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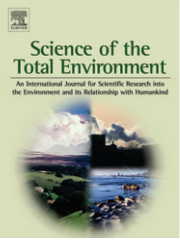

An integrated colloid fractionation approach applied to the characterisation of porewater uranium–humic interactions at a depleted uranium contaminated site

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ABSTRACT

Methods for the fractionation of aquatic colloids require careful application to ensure efficient, accurate and reproducible separations. This paper describes the novel combination of mild colloidal fractionation and characterisation methods, namely centrifugal ultrafiltration, gel electrophoresis and gel filtration along with spectroscopic (UV–visible) and elemental (Inductively Coupled Plasma-Optical Emission Spectroscopy, Inductively Coupled Plasma-Mass Spectrometry) analysis, an approach which produced highly consistent results, providing improved confidence in these methods. Application to the study of the colloidal and dissolved components of soil porewaters from one soil at a depleted uranium (DU)-contaminated site revealed uranium (U) associations with both large (100 kDa–0.2 μm) and small (3–30 kDa) humic colloids. For a nearby soil with lower organic matter content, however, association with large (100 kDa–0.2 μm) iron (Fe)–aluminium (Al) colloids in addition to an association with small (3–30 kDa) humic colloids was observed. The integrated colloid fractionation approach presented herein can now be applied with confidence to investigate U and indeed other trace metal migration in soil and aquatic systems.

1. Introduction

Humic substances are commonly found in soils, sediments and natural waters and comprise the brown-coloured, polyelectrolytic organic macromolecules arising from the decomposition of plant-, animal- and microbially derived organic matter (Stevenson, 1994; Tipping, 2002). Molecular weights ranging from 0.1 to 1000 kDa have been reported, but solid phase humic molecules are generally considered to be larger than their aquatic counterparts (Stevenson, 1994; Tipping, 2002). Indeed, aquatic humic substances are commonly classified as colloids (e.g. Choppin, 2006; Ranville et al., 2007), although several authors note that some may be classed as truly dissolved as a consequence of their polyelectrolyte behaviour (Thurman et al., 1982; Gustafsson and Gschwend, 1997; Wilding et al., 2004).

More generally, the widespread occurrence of colloids, including humic substances, in riverine, coastal and marine environments (e.g. Guo et al., 1994; Mopper et al., 1996) and in groundwaters, including near surface soil porewaters, is well documented. Their composition and behaviour have been central to many investigations (e.g. Gustafsson et al., 1996). Also of major importance has been their role in the environmental transport and fate of metals such as the actinides (Santschi et al., 2002; Singhal et al., 2005; Choppin, 2006;

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Ranville et al., 2007), including uranium (U) at contaminated sites (Mibus et al., 2007). Significantly, colloid-associated metals may behave very differently from truly dissolved metals (Choppin, 1999; Crancon and van der Lee, 2003; Singhal et al., 2005; Mibus et al., 2007). Special interest in aquatic humic colloids stems from their ability to participate in electron transfer as well as ensuing complexation reactions. The resulting changes in metal oxidation state and solubility may lead to enhanced metal mobility in surface soil environments.

Field-based aqueous phase speciation studies usually involve initial separation of the particulate and "dissolved" fractions with various cut-off diameters, e.g. 0.2, 0.45, 1, 10 and 20 μm, having been used to delineate the particle-"dissolved" boundary. The last three are more commonly used in groundwater studies although some workers do classify the 1–20 μm size range as small particulate matter that can be stabilised in aquatic systems during higher energy events, e.g. storm flow conditions (Jacobsen et al., 1997; Ryan et al., 1998; Graham et al., 2006). The 0.2 μm cut-off value excludes bacteria as well as particulate matter (e.g. Pearce, 2007) and is increasingly being used in aqueous phase metal speciation studies (e.g. Dupré et al., 1999).

