Optimizing the removal of carbon phases in soils and sediments for sequential chemical extractions by coulometry†

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We have developed a coulometric technique to optimize the removal of the carbonate and organic fractions for sequential chemical extractions of soils and sediments. The coulometric system facilitates optimizing these two fractions by direct real-time measurement of carbon dioxide (CO_2) evolved during the removal of these two fractions. Further analyses by ICP-MS and alpha-spectrometry aided in interpreting the results of coulometry experiments. The effects of time, temperature, ionic strength and pH were investigated. The sensitivity of the coulometric reaction vessel/detection system was sufficient even at very low total carbon content (<0.1 mol kg^{-1}). The efficiency of the system is estimated to be 96% with a standard deviation of 8%. Experiments were carried out using NIST Standard Reference Materials 4357 Ocean Sediment (OS), 2704 Buffalo River Sediment (BRS), and pure calcium carbonate. Carbonate minerals were dissolved selectively using an ammonium acetate–acetic acid buffer. Organic matter was then oxidized to CO_2 using hydrogen peroxide (H_2O_2) in nitric acid. The carbonate fraction was completely dissolved within 120 min under all conditions examined (literature suggests up to 8 h). For the OS standard, the oxidation of organic matter self-perpetuates between 45 and 50 °C, a factor of two less than commonly suggested, while organic carbon in the BRS standard required 80 °C for the reaction to proceed to completion. For complete oxidation of organic matter, we find that at least three additions of H_2O_2 are required (popular methods suggest one or two).

Introduction

Sequential chemical extractions are used often to partition soil and sediment samples into operationally defined fractions. The techniques are designed to obtain indirect evidence of the primary geochemical host phases of metals, radionuclides, and other parameters. The sequential extraction approach is applied not only to study stable and radioactive-metal contaminants in the environment,[1–5] but also to investigate natural cycling of metals[6–9] and the geochemical partitioning of phosphate.[10] Variations in the experimental conditions for sequential extraction techniques are numerous: choice of reagents, reagent concentrations, reaction temperatures and reaction times vary widely. However, certain fundamental procedures are employed consistently to apply such methods. In general, a soil or sediment is subjected to a series of chemical treatments, each designed to attach a unique geochemical phase of the sample. In each step, the sample and reagent are shaken or agitated in some way (at a specified temperature) for some period of time. Following this reaction period, the solid and aqueous phases are separated by centrifugation and/or filtration. The residual solid material is reserved for the next reaction step in the sequence and the fluid phase is analyzed for the analyte(s) of interest (Fig. 1).

Perhaps the most confounding challenge to developing reliable sequential extraction methods is optimizing the selectivity of the extractions. The ideal sequential extraction protocol maximizes the selective destruction of each target phase in
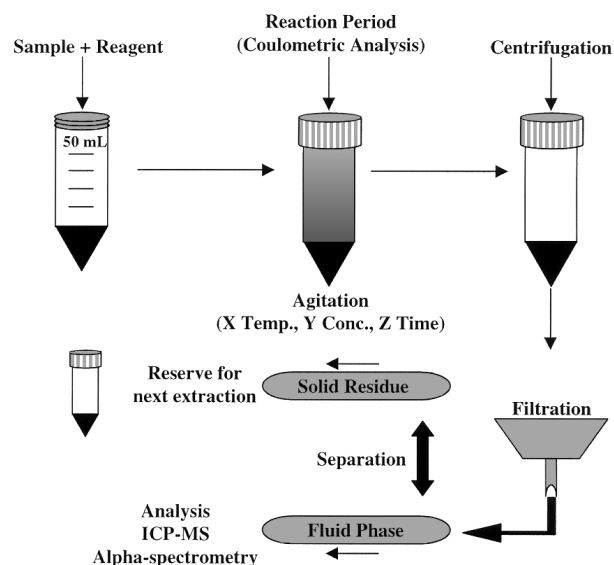

Fig. 1 Illustration of a single sequential extraction step.

the sequence and minimizes the attack on remaining soil or sediment components; the selectivity of the reaction conditions is critical. We make a critical distinction here between the terms 'fractionation' and 'speciation'. Speciation refers to specific physico-chemical forms. For example, one could refer to a polymeric aqueous Pu oxyhydroxyl complex $(PuO_2)_2(OH)_2^{2+}$ or a crystalline $Am(OH)_3$ solid. Fractionation in sequential extractions, on the other hand, refers to the process of operationally defined selective dissolution of 'macro-scale' geochemical components of solid-phase natural-matrix materials, e.g., carbonate minerals or oxides of Fe and Mn, combined with radiometric measurements of radioactive analyte nuclides released during the dissolution reaction. Although complete phase-specificity of the reaction steps is unlikely, the results of these experiments represent indirect evidence of possible and/or probable geochemical associations of the radioactive element in question with these macro-scale soil or sediment components and thus provide valuable information concerning the conditions under which the element may be released to the environment.

