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Development and application of functional assays for freshwater dissolved organic matter

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Abstract

A series of 11 standardised, reproducible, assays have been developed of physico-chemical functions of dissolved organic matter (DOM) in freshwaters. The assays provide quantitative information on light absorption, fluorescence, photochemical fading, pH buffering, copper binding, benzo(a)pyrene binding, hydrophilicity and adsorption to alumina. To obtain DOM for the assays, a 45 L sample of filtered freshwater was rotary-evaporated to reduce the volume to ca. 500 cm^3. The concentrate was then passed through a strong cation exchanger, in the Na^+ form, to remove alkaline-earth cations, and then through 0.7 and 0.2 μm filters. Eight samples, two each from a lake and three streamwaters, were processed. The yields of dissolved organic carbon (DOC) ranged from 70% to 107% (average 91%). The samples of DOM, stored in the dark at 4 °C, retained their functional assay characteristics for up to 7 months. When assaying the concentrates, parallel assays were performed with Suwannee River fulvic acid (SRFA), as a quality control standard. For most of the assays, the results for eight freshwater DOM samples are similar to those obtained with SRFA, the chief exception being the greater hydrophilicity of the DOM samples. For eight of the assays, variability among the DOM samples is significantly ($p<0.01$) greater than can be explained by analytical error, i.e. by comparison with results for the SRFA quality standard; the three exceptional assays are photochemical fading, copper binding and benzo(a)pyrene binding. The two lakewater samples studied gave the most extreme assay results, probably because of the influence of phytoplankton-derived DOM. Significant correlations of hydrophilicity and adsorption with optical absorbance may mean that some DOM functional properties can be predicted from comparatively simple measurements.

Keywords: Assays; Dissolved organic carbon; Dissolved organic matter; Characterisation; Humic substances; Natural waters

1. Introduction

The term "dissolved organic matter" (DOM), as applied in environmental science, refers to the collection of organic compounds present in solution in surface waters, soil waters and ground waters. In freshwater and terrestrial systems the major constituents are humic substances (fulvic, humic and hydrophilic acids), while the minor components include carbohydrates, amino acids, carboxylic acids, hydrocarbons, sterols, alcohols, ketones, ethers, pigments and anthropogenic organic contaminants (Thurman, 1985).

It is increasingly recognised (Perdue and Gjessing, 1990; Kullberg et al., 1993; Hessen and Tranvik, 1998) that DOM has a number of important ecological and

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geochemical functions, including light absorption, proton binding, binding of heavy metals, aluminium and radionuclides, binding of organic contaminants, adsorption at surfaces, aggregation and photochemical reactivity. Information about these functional properties has been obtained largely from laboratory experiments with isolated fractions, especially humic and fulvic acids. These studies have involved the use of materials from different natural environments, obtained by a variety of isolation methods. However, the body of available information, although substantial, is not demonstrably representative, either of the isolated fractions or of DOM as a whole. Neither is there a rational basis on which to select an average property, nor to express uncertainty, i.e. variability, in that property. Thus, it is difficult to apply the detailed laboratory-based information to provide quantitative understanding and prediction of the environmental roles of natural organic matter. Therefore, it would be useful to have a systematic means to determine the variability in the key functional properties of DOM.

The most systematic and extensive comparative study of DOM to date was the ''NOM typing project'' (Gjessing et al., 1999), in which DOM was isolated by both reverse osmosis and evaporation from nine locations in southern Norway, and subjected to a range of measurements by researchers from different laboratories. The study sought mainly to compare properties of relevance to water treatment, but many of the results also relate to the ecological effects of DOM, and these are included in Table 1. The table also includes other comparative studies; these have largely focused on a

Table 1
Comparative studies of the functional properties of natural organic matter

Property measured	Reference
Molar absorptivity of aquatic HS	Chin et al. (1994)
Specific spectral absorbance of NOM[a]	Abbt-Braun and Frimmel (1999)
Molar absorptivity of NOM[a]	Egeberg and Alberts (2002)
Molar absorptivity of DOM	Maurice et al. (2002)
Fluorescence of NOM[a]	Abbt-Braun and Frimmel (1999)
Fluorescence of NOM[a]	Blaser et al. (1999)
Acid–base properties of whole water HS	Hongve et al. (1989)
Acid–base properties of DOM in surface waters	David and Vance (1991)
Proton binding by different FA	Ephraim et al. (1995)
Proton capacity of NOM[a]	Abbt-Braun and Frimmel (1999)
Proton binding by soil FA	Fiol et al. (1999)
Proton binding of NOM[a]	Takács et al. (1999)
Proton binding by isolated DOM fractions	Ma et al. (2001)
Proton binding by HS	Ritchie and Perdue (2003)
Cu binding by FA	Cabaniss and Shuman (1988)
Cu binding by FA	McKnight et al. (1983)
Cu complexation capacity of NOM[a]	Abbt-Braun and Frimmel (1999)
Cu binding by NOM[a]	Takács et al. (1999)
Cu and Cd by HS and lakewater ligands	Xue and Sigg (1999)
Cu binding by isolated DOM fractions	Ma et al. (2001)
Cu binding to DOM in natural water samples	Bryan et al. (2002)
Pyrene binding by DOM from soil and surface water	Patterson et al. (1996)
Pyrene binding by aquatic HS	Chin et al. (1997)
B(a)P and pentachlorophenol binding by HS	De Paolis and Kukkonen (1997)
DOM–PAH interactions	Raber and Kögel-Knabner (1997)
Lipophilicity of NOM, by octanol solubility[a]	Gjessing et al. (1999)
Hydrophobicity of NOM, by chromatography[a]	Egeberg and Alberts (2002)
Aggregation of aquatic HS	Tipping and Ohnstad (1984)
Coagulation of NOM by alum[a]	Abbt-Braun and Frimmel (1999)
Coagulation of NOM by alum[a]	Ratnaweera et al. (1999)
Adsorption of aquatic HS by goethite	Tipping (1981)
Ads of hydrophobic and hydrophilic soil organic acids	Kaiser and Zech (1997)
Adsorption of NOM by activated carbon[a]	Abbt-Braun and Frimmel (1999)
Adsorption of NOM by alumina[a]	Fettig (1999)
Adsorption of DOM by activated carbon[a]	Fettig (1999)

DOM, dissolved organic matter; FA, fulvic acid; HS, humic substances; NOM, natural organic matter.
[a]NOM typing project.

single DOM attribute. In all cases studied to date, differences among samples have been found, and it can be concluded that DOM does vary physically, chemically and functionally from site to site and in time. However, we do not have sufficient quantitative definition of the variation, and there is a need for further systematic study of functional variability among DOM samples, if useful and reliable predictions of the effects of DOM are to be made. Moreover, it is of interest to determine whether there are correlations among the different functional properties.

In the present paper, we describe a set of functional assays for DOM, by which we mean simple, reproducible measurements that provide information about the environmental roles of DOM, rather than its more basic physico-chemical properties. A key aspect of the approach is the use of a quality control standard, Suwannee River fulvic acid (SRFA), that is repeatedly put through the suite of assays in order to characterise their reproducibility. We applied the assays to two samples each from four surface waters, one lake and three streams, in order to explore variability in the functional properties of freshwater DOM.

