrosart® membrane cassette (Sartorius). When the volume of unprocessed water left reached 500 ml, the larger feed reservoir was filled with 0.01 M ammonium chloride (made up to pH = 8.4 by mixing ultrapure ammonia and hydrochloric acid reagents) and coupled to the polysulfone reservoir and the system was operated in the diafiltration mode until > 99% of the sample solution had been replaced with this ultrapure, low ionic strength buffer solution. In the final stage of processing, the system was operated in the concentration mode to achieve a colloid concentration factor of 10 for a river sample or 80 for a coastal seawater sample.

Prior to equilibration with a sample made up of natural colloids in 0.01 M buffer, pH = 8.4, the Quantomix™ capsule membrane was coated with Poly-L-Lysine, a positively charged polymer. This was found to be suitable for attaching the negatively charged humic colloids and macromolecules. Conditioning time was 4 h with 0.1% Poly-L-lysine in water, followed by a 12 h equilibration with the sample. The solution was then removed with an MA-4 Multi-well Aspirator (designed to safely suck out the sample without rupturing the membrane) and replaced with QX-102 Imaging Buffer prior to SEM imaging.

The instrument parameters used for SEM imaging were:

- Robinson detector (backscattered electrons) in use with a working distance of 10 mm
- Vacuum = 30 (on the high side for wet mode SEM, could probably be pushed higher)
- Acceleration voltage = 15 kV
- Spot size = 10
- Tungsten filament current optimised for brightness at about 60% of maximum current

RESULTS

The Calibration Capsule was imaged fist to confirm that it contained gold and silica nanoparticles (40 nm and 500 nm in size, respectively) and to find the optimal imaging conditions for the liquid samples. In both river water (Figs 3 & 4) and seawater derived samples (Figs 5 & 6), it was possible to focus on individual colloids which showed up as bright, almost spherical blobs with diameter smaller than 0.4 μm. No significant degree of aggregation could be detected despite the lengthy CFF processing which had resulted in higher (10–80 ×) than ambient concentrations. This observation was attributed to steric and electrostatic repulsions between the dispersed humic colloids. It differed markedly from the self-aggregation into chains produced by phytoplankton exudates (not shown) supplied to us by Michelle Campbell, curator of the SAMS algae culture collection. EDXA was carried out on a limited number of individual colloids (data not shown) and revealed an elemental composition (C, O, Si, Fe) typical of iron-rich humic substances, as expected. Interestingly, the November C:O ratio was twice as high as the February C:O ratio. This may have reflected the relative abundance of aliphatic acids in the autumn flush, as opposed to polyphenolic compounds originating from deeper in the peat soil in the winter.

EXPENDITURE SUMMARY

The funds received (£2062) were spent entirely on the QX-102 starter kit from Quantomix™, which includes 24 cell biology liquid sample capsules, a multi-well aspirator, 2 vials of imaging buffer and 2 calibration capsules. In addition, four 5-kDa Hydrosart® cassettes for our Sartorius CFF system (Fig. 2) were purchased by Professor Chon-Lin Lee, NSYSU, Taiwan, with funding from the Taiwanese Ministry of Education.

OPPORTUNITIES FOR COLLABORATION WITH OTHER MASTS PARTNERS

This work confirms our earlier concept that peat-derived organic substances (humics) are effective iron carriers, not in stream and rivers but also in coastal waters as the organic-iron associations are transported offshore and mix with ambient ocean water. The role and fate of solid mineral micro-particles, which may become stabilised by humic substances, remain to be established for the land-ocean continuum of the Flow Country. To this end, I have entered into collaboration with another MASTS member, Dr Christian Schröder (University of Stirling), with a view to examining this iron-rich colloidal material by Mössbauer spectroscopy. Dr Schröder is currently establishing a Mössbauer spectroscopy centre at the University of Stirling. Together with synchrotron based Mössbauer spectroscopy, which he has started to experiment with, this has the potential to extend the present work to inorganic forms of colloidal iron. How these may interact with humic substances to make iron bioavailable in seawater is poorly understood at present.