We are developing a standard sequential extraction protocol for determining the fractionation of radionuclides in soils and sediments. The method is being developed using NIST Standard Reference Materials SRMs. The proposed protocol is to consist of six operationally defined fractions (Table 1). As part of the method development, we are optimizing the extraction of U and Pu in a marine sediment standard (NIST Standard Reference Material 4357 Ocean Sediment—OS). In this context, optimum conditions are defined as those which allow for maximum extraction of the intended geochemical phase with minimum attack on non-targeted fractions. The phase specificity of the extractions is monitored generally by measuring the extraction of stable metals associated with the 'macro-scale' fraction in question. For example, the dissolution of reducible Fe and Mn oxides can be monitored by extraction of Fe and Mn into the fluid phase. Elements such as Al, Ti, and Si, on the other hand, would indicate the dissolution of more refractory phases. The experimental variables being tested are time, temperature and reagent concentration. The choice of reagents, reaction temperatures, and reagent concentration ranges, as well as the development of our experimental design, are described elsewhere.[11–13] In this paper, we present a novel coulometric approach to optimizing the dissolution of carbonates and the oxidation of organic matter (of soils and sediments) for sequential extractions by using real-time measurements of evolved carbon dioxide (CO_2). In addition (for the carbonate fraction), we provide stable-element data (by ICP-MS) and alpha-spectrometry results to assist in understanding the coulometry experiments. Our results provide valuable information for optimizing these two important fractions of typical sequential extraction protocols.

Carbon fractions

Carbonate fraction

'Carbonate' phases represent an important sink for radionuclides and trace metals in the environment. Trace and radioactive metals may absorb and/or co-precipitate with carbonates inorganically and biogenically.[7] For example, U is known to coprecipitate biogeochemically (with calcium Ca) from sea-water in the formation of calcium carbonate shell material.[14] Similarly, Am adsorbs strongly to the surface of calcite.[15] Carbonates may be present in soils and sediments in variable chemical composition, degree of crystallinity and particle size. The forms commonly found in soils and sediments include aragonite, calcite, dolomite, magnesite, and siderite.

A number of similar methods are recommended for the selective dissolution of carbonate minerals in soils and sediments.[1–10,16] Most recently, many of these approaches are based on a protocol presented by Tessier et al.,[6] who used a 1 mol L^{-1} sodium acetate CH_3COONa (NaAc) extraction of carbonates for 5 h, adjusted to pH 5 with acetic acid CH_3COOH (HAc). Ideally, the acetate buffer reacts with solid calcite (and other carbonate forms) in the sample to produce free-aqueous Ca^{2+}, gaseous CO_2, and liquid water H_2O by the reaction:

$$CaCO_3(s) + 2H^+(aq) \rightarrow Ca^{2+}(aq) + CO_2(g) + H_2O(l) \quad (1)$$
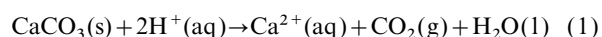
In the Tessier et al. study,[6] the authors assessed the completeness of the reaction by monitoring the dissolution of stable Ca. Although the reaction is designed to selectively dissolve carbonates, the dissolution of amorphous and easily dissolved (poorly crystalline) metal oxides/oxyhydroxides may also occur during this reaction (specifically adsorbed metals may also be extracted during this reaction step). For example, Miller et al.[16] suggested a 0.44 mol L^{-1} HAc (calculated pH 2.5) extraction for 8 h and refined their interpretation of the target 'phase' to 'some poorly crystalline hydroxy- and carbonate-metal phases'. Salbu et al.[4] modified the Tessier procedure for selective dissolution of carbonates by reducing the time of the reaction to 2 h. Our approach was to monitor the dissolution reaction, not only indirectly (by measuring stable metals in the extractant solutions), but also directly via evolved CO_2.

Organic matter fraction

Organic matter is also recognized as an efficient sink for radionuclides and metals in the soils and sediments.[17,18] Examples of organic compounds found in the environment include humic and fulvic acids. Organic-metal binding is often described by specific functional group substructures in humic and fulvic substances (such as citrates, carboxylates,

Table 1 Test fractions for developing the NIST standard sequential extraction protocol. Further details concerning the development of this standard protocol may be found in Schultz et al.[11,12]

| | | Test settings | | |
| | | | | |
Reagent	Target phase	Temperature/°C	Time/h	Concentration/M
MgCl₂	Exchangeable ions	25/50/90	1/2/4	0.1/0.4/1.0
NH₄Ac–HAc (pH 5)	Carbonates	25/50/90	1/4/16	0.5/1/2
NH₂OH·HCl (pH 2)	Reducible oxides	25/50/90	1/4/16	0.01/0.04/0.1
H₂O₂–HNO₃ (pH 2)	Organic matter	25/50/90	1/4/16	30% H₂O₂[a]
HNO₃	Resistant oxides and sulfides	25/50/90	1/4/16	4/8/16
Fusion[b]	Residual	Total dissolution		

[a]Parameter tested was the number of additions of 30% H_2O_2 needed to oxidize all organic matter. [b]Residual fraction is total dissolution of most refractory fraction of material by fusion.

phthalates, and phenols) that form moderately to highly stable metal complexes.[19]

Three common methods have been suggested for the oxidation or extraction of organic matter in soils and sediments: (1) oxidation by sodium hypochlorite NaOCl; (2) extraction by alkali [sodium (Na) or potassium (K) pyrophosphate ($Na_4P_2O_7$ or $K_4P_2O_7$)]; and (3) oxidation by hydrogen peroxide H_2O_2.

Sodium hypochlorite. NaOCl was first used by Anderson[20] to oxidize organic matter in order to pre-treat soil for mineralogical characterization. The reaction, carried out at pH 9.5, destroys soil organic matter with little non-targeted dissolution of oxides/oxyhydroxides of Mn and Fe.[21] However, the technique is designed for extraction of soils of negligible carbonate content.[22] In addition, the high pH of this reaction may cause analyte–metal hydrolysis and readsorption to remaining solid-phase surfaces prior to separation of solid and aqueous phases.[11]

Alkali pyrophosphates. Organic matter in soils and sediments is charge stabilized by calcium, iron, aluminium and other metal cations. Solutions containing anions that can remove these stabilizing metals, by precipitation, the formation of chelates or coordination complexes, are very effective in the extraction of humic materials. Extraction using alkali pyrophosphates at pH 10 has been used in a number of studies to identify organically bound metals in soils and sediments.[5,16,23] Unfortunately, alkali pyrophosphates have been shown to dissolve oxides of Fe and Mn[22] and the high pH leads to the same problems as mentioned for the NaOCl extractions.