2. Methods

2.1. Field sites

Esthwaite Hall Beck (EHB, National Grid Reference, NGR SD 357 957) is a stream draining a catchment of area ca. 1 km^2 comprising brown earth soils overlying Silurian slates (Palaeozoic slaty mudstone and siltstone). The catchment land cover is mainly mixed woodland, with some pasture. Esthwaite Water (EW, NGR SD 362 968) is a eutrophic lake of catchment area 17.1 km^2, surface area 1.00 km^2 and mean depth 6.4 m (Ramsbottom, 1976). The lake thermally stratifies in summer, and then has an anoxic hypoliminion. Samples were taken from the outflow or shore and represent epilimnetic water. The catchment soils consist of brown earths, cambic stagnohumic gleys and brown podzols, overlying Silurian slates (Palaeozoic slaty mudstone and siltstone). The catchment land cover is mixed woodland and pasture. The catchment includes EHB (see above). Gais Gill (GG, NGR NY 716 011) is a stream draining ferric stagnopodzols overlying Palaeozoic slaty mudstone and siltstone. The catchment area is moorland with an area of ca. 1 km^2. Rough Sike (RS, NGR NY 756 326) is a stream draining blanket peat of total depth 1–4 m, that has accumulated on glacial clay till overlying Carboniferous limestone, sandstone and shale (Heal and Smith, 1978). The catchment has an area of ca. 1 km^2. The vegetation is principally *Eriophorum-Calluna* and *Sphagnum*.

2.2. Isolation and concentration of DOM

Water samples (20–50 L) were collected in thoroughly rinsed 10 L plastic containers, that had been used numerous times previously for water collection; therefore ''bleeding'' of DOM would have been minimal. On return to the laboratory, the sample was filtered through Whatman GF/F filters (nominal pore size 0.7 μm), and then stored in the dark at 4 °C. Sub-samples were taken for analysis of dissolved organic carbon (DOC) (TOC-VCPN/CPN analyzer, Shimadzu, Kyoto, Japan), pH (glass electrode), alkalinity (Gran titration), conductivity (Jenway 4510 m) and major cations (ICP-OES, Perkin Elmer Optima 4300 DV). The remaining sample was concentrated to approximately 500 cm^3, using a Büchi Rotavapor R-220, operating with a water bath temperature of 45 °C, and a vacuum of 10 mbar. The system was configured so that fresh unconcentrated sample could be added incrementally to the rotating (brown glass) source flask without loss of vacuum. The rate of removal of water was 2 $L\,h^{-1}$. During the several days required for the concentration process, the concentrate was stored overnight at 4 °C in the dark.

The exact volume of concentrate was determined, a sample taken for DOC analysis, and the remainder passed through a 100 cm^3 column of Amberlite IR-120 resin that had been converted to the sodium form by the application of 5 L of 0.1 M $NaNO_3$ at a flow rate of 5 $cm^3\,min^{-1}$. The eluate volume was measured, a sample taken for DOC analysis, and the remainder filtered sequentially through Whatman GF/F and Millipore 0.22 μm filters. The final isolate was analysed for pH and, after suitable dilution, major cations.

The isolates were stored at 4 °C in the dark. They comprised solutions of DOM (148–599 $mg\,L^{-1}$ DOC) in an electrolyte medium consisting of Na^+, together with strong acid anions (Cl^-, NO_3^-, SO_4^{2-}) and HCO_3^-. The electrolyte concentration varied among samples (since it depended upon the initial water composition), and this was taken into account in preparing assay solutions, in order to achieve standard conditions.

2.3. Functional assays

In all the assays, SRFA, purchased as a reference material from the International Humic Substances Society (IHSS), was used as a quality control standard. A stock solution of SRFA was prepared by adding 0.0445 g of solid SRFA to 200 cm^3 of ultra-pure water. This was then assayed in parallel with the field samples. The numbering and naming system for the assays, and nature of each assay result are given in Table 2.

2.3.1. Optical absorbance

Solutions containing 10 $mg\,L^{-1}$ DOC were prepared in duplicate, in an electrolyte solution of 0.05 M NaCl

Table 2
Name of each assay, the nature of the assay result, and the abbreviated designation

No. Abbreviation	Assay	Assay result
1 Optical absorbance 280 nm	Extinction coefficient at 280 nm ($L\,g\,C^{-1}\,cm^{-1}$)	E_{280}
2 Optical absorbance 340 nm	Extinction coefficients at 340 nm ($L\,g\,C^{-1}\,cm^{-1}$)	E_{340}
3 Fluorescence (325/450)	Peak intensity with excitation at 325 nm and emission at 450 nm, per mg DOC L^{-1}	$F_{DOC/325/450}$
4 Photochemical fading	% loss in DOM absorbance at 340 nm	A_{340} loss%
5 Buffering capacity	Acid groups titrated between pH 4 and 9 (meq/g C)	Ac_{4-9}
6 Copper binding	Conditional stability constant ($L\,g\,C^{-1}$)	$\log K_c$
7 Benzo(a)pyrene binding	Partition coefficient ($cm^3\,g\,C^{-1}$)	$\log K_p$
8 Hydrophilicity (DOC)	% of DOC not adsorbed by XAD-8 or DAX-8 resin at pH 2	$Hyphil_{DOC}$%
9 Hydrophilicity (absorbance)	% of DOM absorbance (340 nm) not adsorbed by XAD-8 or DAX-8 resin at pH 2	$Hyphil_{A340}$%
10 Alumina adsorption (DOC)	% of DOC adsorbed at pH 4	Ads_{DOC}%
11 Alumina adsorption (absorbance)	% of DOM absorbance (340 nm) adsorbed at pH 4	Ads_{A340}%

and 0.075 M phosphate buffer at pH 7.0. Absorbance was measured over the wavelength range 220–600 nm, using a Hitachi U-2000 Spectrophotometer. Electrolyte-only blanks were also run. The samples contained nitrate and sulphate, both of which absorb light at wavelengths 280 and 340 nm, used as assay outputs. However, tests showed the contributions of the two inorganic ions to the measured absorbance values to be negligible (<1%).

2.3.2. Fluorescence

Solutions containing 10 mg L^{-1} DOC were prepared in duplicate, in a background of 0.1 M NaCl and 0.001 M phosphate buffer (pH 7). Fluorescence was measured in 4 cm^3 capacity cuvettes using a Varian Cary Eclipse fluorescence spectrophotometer, equipped with a multicell holder with Peltier temperature controller enabling the measurement of excitation–emission matrices (EEM) at 20.0±0.1 °C. Each EEM was generated by scanning excitation wavelengths from 200 to 400 nm in 5 nm steps, and detecting the emitted fluorescence between 280 and 500 nm in 2 nm steps. Scan speed was 9600 nm min^{-1}, permitting collection of a complete EEM in ~60 s. The assay output ($F_{DOC/325/450}$) is the fluorescence intensity of the peak observed between 300 and 340 nm excitation wavelength and 400 and 460 nm emission wavelength, divided by the DOC concentration; the fluorophore in question is often attributed to fulvic-like substances. The Raman signal due to water (excitation 348 nm, emission 395–400 nm) was also measured, as a quality control; this averaged 20.51 ($n = 40$) on analysis days, in good agreement with long-term instrument performance (mean 20.48, standard error 0.05, $n = 365$). Note that for all samples except EW1 and RS1, the DOC concentrations in the samples sent for fluorescence measurements were measured directly, and the measured values used to calculate $F_{DOC/325/450}$. But for EW1 and RS1, $F_{DOC/325/450}$ was estimated from the dilution factor and the concentration of the stock solution.

2.3.3. Photochemical fading

Solutions of 5 mg L^{-1} DOC were prepared in a background of 0.1 M NaCl and 0.001 M phosphate buffer (pH 7). Triplicate 3 cm^3 aliquots were pipetted into 1 cm path length UV Quartz Macro cuvettes (6030UV, Fisher Scientific) with lids, and positioned in a circle of radius 8 cm around a Pen-Ray mercury lamp (90-0003-01, Ultraviolet Products, Cambridge), with a primary output at 254 nm. The samples were irradiated for 3 h in a temperature-controlled dark room (20±1 °C). Following irradiation, optical absorbance at 340 nm was determined on the samples, and on control solutions that had been kept in the dark at 20 °C, and the percentage loss of absorbance due to irradiation was calculated.