Fig.1

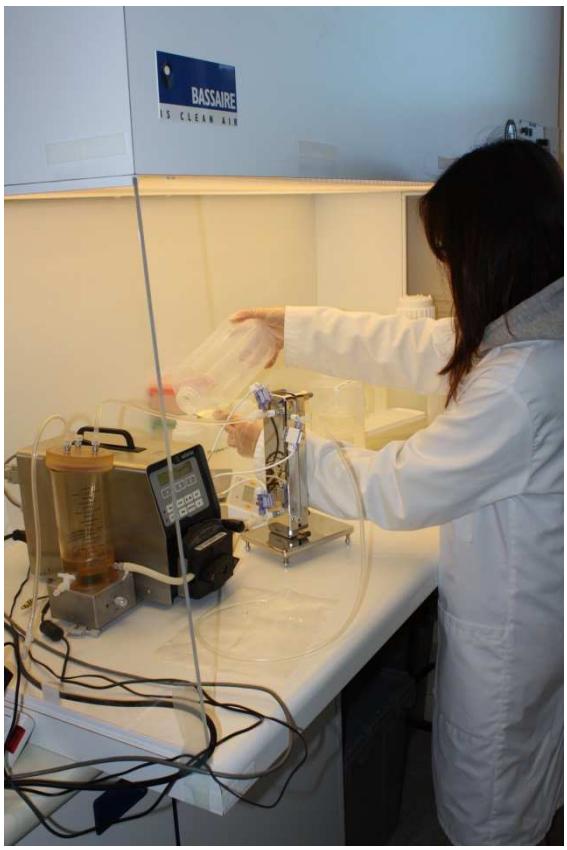

Fig. 2

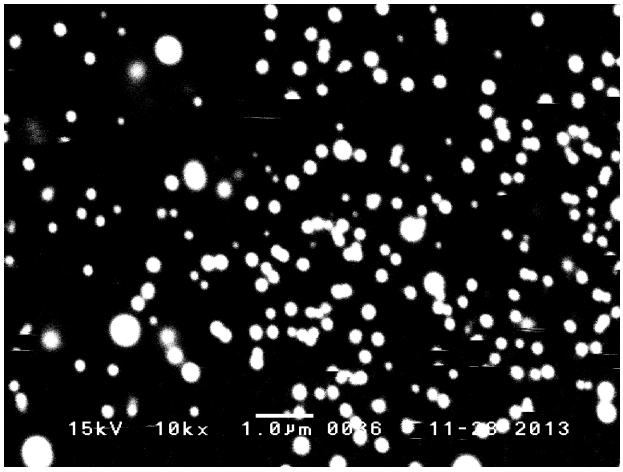

Fig. 3

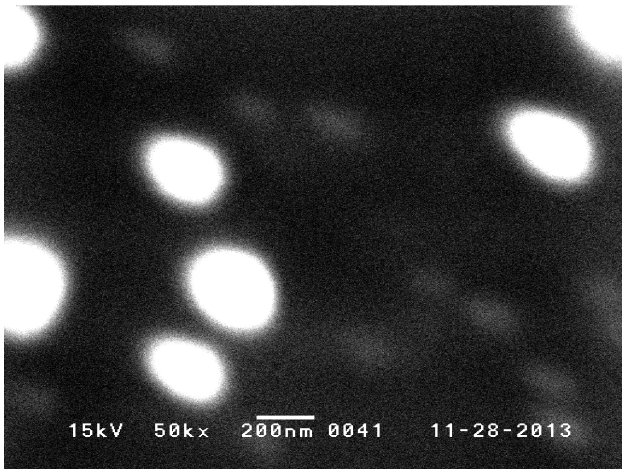

Fig. 4

Fig. 5

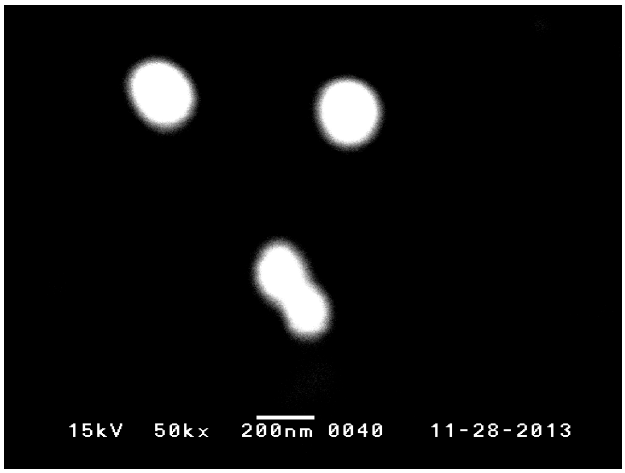

Fig. 6