Hydrogen peroxide. An alternative to the high pH NaOCl and pyrophosphate methods was advanced by Tessier *et al.*[6] The strategy introduced by these authors was to oxidize soil or sediment organic matter following the dissolution of carbonates and the reductive dissolution of the oxide phase. This strategy is advantageous (over the hypohalous reagents and alkali pyrophosphates) for determining organically bound metals and radionuclides. Most importantly, a lower pH (2) reduces the hydrolysis and readsorption of metallic elements to remaining solid surfaces. This point is critical for the optimization of our sequential extraction protocol; for example, Pu^{4+} hydrolysis and adsorption to solid surfaces begins at just above pH 2.[24] In addition, the specificity of this approach appears to be superior to NaOCl and pyrophosphate techniques. First, incorrect identification of inorganically sorbed analytes is minimized by a change in extraction order: the 'carbonate' and 'reducible oxide' fractions are extracted prior to the oxidation of organic material. Second, the non-targeted dissolution of carbonates by NaOCl is eliminated. Third, our coulometry experiments indicate that dissolution of reducible oxides by $NH_2OH \cdot HCl$ (pH 2, HNO_3) proceeds with no apparent oxidation of organic matter.

The approach to optimizing the selectivity of the organic matter fraction (for sequential extractions) has been, in general, to monitor the dissolution of non-targeted phases (*i.e.*, stable element analyses of extracts). However, no stable metal exists that can be used to indicate unambiguously the oxidation of organic matter for this application. In the present study, we offer a novel approach to optimizing the carbonate and organic matter fractions by monitoring evolved CO_2 produced by these reactions. This approach provides a clear picture of the rates and completeness of the reactions for these two important fractions in a sequential extraction sequence.
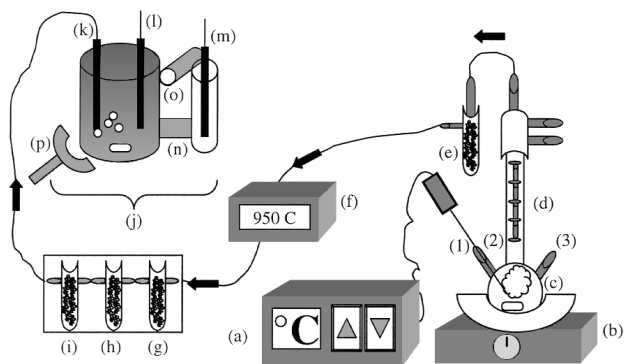
Experimental

Reagents and dilutions

All reagents used for these experiments were ACS Reagent Grade. Extractant solutions were prepared by volumetric dilutions (uncertainty of $\pm 4\%$, 95% confidence level). Mass measurements of sediment samples were made to 10 µg. Dilutions for stable element analyses (where necessary) were prepared either by volumetric dilution using 100 mL or 50 mL volumetric flasks with a stated measurement uncertainty of $\pm 2\%$ (95% confidence level) or gravimetrically using a Mettler PE 360 digital balance to 1 mg. We estimate the uncertainty for mass measurements to be $\pm 0.2\%$ (mechanical) and $\pm 1\%$ (digital) (95% confidence level).

Extractions

Sediment standards used for this study were oven-dried at 50 °C (for at least 12 h) and stored in a desiccator until use. All carbonate extractions (unless otherwise stated) used a 15 : 1 reagent (mL) to sample (g) ratio (150 mL reagent: 10 g sediment). For the stable-element analysis experiments, NH_4Ac solutions were adjusted to pH 5 by dropwise addition of 25% acetic acid. For the coulometry experiments, the pH of the solutions was varied by adjusting the concentration of the NH_4Ac in a 25% HAc solution until the desired pH was achieved. For all carbonate experiments, sediments were pre-extracted with 150 mL 0.5 mol L^{-1} magnesium chloride ($MgCl_2$) (15 mL reagent: 1 g sample, 1 h, 25 °C). For the organic matter coulometry experiments, 10 g samples were pre-extracted with 150 mL 0.1 mol L^{-1} NH_4Ac in 25% HAc for 120 min. All pre-extractions were followed by two rinses with 50 mL deionized distilled water (DDW). The sediment aliquots were separated from the solution phase by centrifugation (2500 rpm for 30 min) and the supernatant solution was either discarded or reserved for stable element analysis. For the organic fraction, the residues from these pre-extractions were then transferred to the reaction vessel using 0.1–0.001 mol L^{-1} HNO_3. Organic matter was oxidized by the addition of 30% hydrogen peroxide (H_2O_2), in 20 mL aliquots (2 mL 30% H_2O_2: 1 g sample), using a graduated glass syringe. At least three additions of H_2O_2 were required for complete oxidation of organic matter. No effect on evolved CO_2 of solution pH (from pH 1 to pH 3) was observed for these experiments. In separate experiments, the evolution of CO_2 was monitored for the 'exchangeable' $MgCl_2$ extraction and the 'reducible' $NH_2OH \cdot HCl$ fractions: no evolution of CO_2 was observed for either of these reaction steps.