2.3.4. Buffer capacity

Duplicate solutions were prepared, with a DOC concentration of 100 mg L^{-1}, in a background electrolyte of 0.5 M NaCl. Before performing the titration, the solution was adjusted to pH 3.5 using 1 M HCl and bubbled with wet CO_2-free air, for 3 h to expel CO_2. A Radiometer ABU 80 autoburette was used to add CO_2-free 0.1 M NaOH, the solution being blanketed with wet CO_2-free air, and thermostatted at 20 °C. The pH was measured with a Radiometer GK2401C combination glass electrode, calibrated with pH 4 and 7 buffers. The base additions were made until the pH exceeded 10.0.

2.3.5. Copper binding

Triplicate calibration solutions (volume 20 cm^3) containing Cu^{2+} at concentrations of 10^{-6}, 10^{-5} and 10^{-4} M were prepared, in an electrolyte solution of 0.1 M $NaNO_3$ and 0.001 M HNO_3. Nine sample solutions (volume 20 cm^3) containing 40 mg L^{-1} DOC and 2.5×10^{-6} M Cu were made up in 0.1 M $NaNO_3$ and 0.001 M 2-(N-morpholino)ethanesulphonic acid buffer (pH 6), and stored overnight at 4 °C. The stored solutions were brought to 20 °C by immersion in a water bath, and then three of the sample solutions were adjusted to pH~5.7, three to pH~6, and three to pH~6.3, the exact pH value being recorded in each case. The potential of each standard and sample solution was determined with an Orion cupric solid state half-cell electrode and an Ag/AgCl double junction reference electrode attached to an Orion Research Microprocessor Ionanalyzer 901, readings being taken after 30 min. The voltage readings and logarithms of the Cu^{2+} concentrations for the standards were used to construct linear calibration curves, which had r^2 values >0.997, and slopes that ranged from -26.2 to -28.6, in agreement with the theoretical value of -28.1. The Cu^{2+} concentrations for the samples were then determined for the three pH values, and a linear interpolation was performed to obtain the concentration at pH 6.0. At pH 6.0, Cu hydrolysis is negligible, and there is little complexation of Cu by the inorganic anionic components of the solution. Therefore, the amount of Cu bound by the DOC could be calculated by subtracting the Cu^{2+} concentration from the total added Cu. The conditional stability constant for Cu binding by the DOM was then calculated (K_c = moles Cu bound per g DOC/[Cu^{2+}]). The maximum concentration of chloride ion in the sample solutions was 0.0075 M, which is below the value of 0.01 M found by Bryan (2001) to be the concentration at which chloride interference effects began to be noticeable. Note that the Cu:DOC ratio in the original surface waters is $\leqslant 5 \times 10^{-6}$ mol g^{-1} (A.J. Lawlor, personal communication), and if all this metal is assumed available to participate in complexation by DOM, the $\log_{10} K_c$ values increase by 0.02 at the most, which can be considered negligible.

2.3.6. Benzo(a)pyrene binding

Ten solutions (volume 10 cm^3) containing 10 mg L^{-1} DOC, 0.1 M NaCl and 0.001 M phosphate buffer (pH 7) were placed in glass bottles (volume 20 cm^3), and 10 DOC-free solutions were also prepared. A stock solution of [7, 10^{-14} C]benzo(a)pyrene was prepared in ethanol, at a concentration of 2.78×10^7 Bq L^{-1}, or 1.23×10^{-5} M. Ten microliters of the stock were added to each assay solution to give a final concentration of 1.23×10^{-8} M. The bottles were capped, shaken gently and stored in a refrigerator at 4 °C for 24 h, to allow the benz(a)pyrene to partition between the solution and the glass walls of the bottle. After equilibration, 1 cm^3 of solution from each glass vial was transferred into a clean scintillation vial, 8 cm^3 of Ecoscint A Scintillation Solution (National Diagnostics, USA) was added, and the radioactivity of the sample determined using a Packard Tri-Carb liquid scintillation analyser (Model 2200CA), Quench corrections were made, but they were very small. The glass-solution partition coefficient of benzo(a)pyrene was determined from the results of the DOC-free experiments, and used to calculate the concentration of unbound benzo(a)pyrene in the solutions containing DOC. This concentration was then subtracted from the total dissolved benzo(a)pyrene concentration to obtain the concentration bound to the DOC, and hence the partition coefficient, defined as the amount of benzo(a)pyrene bound per g DOC divided by the free concentration.

2.3.7. Hydrophilicity (column method)

The non-ionic resin XAD-8 (Sigma) was prepared for use by washing in 0.1 M NaOH, rinsing with distilled water, and then sequentially extracted with methanol and acetonitrile, each for 48 h, in a Soxhlet apparatus. The extraction was repeated and the resin stored under methanol. A glass column (volume 3 cm^3) connected to a peristaltic pump, was filled with the resin slurry (ensuring the resin did not dry out) and sequentially washed with 20 cm^3 of 0.1 M NaOH and 0.1 M HCl, at a rate of 1.7 cm^3 min^{-1}. The washing procedure was then repeated twice with a final wash of 50 cm^3 of 0.03 M HCl. Test solutions (volume 20 cm^3) comprising 10 mg L^{-1} DOC and 0.1 M NaCl, were prepared in triplicate and adjusted to pH 2 with HCl. Acidified blank solutions (0.3 cm^3 of HCl per 20 cm^3 of deionised water) were prepared in duplicate. The blank and sample solutions were then sequentially passed through the column, discarding the initial sample and blank solutions to prevent carry-over, with the subsequent solutions collected for analysis of DOC and optical absorbance at 340 nm; for the absorbance readings, samples were mixed 1:1 with KH_2PO_4 buffer, pH 7. The hydrophilic fraction, based on DOC, was calculated as the ratio of DOC concentration in the solution leaving the column to that in the input solution, correcting for the small amount of DOC "bleed" (ca. 0.7 mg L^{-1}) from the resin. A similar procedure was followed to calculate the hydrophilic fraction based on absorbance.

2.3.8. Hydrophilicity (batch method)

The non-ionic resin Supelite DAX-8 (Rohm and Haas) was prepared for use by soaking in methanol for 24 h, and rinsing with ultra-pure water. The resin was then transferred to a 100 cm^3 column and washed in the following sequence: (i) 5 L of water, (ii) 500 cm^3 0.1 M NaOH, (iii) 500 cm^3 0.1 M HCl, (iv) 500 cm^3 0.1 M

NaOH, (v) 500 cm^3 0.1 M HCl, (vi) 5 L water. The test solutions (volume 25 cm^3) contained 20 mg L^{-1} DOC and 0.1 M NaCl, and were adjusted to pH 2 with HCl. They were added to the DAX-8 resin, equivalent to 1 g dry weight, in Beckman polycarbonate centrifuge tubes. Controls were prepared in which either the resin or the DOC was omitted. The centrifuge tubes were shaken (200 rpm) at 20 °C for 3 h, then centrifuged for 30 min at 10,000 rpm, using a Beckman model J2-21 centrifuge. The supernatants (containing the hydrophilic acid fraction) were removed for the determination of DOC and optical absorbance at 340 nm. The hydrophilic fraction, based on DOC, was calculated as the ratio of the DOC concentration in the presence of resin to that in its absence, correcting for DOC "bleed". A similar procedure was followed to calculate the hydrophilic fraction based on absorbance.