Importantly, separation techniques including capillary electrophoresis, field-flow fractionation, size exclusion chromatography and ultrafiltration have shown that the "dissolved" fraction contains a wide range of colloidal and "truly" dissolved species that may have varying metal-binding capabilities. Ultrafiltration is often the preferred method because it can both isolate and fractionate colloidal species and because equipment and operator skill requirements are lower. There have been concerns, however, about its efficacy: (i) the resulting fractionation may be operator- and equipment-dependent (e.g. Buesseler et al., 1996); (ii) processes occurring during separation may generate artifacts, e.g. membrane retention of dissolved metal species due to charge effects (Schäfer et al., 2002; Wilding et al., 2004; Susanto and Ulbricht, 2006; Guo et al., 2007); (iii) the size ranges associated with some components of the colloidal and "truly" dissolved phases may overlap, meaning that a 3 kDa molecular weight cut-off (MWCO) value cannot separate large dissolved compounds such as non-globular proteins and carbohydrates (≤1000 kDa) from colloidal species such as humic substances (Gustafsson and Gschwend, 1997). Analytical procedures can be optimised to eliminate/minimise (i) and (ii) but the final point (iii) presents a significant limitation when it comes to extrapolating ultrafiltration-derived metal associations to the potential fate of metals in aquatic systems. In comparison with humic substances, which have multiple metal-binding sites on each molecule, many of these non-globular proteins and carbohydrates are likely to be relatively minor metal-binding components (Stevenson, 1994). Exceptions to this may be the mixture of polyanionic polysaccharides and proteins produced by bacteria such as *Ps. fluorescens* Biovar II, which are reportedly excellent biosorbents for heavy metals and actinides, and which may be ≤8 kDa in size (Hung et al., 2005). Thus a MWCO value of 3 kDa may give a good indication of metal distributions between "truly" dissolved and colloid-bound phases in many natural waters but, where bacterial production of extracellular polysaccharides is of high significance, it may be necessary to undertake careful compositional characterisation of the >3 kDa fraction.

Although ultrafiltration may not separate humic colloids from other similar-sized components present in aqueous samples, it does avoid the harsh chemical conditions employed in traditional methods, e.g. adsorption on non-ionic macroporous ion exchange resins such as DAX-8 and XAD-4, for isolating humic colloids from natural waters (e.g. Aiken et al., 1992). In those methods, adsorption is achieved by adjusting the pH of the resin to 2; elution of the isolated colloids is then effected by increasing the pH to 13. Several studies have reported alteration of the physical and chemical properties of the humic colloids upon exposure to highly acidic and/or to highly alkaline conditions (Graham, 1995; Shin et al., 1999).

Important consideration should therefore be given to other colloid fractionation methods, including gel electrophoresis and gel filtration (or size exclusion) chromatography, which can be used in combination with ultrafiltration. One of the advantages of gel electrophoresis is that fractionation is achieved according to charge as well as size. Thus the strongly negatively charged humic colloids may be distinguished from neutral and/or positively charged colloidal species, large dissolved species of lower charge, as well as small dissolved species. Gel filtration chromatography, where applied independently to achieve size separation, is complementary to both ultrafiltration and gel electrophoresis. Here we present an integrated approach involving ultrafiltration, gel filtration and gel electrophoresis; combined with metal concentration measurement by Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES) and/or Inductively Coupled Plasma-Mass Spectrometry (ICP-MS), this represents a mild yet powerful way to investigate metal associations in aquatic systems.

A small number of representative soil samples were selected and their porewaters, isolated by centrifugation and 0.2 μm-membrane filtration, were used to:

(i) compare colloidal/dissolved separations achieved by gel electrophoresis, ultrafiltration and gel filtration methods;
(ii) carry out laboratory experiments to investigate possible organic artifact generation during gel electrophoresis and ultrafiltration;
(iii) obtain ICP-OES elemental concentration data to investigate possible inorganic artifact generation during gel electrophoresis and ultrafiltration.

These isolation and fractionation methods in combination with ICP-MS were then used in a scoping study of U associations with humic colloids in the soil porewaters from a depleted uranium (DU)-contaminated site.

2. Materials and methods

2.1. Soils

Soils were collected during May 2006 from the Dundrennan Firing Range, SW Scotland, as part of a wider research programme on environmental dispersal and fate of DU. Location and historical details of DU munitions testing by the UK Ministry of Defence and an initial assessment of the extent of contamination have been published (Oliver et al.,