Carbon measurements by coulometry

A variety of thermal and wet-chemical procedures has been developed for the measurement of total carbon (TC), organic carbon (OC) and inorganic carbon (IC). Total carbon determinations are commonly made *via* coulometry, manometry, infrared gas analysis, and gas chromatography. For this work, a coulometer was chosen to make CO_2 measurements.[25] Coulometric techniques have been used in a number of studies concerning both the TC content and the partitioning of IC and OC in sediments and sea-water.[26–28] For our purposes, the method provided the necessary sensitivity, the ability for real-time CO_2 measurements, and the flexibility to be incorporated in-line with a reaction vessel. Reactions were carried out in a 250 mL glass reaction flask, equipped with a temperature monitor/control unit (Fig. 2). A Teflon-coated thermometer was inserted into port one and sealed with a Teflon stopper. To begin an analysis, samples were transferred to the reaction vessel *via* port 3 with a minimum amount of distilled deionized water. The port was then sealed and a high-purity gaseous oxygen (O_2) carrier stream was introduced. Airtight seals were

Fig. 2 Experimental setup for coulometry experiments: (a) temperature controller/monitor; (b) magnetic stirring plate, with heating mantle; (c) reaction vessel with three ports and magnetic stirbar, Teflon coated thermometer inserted through a Teflon stopper in port 1, samples and reagents introduced through port 3; (d) water cooled condenser; (e) Drierite water vapor scrubber; (f) in-line furnace; (g) magnesium perchlorate water vapor scrubber; (h) acid dichromate NO_x scrubber; (i) MnO_2 NO_x scrubber; (j) coulometry cell; (k) CO_2 inlet port; (l) electrode; (m) silver electrode; (n) salt bridge; (o) light source; (p) photo-detector.

maintained by the use of Teflon sleeves at each port/fitting interface and spring-held glass flanges at each portal. The O_2 flow rate to the coulometer was maintained at a constant $100 \, \text{cm}^3 \, \text{min}^{-1}$. The system was allowed to purge until a constant minimum background level was achieved (1 µg C min^{-1} to 5 µg C min^{-1}). Background minima were reached within about 15 min. Reagents were added through a septum mounted at the top of port 3 using a 20 mL glass syringe. Once the reaction began, evolved CO_2 was carried through a series of purification scrubbers prior to entering the coulometer. The gas stream passed first through a water-cooled condenser followed by a Drierite tower to remove water and acid vapor. This was followed by thermal treatment of the gas stream in an in-line combustion furnace at 950 °C to assure complete oxidation of C to CO_2 [possible trace quantities of carbon monoxide (CO) are oxidized at this step]. Following combustion, final purification was achieved *via* a magnesium perchlorate ($MgClO_4$) tower (designed to remove remaining water vapor) and an acid dichromate–MnO_2 NO_x scrubber to remove acid impurities. Once purified, the gas stream enters the coulometer cell and is measured.

The coulometer cell is filled with a partially aqueous medium containing monoethanolamine ($HOCH_2CH_2NH_2$, MEA) and a colorimetric indicator. A platinum cathode and a silver electrode are positioned in the cell and the assembly is positioned between a light source and a photodetector. When a gas stream passes through the solution, CO_2 is quantitatively absorbed, reacting with the MEA to form a titratable acid (hydroxyethylcarbamic acid, $HOCH_2CH_2NHCOOH$). This acid causes the indicator color to fade. Photodetection monitors the change in the color of the solution as a per cent. transmittance (%T). As the %T increases, the titration current is automatically activated to electrochemically generate base at a rate proportional to the %T. When the solution returns to its original color, the current stops. CO_2 introduced to the cell is thus determined as a proportion to the titration current.

The efficiency of the system was determined by: (1) reacting pure calcite with the acetic acid buffer (pH 4); and (2) by measuring evolved CO_2 from the carbonate and organic matter fractions of the NIST SRM 2704 Buffalo River Sediment BRS (certified for total C). Based on these experiments, the efficiency of the system is 96% with a standard deviation of 8%. A literal interpretation of the reaction rates is not suggested here (the rate of CO_2 introduced to the coulometer cell is partly a function of the in-line O_2 flow rate). However, the

results of these experiments describe unambiguously the dissolution of carbonates and the oxidation of organic matter for optimizing the conditions for the sequential extraction of these two fractions.

Stable metal analyses by ICP-MS

In addition to CO_2 measurements, stable element analyses by ICP-MS were conducted for a separate set of carbonate extractions in this study. The intention of these experiments was to use Ca dissolution (as well as stable Al, Fe, K, Mn, Sr, and Ti) to assess the selectivity of the carbonate reaction with changes in the reaction conditions. The reaction variables tested were: (1) time of extraction; (2) temperature of extraction; and (3) reagent concentration (ionic strength). These experiments were conducted according to a full-factorial experimental design, based upon the principles presented in Box *et al.*[29] The full-factorial experimental design assesses the main effects of each of these three variables (and interactions between variables) in a minimum number of experimental runs (at predetermined variable settings). We illustrate the technique using a three-dimensional figure (Fig. 3), with the axes representing independent variables time (x_{tm}), temperature (x_{tp}), and concentration (x_c). The origin represents experiments conducted at the 'low' setting (coded '−') for each variable (1 h, 25 °C, 0.1 M NH_4Ac). The opposite end of each axis thus represents the high setting (coded '+') of each variable (4 h, 90 °C, and 1 M NH_4Ac, Fig. 3). A midpoint experiment was included to account for curvature in the relationship between response y (% metal extracted) and changes in x_i. The experimental uncertainty can be estimated by conducting duplicate extractions at tetrahedral positions on the cube. By conducting experiments at each of the indicated points on the cube, a mathematical relationship is established which describes a possible model for the response y with changes in x_i. If all variables and interactions are important, the equation that describes this model is