2.3.9. Adsorption by alumina

A stock suspension (10 g L^{-1}) of γ-Al_2O_3 (Alfa Aesar, 99.997% pure, specific surface area 60 $m^2 g^{-1}$) in deionised water was subjected to ultrasonic dispersion for several hours. The assay suspensions (25 cm^3) were prepared in Beckman polycarbonate centrifuge tubes and contained 0.4 g L^{-1} alumina, 10 mg L^{-1} DOC and 0.1 M NaCl, adjusted to pH 4. Blanks were also prepared, either containing no alumina or no DOC. The suspensions and blanks were equilibrated by shaking (200 rpm) at 20 °C for 24 h. After equilibration, the pH was measured; the average value was 4.1 (SD 0.1). Then the suspensions and blanks were centrifuged for 60 min at 10,000 rpm. The supernatants were removed for DOC analysis and absorbance at 340 nm (in pH 7 phosphate buffer). The amount of adsorption expressed in terms of mass of DOC was calculated by subtracting the supernatant DOC concentration from that of the alumina-free control, taking account of any background DOC (ca. 0.8 mg L^{-1} DOC), and a similar approach was used to calculate adsorption in terms of optical absorbance.

3. Results

The chemical characteristics of the eight water samples are shown in Table 3. The samples all had near-neutral pH values, but varied in DOC concentration—[DOC]—and alkalinity. The two samples from EHB were taken a year apart, and have very similar chemistries. The first EW sample was taken at the time of the lake's overturn in 2003. The highest concentrations of DOC were found for Rough Sike, the stream draining the blanket peat catchment; both samples were taken in the summer, when [DOC] is relatively high, although these are not the highest values; concentrations in excess of 30 mg L^{-1} have been observed on some occasions at this site.

The isolation method gave a high yield of DOC, the overall average for the eight samples being 91%. The lowest recovery (70%) was for sample EW1, from which a precipitate of calcium carbonate formed during the rotary evaporation and probably removed some DOM by adsorption or co-precipitation. Losses of DOC from the other samples were minor, and again mostly occurred during rotary evaporation; losses to the cation exchange column or in the final filtration step were $\leqslant$2%. Although the water bath of the rotary evaporator is set at 45 °C during the concentration step, the temperature of the sample was measured to be 20 °C, due to the evaporation process. Therefore, the method does not involve the exposure of the DOM to high temperature.

Metal analyses of the isolates showed that the cation-exchange step removed more than 97% of the alkaline-earth cations (Mg, Ca) present in the original sample. There was, however, little removal of either Al or Fe, which were therefore present in the assay solutions, at concentrations of up to 0.3 mmol Al g C^{-1} and up to 1.4 mmol Fe gDOC^{-1}.

In the following text, we describe the performance of each assay, the results, and whether the variability in the samples is greater than that in the SRFA standards. The plots in Figs. 1–4 show the results for both the samples

Table 3
Raw sample chemical data

Site	Date	Sample code	[DOC] mg L^{-1}	pH	Alk μeq L^{-1}	Cond μS cm^{-1}
Esthwaite Hall Beck	17/11/03	EHB1	4.0	7.15	160	81
	26/11/04	EHB2	4.9	6.94	110	78
Esthwaite Water	09/10/03	EW1	3.9	7.38	620	119
	27/07/04	EW2	3.7	7.85	490	106
Gais Gill	08/01/04	GG1	6.0	7.25	270	56
	11/10/04	GG2	3.4	7.83	530	84
Rough Sike	27/08/03	RS1	8.3	7.67	630	118
	02/06/04	RS2	12.9	7.21	260	69

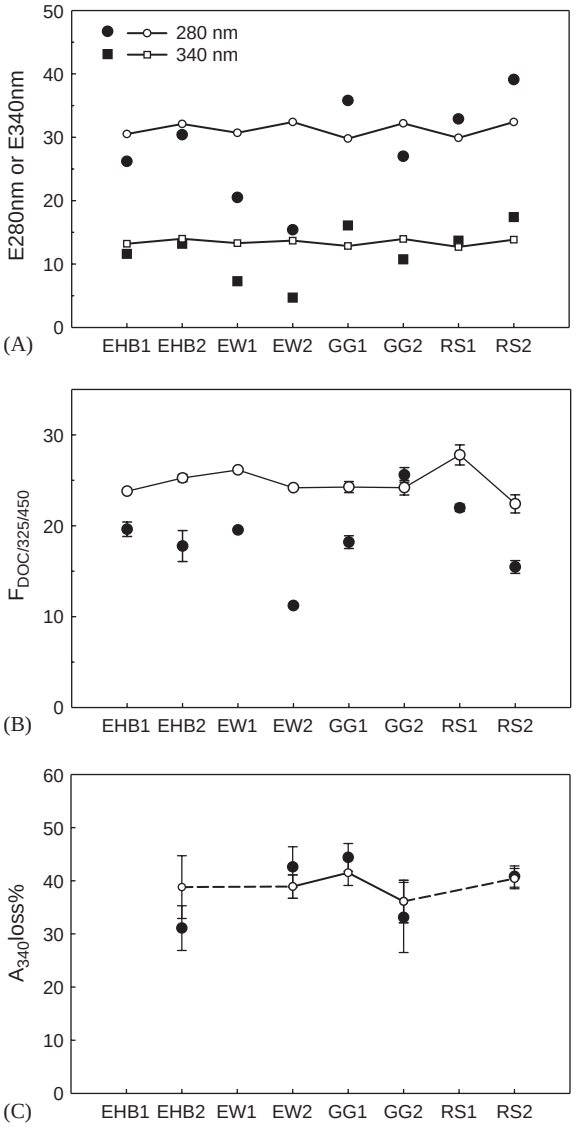

Fig. 1. Results of assays of optical properties. (A) Optical absorption (assays 1 and 2); (B) fluorescence (assay 3); (C) photochemical fading (assay 4). In these and the other diagrams, results for the samples are shown by filled symbols, and those for the SRFA quality control standard by open symbols. Error bars indicate one standard deviation; where error bars are absent, their range is smaller than the points.

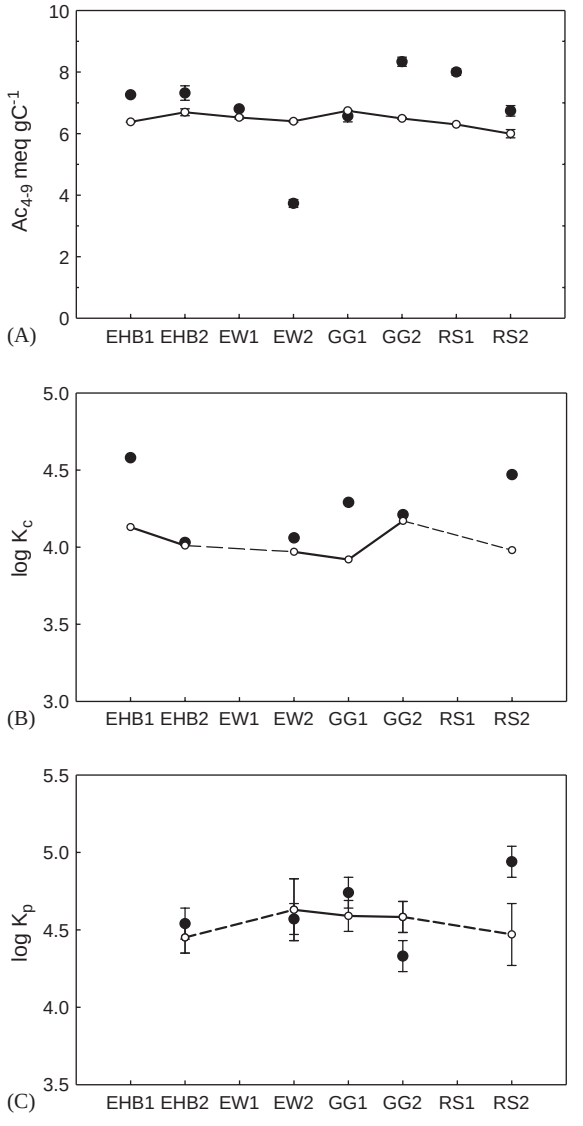

Fig. 2. Results of assays of interactions with solutes. (A) buffering capacity (assay 5); (B) copper binding (assay 6); (C) benzo(a)pyrene binding (assay 7).

and the SRFA standard, but it should be noted that the order of presentation is alphabetical by sampling site, not chronological, and so any apparent trends in the QC results do not reflect systematic variation. In fact, only two of the assays showed a trend with time in the QC standard result; the extinction coefficients at 280 and 340 nm of SRFA increased by 7% over the period of the study, the increase being significant at the 5% level. The assay results shown in the figures are those obtained soon after sampling, although repeat measurements were made in 18 of the 58 sample–assay combinations (see Table 4). Statistical evaluation (Table 5) was performed using the one-tailed F test (Snedecor and Cochran, 1967) to determine whether the standard deviation of the assay results for a given assay was significantly greater, at the 1% level, than that of the repeat results for each standard. Where appropriate, comparisons are made with published data.

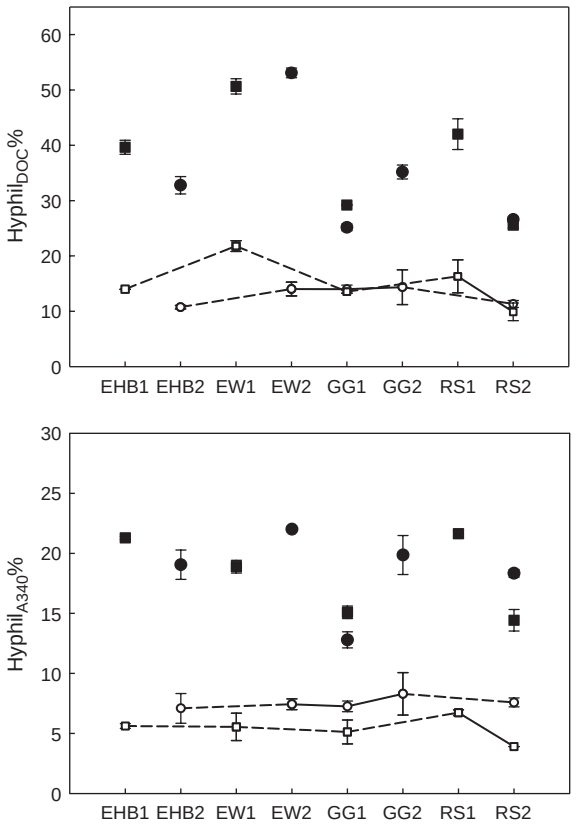

Fig. 3. Results of hydrophilicity assays (8 and 9), based on [DOC] (upper panel) and absorbance at 340 nm (lower panel). Circles refer to the column versions of the assays, and squares to the batch versions.

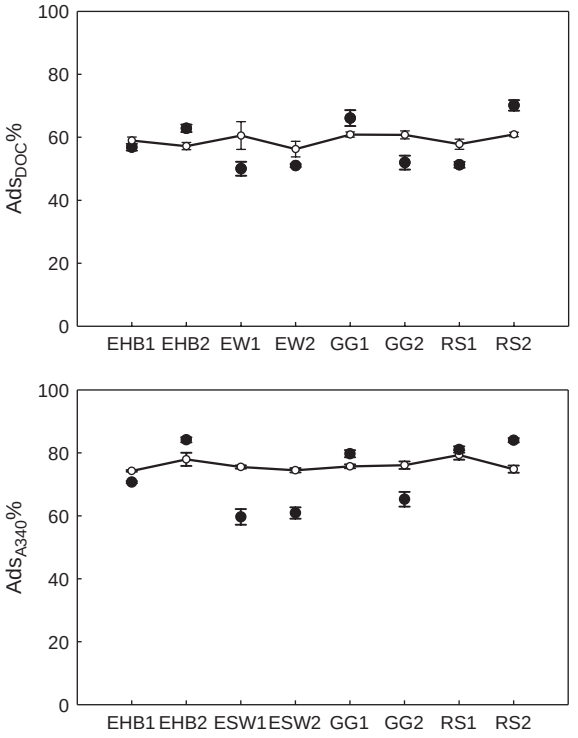

Fig. 4. Results of adsorption assays (10 and 11), based on [DOC] (upper panel) and absorbance at 340 nm (lower panel).

3.1. Optical absorbance

Fig. 1A shows that the extinction coefficients at 280 and 340 nm are strongly correlated ($r^2 = 0.98$). The values for the lakewater samples (EW1 and EW2) are noticeably lower than those of the streamwater samples, especially for the sample taken in late June, and this leads to lower standard deviations if the lakewater results are excluded. The variability among samples is significantly greater than that among the results for the SRFA standard (Table 5), whether or not the lakewater results are excluded. Repeat measurements were made in five cases (Table 4), and the average differences in extinction coefficients were 1% at 280 nm and 2% at 340 nm, in each case the same as the average difference in the SRFA values.

Values can be compared with two previous studies. The molar absorptivities at 280 nm reported by Chin et al. (1994) and Maurice et al. (2002) for whole-water NOM or reverse osmosis isolates correspond to extinction coefficients in the range 12.5–42.4 L g C^{-1} cm^{-1}; for the total of six samples, the mean was 29.9 and the SD 9.7, which are similar to our own results (Table 5).

3.2. Fluorescence

Fig. 1B shows that $F_{DOC/325/450}$ is generally lower for the samples than for the SRFA standard. Two samples stand out; EW2 had an especially low fluorescence, while GG2 had a high fluorescence. The fluorescence of the samples varies significantly more than that of the standard, when the lakewater results are included, but not when only the streamwater samples are considered. Repeat assays were made in two cases (Table 4); for EHB1, the second value of $F_{DOC/325/450}$ was 3% higher than the first, while for GG1 the second was 3% lower.

Blaser et al. (1999) measured total luminescence of 11 samples from the NOM typing study, isolated by reverse osmosis. For excitation at 340 nm and emission at 440 nm, similar wavelengths to those reported by us, they found a relative standard deviation (RSD) of 17%, appreciably less than the value of 27% that can be derived from the data in Table 5. This suggests that our waters are more dissimilar than the NOM typing project ones. (Note that absolute fluorescent intensities cannot be compared because the results are in arbitrary units, i.e. they differ from one instrument to another.)

Table 4
Times (days) between sampling and assay

Assay no.	EHB1	EHB2	EW1	EW2	GG1	GG2	RS1	RS2
1 and 2	17, 109	10	21	13, 108	25, 256	17	9, 112	16, 90
3	17, 77	13	18	21	25, 222	18	19	19
4	—	10	—	9, 108	162	11	—	9, 216
5	16	13, 14	20	10	22	25	14	12, 225
6	198, 322	18	—	21	110, 228	24	—	7
7	—	11	—	22	231	14, 72	—	79
8 and 9 C[a]	22	—	47, 138	—	33	—	33	27
8 and 9 B[a]	—	18	—	15, 169	190	23	—	48
10 and 11	24	12	26	14	29, 224	24	27, 112	14

[a]C, column; B, batch.