$$y = \frac{1}{2}\left[\begin{array}{l} \mu + \beta_{tm}x_{tm} + \beta_{tp}x_{tp} + \beta_c x_c + \beta_{tm\cdot tp}x_{tm}x_{tp} + \beta_{tm\cdot c}x_{tm}x_c \\ + \beta_{tp\cdot c}x_{tp}x_c + \beta_{tm\cdot tp\cdot c}x_{tm}x_{tp}x_c \end{array}\right] \quad (2)$$

where y is equal to the response of the dependant variable (% extracted) and μ represents the grand mean of all responses. The β_is are empirically obtained relational constants which describe the change in y (effect) as we move from the (−) to the (+) setting for each variable individually. Interactions between independent variables x_{tm}, x_{tp}, and x_c are described by terms 5, 6, 7, and 8 in eqn. (2). The main effect, *i.e.*, β_i, of each individual independent variable is determined by the difference between the average of the response y for experi-
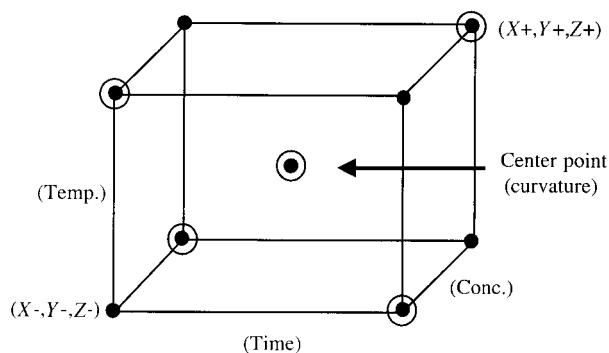
Fig. 3 Cubic representation of the full-factorial experimental design. Main effects of each variable (time, temperature, and concentration) are determined by the difference between the average response of analytes (% extracted) at the high setting (4 experiments) and the low setting (4 experiments).

ments conducted at the (−) level and experiments conducted at the (+) level., *i.e.*, $\beta_i = \bar{y}_+ - \bar{y}_-$.

We monitored the response of stable elements Al, Ba, Ca, Fe, K, Mn, Pb, Sr, and Ti at two pre-specified levels for each variable and a midpoint experiment was included to account for curvature in response (Table 1). Concentrations of stable nuclides were measured with an ELAN 5000 inductively coupled plasma mass spectrometer (ICP-MS) from PE-Sciex (Thornhill, Ontario, Canada). The TotalQuant mode, which quantifies the spectral information for elements of the entire periodic table, was used for all measurements. The instrument was calibrated using a solution containing 20 ng g^{-1} for each of 25 elements (Be, Mg, Al, Sc, V, Cr, Mn, Co, Ni, Cu, Zn, As, Se, Sr, Mo, Ag, Cd, Sb, Ba, La, Eu, Yb, Tl, Pb, Th). This solution was prepared by diluting (with 1.5% high purity HNO$_3$) a multi-element standard purchased from High Purity Standards (Charleston, SC). NIST SRMs 1640 'Trace Elements in Natural Water' and 1643d 'Trace Elements in Water' were used as quality controls for instrument calibration and sample analysis. Five replicate measurements were made for each sample (with a dwell time of 0.5 s at each nuclide mass).

Alpha-spectrometry

Uranium and Pu isotopic analyses were performed by methods similar to those presented by Burnett *et al.*[30] Radiochemical separations were performed using a tandem column arrangement, with extraction chromatography resins UTEVA and TRU (manufactured by EiChrom Industries, Darien, IL, USA). Counting sources were prepared by cerium fluoride microprecipitation/filtration and counted in vacuum-controlled alpha-spectrometry chambers using solid-state silicon semiconductor detectors.

Results and discussion

Carbonate fraction

Based on our results (from coulometry and ICP-MS analyses of the extracts from the full-factorial experiment), reaction temperature and time of extraction are important variables for determining optimum conditions for the carbonate fraction. We observed little effect on the extraction of stable elements Al, Ca, Fe, K, Mn, Sr, or U and Pu isotopes resulting from changes in ionic strength (from 0.5 mol L^{-1} NH$_4$Ac to 2 mol L^{-1} NH$_4$Ac, at pH 5, Table 2). The effects of reaction temperature and time of extraction were observed most clearly

for Ca, Mn, Sr and U. Our coulometry results clearly show that the reaction of the acetate buffer with carbonate in the OS standard proceeds to completion (independent of acidity from pH 3 to pH 5) within 2 h (Fig. 4 A,B). 'Completion' is defined here as the point where the reaction rate slows to less than one per cent. of the total-integrated evolved C. For pure calcite, the reaction is complete within about 1 h and is complete in less than 90 min for the BRS (Fig. 5). As mentioned earlier, the TC content of the OS standard is very low. Estimates by thermal techniques suggest a TC content of approximately 3.5 mg g^{-1} or 0.35%. The total Ca concentration in the OS (Ca$_T$) is about 6.3 mg g^{-1}.[31] If evolved CO$_2$ represents dissolution of CaCO$_3$, one can calculate (stoichio-
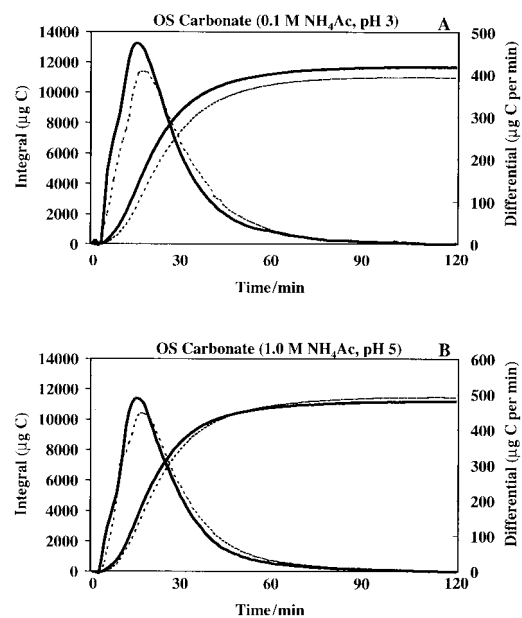
Fig. 4 Real-time measurement of evolved CO$_2$ (as µg C) for the carbonate fraction of the OS standard at pH 3 (A) and pH 5 (B). Right axis shows the reaction rate (µg min^{-1} of C). Left axis shows the total integrated CO$_2$ (as µg C) over time. Duplicate runs (solid and broken lines) are shown in this figure to highlight reproducibility. In both A and B, the reaction rate slows to background in less than 2 h.

Fig. 5 Experimental results for (A) pure calcite and (B) the carbonate fraction of the BRS standard at pH 5. The reaction rate slows to background in less than 2 h for both experiments.

Table 2 Main effects of changes in temperature, concentration, and time for extraction of select elements for the carbonate fraction of the OS standard
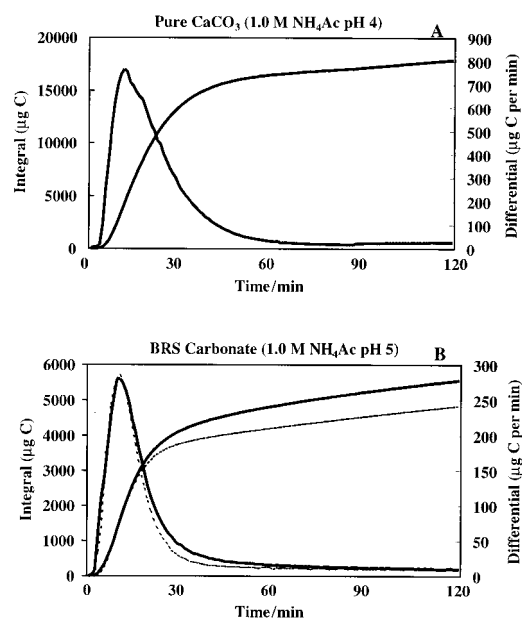
	Total extracted (%)[a]		Effect[b]		
Element	Average (μ)[c]	Range	Temp. (x_{tm})	Conc. (x_c)	Time (x_{tm})
Al	0.0	0.0–0.01	<0	<0	<0
K	2.4	1.0–4.8	1.7	0.4	0.5
Ca	33	10–50	17	<0	10
Mn	6.9	1.3–15	7.6	0.06	3.8
Fe	0.3	0.0–0.1	<0	0.18	<0
Sr	8.5	3.4–15	3.4	<0	2.6
U	6.6	1.8–14	5.2	<0	2.2
Pu	0.3	0.0–0.1	<0	0	<0

[a]Total concentrations of elements in the OS are based on estimates (given for information purposes) provided in the NIST certificate for SRM. [b]The main effects are calculated as the difference between the average per cent. extracted at the high and low settings for each variable. [c]The average corresponds to μ in eqn. 2.

metrically) an expected value for extracted Ca (under the same conditions) of about 36 mg. We ran separate carbonate extractions ($n = 4$ replicates, 1.0 mol L^{-1} NH$_4$Ac, 2 h, 25 °C, pre-extracted with MgCl$_2$) to compare the extracted Ca value with the value obtained from coulometry measurements. The value was somewhat low (33 mg Ca, standard deviation of 3 mg Ca), but within one standard deviation of the value for Ca calculated by the IC measured by coulometry.

A substantial portion of a Mn-bearing phase is dissolved when the extractions are carried out at 90 °C, the effect is enhanced for extractions carried out at 90 °C for 16 h. Uranium exhibits similar behavior: as much as 14% is extracted at 90 °C, 16 h, indicating that a correlation may exist between dissolution of Mn and U under these conditions. Further evidence that the lower temperature is more selective (in terms of carbonate dissolution) is found in U:Ca ratios of extraction solutions. If one assumes that the mass ratio of U:Ca in seawater (R_{sw}) is preserved in the formation of pure calcium carbonate shells, then this value can be compared to our extractions as a measure of specificity. For the carbonate extractions carried out at 25 °C, the U:Ca ratio (R_{ex}) agrees remarkably well with that of average R_{sw} ($R_{ex}/R_{sw} = 1.13$). On the other hand, for extractions carried out at 90 °C, the U:Ca ratio R_{90} is roughly two and one half times that of R_{sw} ($R_{90}/R_{sw} = 2.6$). The U:Ca ratio for the midpoint experiment R_{mid} (50 °C, 4 h, 1 mol L^{-1} NH$_4$Ac, pH 5) was elevated from the low settings extractions ($R_{mid}/R_{sw} = 1.6$). These results (when combined with the direct measurement of evolved CO$_2$) suggest that extraction at 25 °C, for about 2 h, is most selective (in terms of carbonate dissolution) at pH 5 using the acetate buffer.