Table 5
Statistical analysis

Assay	Assay output	n	Samples		SRFA		Sig (1%)
			Mean	SD	Mean	SD	
1	E_{280}	8	28.4	7.9	31.2	1.1	s
		6	31.9	5.0			s
2	E_{340}	8	11.8	4.3	13.4	0.5	s
		6	13.8	2.6			s
3	$F_{DOC/325/450}$	8	18.7	5.0	24.8	1.9	s
		6	20.3	4.4			ns
4	A_{340} loss%	5	38.4	5.9	39.1	2.0	ns
		4	37.4	6.3			ns
5	Ac_{4-9}	8	6.8	1.4	6.4	0.2	s
		6	7.4	0.7			s
6	$\log K_c$ (Cu)	6	4.27	0.22	4.03	0.10	ns
		5	4.32	0.22			ns
7	$\log K_p$	5	4.62	0.23	4.54	0.08	ns
		4	4.64	0.26			ns
8	$Hyphil_{DOC}$%	8	38.3	10.0	14.5	3.5	s
		6	33.8	6.5			ns
9	$Hyphil_{A340}$%	8	19.1	2.8	6.6	1.0	s
		6	18.7	3.0			s
10	Ads_{DOC}%	8	57.5	7.8	59.2	1.9	s
		6	59.9	7.7			s
11	Ads_{A340}%	8	71.0	11.0	75.6	1.5	s
		6	78.2	6.9			s

The statistical significance refers to an *F*-test of whether the assay results for the samples are more variable than the repeated values for the SRFA standard. The second line for each assay considers only data for streams.

3.3. Photochemical fading

This assay exhibited relatively high variability in individual measurements, as indicated by the standard deviations plotted in Fig. 1C. Therefore, the assays were performed 8–12 times on each sample–standard pair. Due to the need to refine this assay during the study, results (Fig. 1C) were obtained for only five of the samples. The results for the samples are more variable than those for the standards, but not significantly so at the 1% level. Exclusion of the lakewater sample has no significant effects on the results (Table 5). Repeat assays were performed in two cases (Table 4). For EW2, A_{340} loss% decreased from 44.3 to 42.6, while for RS2 there was an increase from 40.8 to 41.2. The changes in the SRFA standard were slightly greater.

Note that fading assay only refers to the light-absorbing components of the DOM mixture (aromatic, quinone and conjugated structures), and it appears that those components in the different samples behave similarly. The rate of fading is substantially greater than would be observed in the field, because the light intensity is greater—Gao and Zepp (1998) reported a fractional conversion rate (pseudo first order rate constant) of only $0.022\,h^{-1}$ for the absorption coefficient (equivalent to the extinction coefficient) at 300 nm, for absorbing compounds from the Satilla River exposed to simulated solar radiation. The corresponding rate constant derived from our results at 340 nm is $0.16\,h^{-1}$.

3.4. Buffer capacity

The DOM isolation method precludes the determination of absolute charge densities on the DOM, since we cannot precisely determine the net difference between the concentrations of base cations (almost entirely Na^+) and strong acid anions (Cl^-, NO_3^-, SO_4^{2-}). Therefore, the results are expressed in terms of the equivalents of DOM-associated charge titrated between two pH values, i.e. 4 and 9. Fig. 2A shows that for most of the samples, the titratable charge is similar to or slightly higher than that of SRFA. Only in one case (EW2) is the charge content substantially lower. With this sample included, the sample results vary significantly more than those for SRFA (Table 5), and this is also true if only streamwaters are

included. For two of the samples, the determination of Ac_{4-9} was repeated some time after the initial measurements (Table 4). In the case of EHB2, the second value was 5% higher than the first, while for RS2 it was 4% lower. The corresponding determinations of Ac_{4-9} for SRFA, carried out at the same time as the EHB2 and RS2 assays, were 2% lower and 6% higher, respectively.

Our result for SRFA (Table 5) is in reasonable agreement with the value of 6.1 meq g C^{-1} that can be derived by data reported by Ritchie and Perdue (2003) from titrations of the same material in a background of 0.1 M NaCl. Data reported by these authors permit a value of 5.4 meq g C^{-1} to be derived for Suwannee River NOM, which is within the range found by us (Fig. 2A).

3.5. Copper binding

The results of Fig. 2B show that there is some variation in the value of $\log K_c$ among the six samples assayed, but this variation is not significantly statistically greater than that found for the QC standard (Table 5). Whereas the two GG samples gave quite similar results, the two for EHB are rather different. For two of the samples, the determination of $\log K_c$ was repeated some time after the initial measurements (Table 4). In the case of EHB1, the second value was 0.31 log units higher than the first, while for GG1 it was 0.12 lower. The corresponding determinations of $\log K_c$ for SRFA were 0.26 lower, and identical.

McKnight et al. (1983) measured copper binding by 17 fulvic acid samples, isolated mainly from rivers in the USA, at pH 6.25 and with a background electrolyte of 0.001 M KNO_3. McKnight et al. reported equilibrium constants and site densities, derived from the experimental data by fitting a two-site model, and Tipping (2002) used these parameter values to calculate values of v (mol Cu bound per g FA) at different free concentrations of Cu^{2+}. Taking values of v for $[Cu^{2+}]$ of 10^{-8} M, i.e. similar to the value found in the present work with DOM, these values provide values of $\log K_c$ in L g C^{-1} (assuming the FA to be 50% C). The mean $\log K_c$ from the McKnight et al. data is 4.28, with a standard deviation of 0.21, in agreement with the results for DOM given in Table 5.

3.6. Benzo(a)pyrene binding

This assay was found to give reproducible results if a high degree of replication (tenfold) was employed. Fig. 2C shows results for the five samples that were assayed. The values for four of the samples were similar, but the RS2 sample gave a comparatively high partition coefficient (K_p). However, the variability among the results for the samples is not significantly greater than that for the SRFA standard results (Table 5). The value of $\log K_p$ for GG2 remeasured 58 days after the original determination, was 0.23 log units higher than the original value, while the corresponding value for the SRFA standard was 0.06 log units lower.

The values of $\log K_p$ are similar to those determined for the benzo(a)pyrene-DOM interaction by other workers using equilibrium dialysis. Raber and Kögel-Knabner (1997) reported a $\log K_p$ of 4.71 for DOM from compost material, and a value of 4.92 for DOM extracted from soil. De Paolis and Kukkonen (1997) determined $\log K_p$ at three pH values in the range 5–8, for a riverine FA sample, which average to $\log K_p = 4.66$.

3.7. Hydrophilicity

The results, plotted in Fig. 3, are expressed as % hydrophilic, since this was the directly measured quantity. The % hydrophobic is simply 100 minus the % hydrophilic. During the course of the work, two assay methods—column and batch—were used. The batch method was found to be somewhat easier to perform, but the results from the two methods were in good agreement for the two samples (GG1 and RS2) where direct comparisons were possible. Therefore, the results from the two methods were combined.

In all cases, the DOM was more hydrophilic than the SRFA. When expressed in terms of DOC, the variation in hydrophilicity is from ca. 25% to 55%, but when optical absorbance is followed, the values are lower, and the range smaller (13–22%). The least hydrophilic samples, expressed in terms of both DOC and optical absorbance, were from the streamwaters, GG1 and RS2. The lakewater samples EW1 and EW2 were the most hydrophilic, but only when expressed in terms of DOC. Considering all the samples, both $Hyphil_{DOC}$% and $Hyphil_{A340}$% vary to a significantly greater extent than the values for the SRFA standard, but if only streamwaters are considered, the variation in $Hyphil_{DOC}$% is not significantly greater than the values for the standard.

Repeat assays on samples EW1 and EW2 (Table 4) gave slightly lower values of $Hyphil_{DOC}$% (49% and 51% compared to 51% and 53%, respectively), and slightly higher values of $Hyphil_{A340}$% (20% and 24% compared to 19% and 22%).