Organic fraction

The important factors in optimizing the extraction of organic matter are the reaction temperature and the timing of the additions of H$_2$O$_2$. In optimizing the conditions for this reaction (as well as the other extractions in our sequence), the goal is to determine the least harsh conditions under which the reaction proceeds to completion. The protocol suggested by Tessier et al.[6] calls for a temperature of 85 °C and a series of four steps. The sample is first suspended in a 0.01 mol L^{-1} HNO$_3$ solution. At time $t = 0$ an aliquot of 30% H$_2$O$_2$ (2 mL H$_2$O$_2$ per g of sample) is added and the reaction is allowed to proceed for 3 h. At this point, a second aliquot of H$_2$O$_2$ is added and the reaction is allowed to proceed for an additional 2 h. Following this second reaction period, an aliquot of 3.2 mol L^{-1} NH$_4$Ac is added and the sample agitated for an additional 30 min. The addition of the salt solution is intended to prevent the readsorption of released metals to remaining solid phase particles. Salbu et al.[4] modified this overall procedure to a single addition of H$_2$O$_2$ for 5.5 h at a temperature of 80 °C, followed by the addition of the NH$_4$Ac salt solution. Our results, for the extraction of sediment organic matter from the OS standard, indicate that the temperature of the H$_2$O$_2$ reaction with organic matter may be reduced by as much as a factor of two. In our experiments, the Tessier method was simulated and the evolved CO$_2$ monitored via coulometry. Reactions were begun (by adding H$_2$O$_2$) with the HNO$_3$ solution at temperatures of 90, 70 and 50 °C. In a fourth experiment, the reaction temperature was ramped slowly from room temperature until the reaction self-perpetuated. Duplicate experiments were carried out at each temperature. Results of single experiments are shown here for simplicity.

The oxidation reactions were observed to be somewhat exothermic—the reaction vessel temperature self-elevated by as much as 20% from the set temperature while the reaction was in progress. H$_2$O$_2$ was added in aliquots proportional to

the amount suggested by Tessier et al. (20 mL of 30% H$_2$O$_2$ per addition for a 10 g sample size). In every case, at least three additions of H$_2$O$_2$ were necessary for complete oxidation of OC (Figs. 6–8). In each of the figures, Xs mark the addition of H$_2$O$_2$. For the BRS standard, sediment organic matter appears to be partitioned further into temperature dependent reaction phases. In the case of BRS standard, the highest temperature required for the reaction to self-perpetuate was 80 °C. The 80 °C temperature is consistent with that suggested by a number of authors, including Tessier et al.[6] and Salbu et al.[4] However, at least three additions of H$_2$O$_2$ were required for the complete oxidation of OC for both the OS and the BRS.

For the OS at 90 °C, the reaction rate increased rapidly to a maximum for carbon of approximately 1500 µg min^{-1},

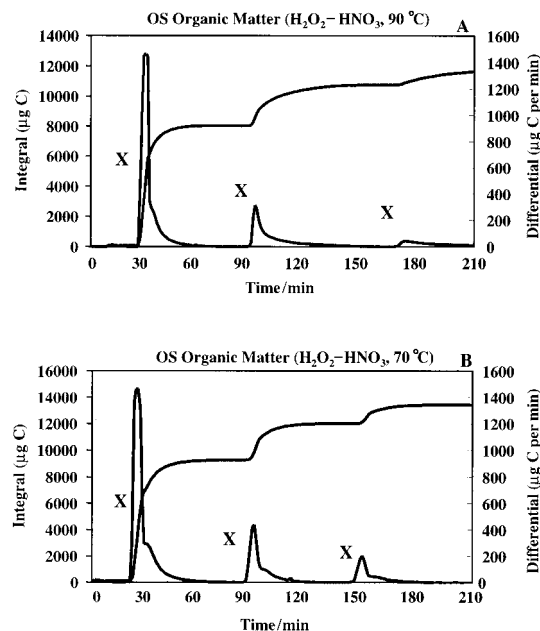**Fig. 6** Real-time measurement of the oxidation of organic carbon in the OS standard by evolved CO$_2$ (as µg C) at (A) 90 °C and (B) 70 °C. Xs mark the addition of 30% H$_2$O$_2$.
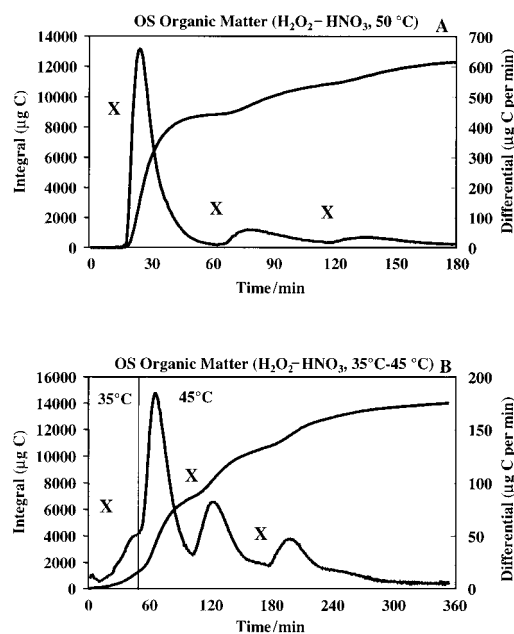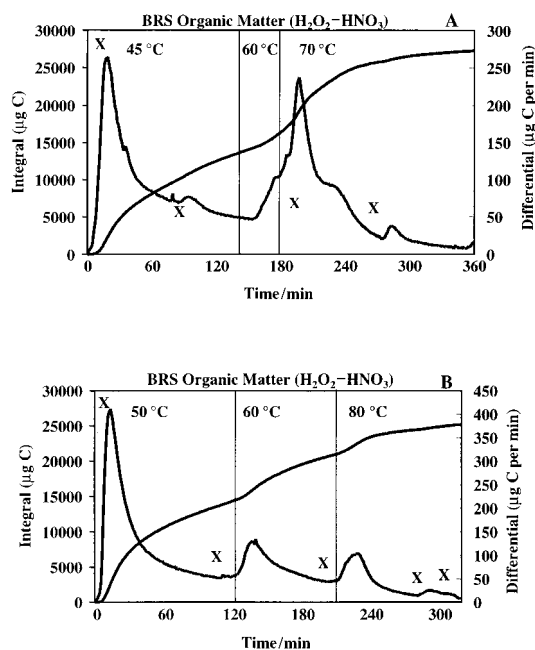
Fig. 7 Experimental results for the oxidation of organic carbon in the OS standard by evolved CO$_2$ (as µg C), at (A) 50 °C and (B) by slowly elevating the set temperature from 35 to 45 °C. X as Fig. 6.