Comparing the results in terms of DOC and absorbance, we see that the light-absorbing material is generally less hydrophilic. Also, taking all eight samples, the RSD in $Hyphil_{A340}$% (15%) is less than that in $Hyphil_{DOC}$% (26%) suggesting that the fractions of the samples that possess chromophores are more similar than DOM as a whole.

Egeberg and Alberts (2002) used a high-performance liquid chromatographic method to determine hydrophobicity, defined in terms of retention on a C18 column. Their experiments were carried out with samples from the NOM typing project. The

chromatograms comprised two major elution zones, and the ratio of the area (in terms of optical absorbance) of the second to the first was defined as the hydrophobicity. Conversion of this variable to % hydrophilicity as defined in the present work leads to an average of 55.4% with a standard deviation of 6.5%. This average is considerably higher value than we report, in terms of absorbance (Table 5), which is probably attributable to the higher pH (4.7) used in their experiments, which leads to a more charged molecule. Gjessing et al. (1999) determined the partitioning of DOM from the NOM typing project between water (pH 1–3) and octanol, as a measure of lipophilicity. Analysis of their reported data indicates that, for the nine samples of the NOM typing project, between 50% and 97% (mean 84%, SD 15%) of the DOM remained in the aqueous phase, and therefore might be termed hydrophilic. Evidently, this is a different measure of hydrophilicity to the sorption approach adopted in the present work.

3.8. Adsorption by alumina

Fig. 4 shows results for the two forms of this assay, adsorption being assessed in terms of both DOC and optical absorption at 340 nm. Between 50% and 70% of the DOC is adsorbed, but between 60% and 85% of the optically absorbing material. The two lakewater samples are the least well adsorbed, by either criterion. Variation in the results for the samples is significantly greater than in those for the SRFA standard. The assays were repeated for two samples (Table 4). The second measurement of Ads_{DOC}% for GG1 was 64% compared to 66% in the original determination, while for RS1 the second value was larger (57% compared to 51%). Values of Ads_{A340}% were both 80% for GG1, while for RS1 the first value was 81% and the second 79%. Light-absorbing material is more strongly adsorbed than DOM as a whole, which may indicate the contribution of hydrophobicity to adsorption (see below).

Davis (1982) reported measurements of the adsorption of lake sediment organic matter (9.4 mg C L^{-1}) by γ-Al_2O_3 (1.03 g L^{-1}, specific surface area 120 m^2 g^{-1}) in 0.01 M NaCl. He reported 60% adsorption at pH 4, very similar to our values (Table 5). However, our results refer to a lower Al_2O_3 concentration (0.4 g L^{-1}) and a lower Al_2O_3 specific surface area (60 m^2 g^{-1}). Since there is relatively little dependence on monovalent electrolyte concentration of DOM adsorption by alumina (Davis, 1982), it appears that the DOM samples studied in the present work adsorb to a noticeably greater extent than the material studied by Davis.

3.9. Inter-relationships among assay results

Data from the different assays were regressed against one another to test for correlations, considered at the 1% level. A strong product correlation between E_{340} and E_{280} ($r = +0.99$) reflects the fact that the spectrum of DOM varies in magnitude but not in shape (cf. Fig. 1). Negative correlations between $Hyphil_{DOC}$% and E_{280} ($r = -0.90$) and E_{340} ($r = -0.92$) are consistent with the positive relation between aromaticity; therefore hydrophobicity, and absorbance (Chin et al., 1994, 1997), and this also explains the positive correlations between Ads_{340}% and E_{280} ($r = +0.89$) and E_{340} ($r = +0.90$), hydrophobic components being more likely to adsorb (Dunnivant et al., 1992; Kaiser and Zech, 1997). Similarly, Ads_{DOC}% is negatively correlated ($r = -0.87$) with $Hyphil_{DOC}$%. Buffer capacity and fluorescence gave a high product correlation coefficient ($r = +0.89$), but the data included a statistical outlier (the point due to EW2), and the rank correlation coefficient ($r = +0.64$) was not significant ($p > 0.05$).

We did not obtain a significant relationship between $\log K_p$ and extinction coefficient for the DOM samples, in contrast to the finding of Chin et al. (1997) that $\log K_p$ for pyrene binding by five aquatic fulvic and humic acids was positively correlated ($p < 0.05$) with molar absorptivity (equivalent to extinction coefficient). However, the samples studied by Chin et al. displayed a wide variation in molar absorptivity, with a RSD of 64%, considerably greater than the variation in the extinction coefficients of the samples studied in the present work (RSD~30%). Therefore, if there is a relationship, our data, also limited to five samples, might not reveal it.

4. Discussion

4.1. Isolation of DOM

The method to obtain DOM samples for assay work is a compromise between a full isolation, with removal of all solutes except the DOM, and a mild method that produces a high yield. The method is mild in that the samples are not exposed to extremes of pH or temperature, and the yields are generally high (>90% on average). In this respect they are similar to, but slightly higher than obtained in the NOM typing project, where RO gave yields of 85–90% in most cases (Gjessing et al., 1999). The waters studied in the present work did not show any evidence of containing significant amounts of inorganic colloidal material, in that no cloudiness was generated during the rotary-evaporation step, except when precipitation of calcium carbonate took place. Therefore, it can reasonably be assumed that the active assayed component of the concentrates is predominantly DOM. However, other raw water samples might contain significant amounts of non-DOM components, the presence of which in the concentrate could lead to misleading assay results.

The final concentrate is a solution containing the dissolved anions present in the original water sample, but with Na^+ replacing the other base cations. This means that the electrolyte media of the assays are uniform with respect to the major cation, and it is a reasonable assumption that differences among samples in the inorganic anions have little or no effect on the assay results. However, the cation exchange procedure used to replace the other base cations with Na^+ was ineffective in the removal of Al or Fe, presumably because the two elements were present in strong DOM complexes, or as colloidal oxides. Strongly bound trace elements such as Cu, Hg and Pb would also survive the cation exchanger, but are unlikely to affect the assays, because their concentrations are low.

The presence of Al and Fe might have influenced the results of the assays of buffer capacity and copper binding. We explored this possibility by performing calculations with WHAM/Model VI (Tipping, 1994, 1998) and SCAMP (Lofts and Tipping, 1998) assuming that the ion-binding properties of DOM can be represented by those of isolated fulvic acid. In the case of the buffering capacity assay, the presence of Al and Fe at the maximum levels observed in the present samples ($0.3\,mmol\,Al\,gDOC^{-1}$ and $1.4\,mmol\,Fe\,gDOC^{-1}$) leads to a 20% increase in the value of Ac_{4-9}, compared to the metal-free situation. The extra base consumption arises mainly from the precipitation of $Al(OH)_3$ and $Fe(OH)_3$, for which solubility products of $10^{8.5}$ and 10^4, respectively, were assumed (Tipping et al., 2002). But the Fe content of $1.4\,mmol\,gDOC^{-1}$ applies only to one of our samples, the remainder having a content of no more than $0.2\,mmol\,gDOC^{-1}$; with this value, the increase in Ac_{4-9} is 14%. In the copper-binding assay, the main effect of Al and Fe is to compete with Cu^{2+} for binding to DOM, thereby increasing the solution activity of Cu^{2+} in the assay. The calculated increase makes $\log K_c$ in the presence of Al and Fe ca. 0.6 log units lower than in their absence. Cu binding to the precipitated Al and Fe hydroxides has negligible effect. It therefore appears that the presence of Al and Fe in the DOM concentrates will indeed influence the assay results, the consequences of which are discussed below.

The work reported here consisted of both assay development and systematic analysis of field samples. Consequently, some of the concentrates had been kept for appreciable periods of time, although stored cold and in the dark, before assays were performed. It should also be noted that the isolation procedure is quite lengthy, taking up to 7 days, and even working at the fastest rate, the last assay is not completed until 24 days after the sampling. Therefore, some repeat measurements were made to see if the assay results showed any time dependence. As noted in the results section, differences between the results of repeat assays and those of the originals were all small, and there were no consistent trends, i.e. no cases where the second results was always greater, or always smaller, than the first. Therefore, we conclude that the functional properties determined by the assays are fairly stable.

4.2. Assay approach

The assays described were designed principally to provide standardised measures of the functional properties of DOM. In most cases there is an obvious connection to environmental function. Thus optical absorbance (assays 1 and 2) is relevant to the light penetration into waters, and this is also connected to photodecomposition (assay 4), which in turn is relevant to photochemical activity, as is fluorescence (assay 3). The interactions assays 5–7 are clearly relevant to natural water chemistry and the transport and bioavailability of essential and potentially toxic metals and hydrophobic organic contaminants. The hydrophilicity assays (8 and 9) are relevant to aggregation, and sorption processes involving cells, mineral surfaces, etc., while the adsorption assays (10 and 11) address this issue for a single mineral. It was beyond the scope of this study to perform molecular characterisations in addition to the functional assays; such measurements are best carried out in more focused work, after the functional assays have identified consistently contrasting DOM sources. The work of Maurice et al. (2002) suggests that data on molecular weight and infra-red spectra would be informative.

The use of IHSS Reference SRFA as a quality standard is an essential part of the functional assay approach, permitting statistical analysis of the variability in the functional properties of the DOM samples. As can be seen from Figs. 1–4 and Table 5, the good reproducibility was achieved in the SRFA results for most of the assays. In most cases the RSD is $\leqslant 5\%$. The fluorescence values vary more (RSD = 8%) and the hydrophilicities are the most variable, with RSDs of 24% and 15% in $Hyphil_{DOC}\%$ and $Hyphil_{A340}\%$, respectively; these latter high values reflect the highly hydrophobic character of SRFA, and it may be helpful to choose an alternative quality control standard for this assay, with a hydrophilicity more similar to the DOM samples.

The assays are each restricted to a single condition, in order that a range of properties can be quantified for a number of samples. The assay results provide comparisons among the samples under conditions that represent compromises between field-relevance and practical measurement. Standardisation, and the need for a mild concentrative technique, mean that the electrolyte media in which the assays are done have higher ionic strengths than the field situations, but are devoid of divalent alkaline-earth cations. However, the samples retain the Al and Fe of the original water samples, and, as

discussed above, these metals probably influence the results of assays involving ion binding, and they may well also influence other functional properties. They are perhaps best regarded as attributes of the DOM material; since they are always strongly associated with DOM in situ (Tipping et al., 2002). However, this is not a completely satisfactory assumption because in some cases, the original water samples may contain ferrous iron, which is only weakly bound to DOM, which may oxidise during or after isolation and thereby contribute an unrepresentatively high Fe(III) content to the DOM. This may have applied to sample RS1 in the present work. Certainly, it is important to measure the Al and Fe contents of the concentrates, so that their possible effects on the assay results can be monitored.

4.3. Variability in the functional properties of DOM

Although the primary purpose of this study was to investigate variability in the functional properties of freshwater DOM, perhaps the first point to make is that, for most of the assays, the results for the DOM samples are similar to those for the SRFA quality standard. Thus, to a first approximation it can be concluded that the properties of DOM as a whole are similar to those of the major, conventionally isolated fraction. This alleviates to some extent the concern expressed by De Haan (1992) that the comparatively harsh isolation procedure for aquatic humic substances may alter their properties. One assay for which there was a consistent and large difference between the results for the DOM samples and that for SRFA was the determination of hydrophilicity, which must arise because SRFA is isolated on the basis of its hydrophobic character, i.e. by adsorption onto XAD-8 resin from acid solution. The finding that the other assay results do not differ greatly from that for SRFA suggests that the hydrophilic fraction of DOM has many functional properties in common with the hydrophobic fraction (although see the discussion about the lakewater DOM samples, below).

Variability in the assay results for the DOM field samples is statistically significantly greater than that of the SRFA standard in eight of the 11 assays, if all DOM samples are considered. The three functional properties that do not vary among the DOM samples are photochemical fading, copper binding and benzo(a)pyrene binding. The most variable property, as judged by the RSD, is hydrophilicity, expressed in terms of DOC (see Fig. 3), but not too much significance should be attached to this variability, since the reproducibility of this assay is relatively poor, as shown by the results for the SRFA standard (see Section 4.2). If only streamwaters are considered, only six of the functional assays give significantly greater variability than the SRFA standard, since now the assay results for neither fluorescence, nor hydrophilicity in terms of [DOC], are significantly more variable than those for SRFA.

The two lakewater (EW) samples, especially EW2, tend to have extreme functional properties, as shown by the reductions in the standard deviations of seven of the 11 assays, when only streamwater samples are considered (Table 5). A likely explanation is that EW2 was sampled during the period of high primary productivity in the lake (July), at which time a significant part of the DOM could be due to the exudates and decomposition products of phytoplankton. Sample EW1 was collected later in the year (October), and probably contained a greater proportion of terrestrially derived material. The streamwater DOM is presumed to be derived almost entirely from the decomposition of terrestrial plants, albeit different ones (trees for EHB1 and EHB2, grasses for GG1 and GG2, heather, cotton sedge and moss for RS1 and RS2).

Although variability among samples can be demonstrated statistically, a more important question is whether the variability has environmental implications. This question cannot be addressed directly, on the basis of the assay results alone. Instead, it would be necessary to formulate environmental models that include DOM functions, then use the models to explore the sensitivities of key outputs to variability in DOM. For example, the Biotic Ligand Model (BLM, Paquin et al., 2000) describes the toxic effects of heavy metals in terms of chemical speciation, including metal complexation by DOM. The BLM could be run with different parameter values for the DOM, to reflect the measured variability in the assays, and the outputs evaluated to determine the sensitivity of the predicted toxic effect, for example in terms of the total dissolved metal concentration required to bring it about. Such an exercise should also consider the degree of certainty with which the model can be parameterised, and sensitivity to other variables, including analytical errors in input data.

5. Conclusions

Eleven assays have been developed to characterise the following functional properties of DOM: optical absorbance at 280 and 340 nm, fluorescence, photochemical fading, buffering capacity, copper binding, benzo(a)pyrene binding, hydrophilicity in terms of DOC, hydrophilicity in terms of optical absorbance, adsorption of DOC by alumina, adsorption of optically absorbing components by alumina.

The 11 assays of the functional properties of DOM are reproducible, as judged by repeated measurements over a period of 15 months on SRFA, which is used as a quality control standard.

Samples of DOM, concentrated by rotary evaporation at 20 °C, treated with a cation-exchange resin to

remove alkaline-earth cations, and passed through 0.7 and 0.2 μm filters, and stored in the dark at 4 °C, retain their functional assay characteristics for up to 7 months.

For most of the assays, the results for eight freshwater DOM samples are similar to those obtained with SRFA. The chief exception is that the DOM samples are appreciably more hydrophilic than SRFA.

For eight of the assays, variability among the DOM samples is significantly ($p<0.01$) greater than can be explained by analytical error, i.e. by comparison with results for the SRFA quality standard. The three exceptions are photochemical fading, copper binding and benzo(a)pyrene binding.

The two lakewater samples studied gave the most extreme assay results, probably because of the influence of phytoplankton-derived DOM.

Significant correlations of hydrophilicity and adsorption with optical absorbance suggest that some DOM functional properties can be predicted from comparatively simple measurements.

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