Fig. 8 Experimental results for the oxidation of organic carbon in the BRS standard by evolved CO_2 (as µg C) for an initial experiment (A) and an 'optimized' extraction (B). Organic C in this sediment is partitioned into temperature dependent fractions. Four additions of H_2O_2 were required to reach a reaction rate of $<1\%$ of the total integrated C. X as Fig. 6.

$300\ \mu g\ min^{-1}$, and $20\ \mu g\ min^{-1}$ following each of three additions of 20 mL 30% H_2O_2 (Fig. 6A). The reactant was quickly used up with each addition as the reaction rate approached zero µg min^{-1} of carbon within about 15 min of each addition of H_2O_2. Our goal in these experiments was to understand the oxidation reaction (over time) as a function of temperature. Clearly, at 90 °C, the organic matter extraction could be completed within about 1 h, with three additions of H_2O_2 at 20 min intervals. Similar results were obtained when the reaction was carried out at 70 °C (Fig. 6B). The reaction rate slowed substantially when the set temperature was lowered to 50 °C (Fig. 7A). In this case, the rate maximum reached about 600 µg min^{-1} rapidly and fell to near zero within about 45 min. The total time needed for complete oxidation of organic matter was approximately 180 min. The ascertain a possible temperature dependent reaction barrier, a fourth experiment was conducted in which H_2O_2 was added initially at room temperature and the temperature was ramped slowly until the reaction began to self-perpetuate. When approached in this way the reaction proceeded slowly, beginning at about 35 °C, and increased rapidly to a rate maximum of about 175 µg min^{-1} of carbon when the solution temperature reached about 45 °C (set temperature of 50 °C). The reaction was slightly exothermic and reached a maximum of 53 °C with each addition of H_2O_2 (Fig. 7B). The reaction went to completeness in about 6 h when carried out in this way.

In contrast to the OS standard, OC in the BRS standard appears to be partitioned into temperature dependent fractions. A reaction maximum (~ 250 µg min^{-1}) was reached rapidly at 45 °C (Fig. 8A) and increased to over 400 µg min^{-1} when the reaction was begun with a set temperature of 50 °C (Fig. 8B). The reaction was then allowed to proceed until oxidation appeared to be at a constant minimum, at which point an aliquot of H_2O_2 was added. When no further reaction was observed (with a system purge period of about 10 min), the set temperature was increased to 60 °C. The reaction began to proceed once again at this set temperature. However, again a constant minimum was observed after about 1 h and addition

of H_2O_2 produced no further reaction at 60 °C. The set temperature was increased a third time (to 80 °C) and the reaction began to proceed, with a fourth addition of H_2O_2 required for completeness. The data show clearly that the organic matter reaction is highly temperature dependant and that at least three additions of H_2O_2 are required to oxidize OC in these two samples (BRS and OS).

Conclusions

For the OS standard, BRS standard, and pure calcite, the carbonate extraction can be accomplished (using the NH_4Ac–HAc buffer, pH 3 to pH 5) in less than 120 min. This contrasts previously suggested reaction times for this fraction of between 5 and 8 h. For the OS standard, our results indicate that increased time and temperature result in dissolution of non-targeted phases (perhaps Mn oxide/oxyhydroxide). Thus, a shorter reaction time at a lower temperature is more selective in terms of carbonate dissolution.

The oxidation of organic matter was highly temperature dependent for the two sediment standards studied. For the OS, the oxidation reaction of organic matter with H_2O_2 self-perpetuates between 45 and 50 °C, and is complete in about 6 h with three additions of 30% H_2O_2 (in 2 h intervals). On the other hand, the reaction can be completed in about 1 h (with three additions of H_2O_2) when conducted at 90 °C. In contrast, the organic carbon in the BRS can be partitioned by the reaction temperature. This sediment consists of an easily oxidized component (50 °C) and two more refractory organic carbon phases, requiring temperatures of 60 and 70 °C for the reaction to proceed. In each case, the temperature required for the reaction to proceed was lower than that commonly suggested for this application.

The timing of the additions of H_2O_2 is also critical for optimizing the selectivity of the organic extraction. At least three additions of H_2O_2 were required for the complete oxidation of organic matter in this study. We suggest that for optimum selectivity the H_2O_2 should be added intermittently as the reaction slows to near zero.

Real-time measurement of evolved CO_2 (by coulometry) is a useful tool for identifying the appropriate number and timing of H_2O_2 additions required for this reaction step. For selective sequential extractions, the lower temperature may be preferred to reduce the attack of the HNO_3 medium on non-targeted geochemical phases. Because of differences in type and quantity of carbon-bearing species in natural soils and sediments, the use of coulometry reduces the uncertainty in setting the 'optimum' conditions for selective chemical extractions.

Disclaimer

Certain commercial equipment, instruments, or materials are identified in this paper to foster understanding. Such identification does not imply recommendation by the National Institute of Standards and Technology, nor does it imply that the materials or equipment are necessarily the best available for the purpose.

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