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RESEARCH ARTICLE

An improved wet oxidation method for radiocarbon analysis of dissolved organic carbon in a simulated freshwater matrix

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Abstract
The analysis of the radiocarbon age of dissolved organic carbon (DOC) is fundamental for understanding the aquatic component of the global carbon cycle, yet the technique is not routinely available at radiocarbon laboratories. This study presents validation experiments for an improved wet oxidation method for ^{14}C-DOC analysis in a freshwater matrix. Emphasis in design protocol for the method was placed on the quantitative removal of inorganic carbon, and a background level consistent with modern accelerator mass spectrometry (AMS) radiocarbon measurements. The method utilizes a pre-oxidized potassium persulfate oxidant in crimp-sealed vials with rigorous multi-stage helium purging to achieve and maintain a sample without atmosphere carbon dioxide and the contamination of modern ^{14}C (^{14}C-free). Method validation of ^{14}C-free samples are demonstrated with procedural blanks, phthalic anhydride (PhA), and an International Atomic Energy Agency Oxalic Acid standard (IAEA-C8).

Introduction

Dissolved organic carbon (DOC) is the largest reactive reservoir of organic carbon in aquatic environments and plays a critical role in global carbon cycling (Battin et al. 2009; Derrien et al. 2019; Hedges 1992). It serves as a crucial source of carbon and energy for aquatic organisms, playing a vital role in regulating biogeochemical processes, nutrient cycling, and pollutant transport within freshwater ecosystems (Battin et al. 2023; Leenheer and Croue 2003). The origins of DOC in water are varied, encompassing recently produced (terrestrial/aquatic) litters and metabolic by-products, soil organic carbon, aged organic carbon from bedrocks, and anthropogenic pollution (Butman et al. 2015; Drake et al. 2019; Nai et al. 2023). Radiocarbon (^{14}C) dating of DOC in freshwater ecosystems could help elucidate the origins of organic carbon and estimate its turnover rate, hence enhancing the evaluation of carbon source-sink dynamics and providing critical insights into the function of DOC within the global carbon cycle (Hood et al. 2009; Marwick et al. 2015; McDonough et al. 2022).

Radiocarbon dating of DOC relies on analytical methods that effectively prevent exogenous carbon contamination. Several methods have been developed for the extraction and analysis of ^{14}C in DOC. Over the years, various methodologies have been developed for the extraction and analysis of ^{14}C in DOC. Williams et al. (1969) first introduced the UV oxidation method for ^{14}C-DOC analysis, which through continuous improvement, has achieved low ^{14}C background values with reduced sample sizes

(Beaupre et al. 2007; Williams and Druffel 1987; Xu et al. 2021; Xue et al. 2015). However, its limited sample throughput and dependence on specialized equipment hinder its broader adoption. Combustion following freeze-drying enables direct ^{14}C measurement, but it is time-consuming and may result in elevated procedural blanks (Burr et al. 2001; Palmer et al. 2001). Ultrafiltration and solid-phase extraction provide advantages in fractionating DOC by molecular weight and chemical characteristics, respectively, but require extensive pre-processing and may introduce biases through selective retention of specific compounds (Druffel et al. 1992; Wassenaar et al. 1991). Leonard et al. (2013) and Lang et al. (2016) proposed a high-throughput, low-cost wet oxidation method for ^{14}C-DOC analysis, achieving effective control over low background values. Here, we present an improved ^{14}C-DOC analytical method based on previous wet oxidation techniques in a simulated freshwater matrix, which features low background values, reduced complexity, and minimized costs. This method enhances accessibility and accuracy in radiocarbon dating of DOC by employing straightforward experimental procedures and minimizing extraneous carbon interference. This method could provide a practical and reliable approach for most radiocarbon laboratories, providing a reliable framework for investigating carbon cycling and residence time in freshwater systems.

Materials and procedures

The wet oxidation method for ^{14}C-DOC (Figure 1) was conducted in a 100 mL crimp-sealed glass vial. A chemical blank, phthalic anhydride (PhA Sigma Aldrich, PN-320064-10 g) and an oxalic acid standard from the International Atomic Energy Agency (IAEA-C8, 0.1513 ± 0.0007 $F^{14}C$) (Clercq et al. 1997) were used as standards to verify the feasibility of the method. Synthetic solutions containing inorganic carbon are formulated with pulverized shell standard, a calcium carbonate powder (0.1503 ± 0.0007 $F^{14}C$, 2553 ± 63 BP, $n = 40$). The measurement of radiocarbon is highly sensitive to extraneous carbon contamination (Lang et al. 2016). The multi-step procedure of converting DOC into CO_2 and final ^{14}C analysis presents the potential to introduce extraneous carbon contamination at various stages. These potential sources of extraneous carbon can originate from the residual inorganic carbon (i.e. atmospheric CO_2) in the crimp-sealed glass vials, or as impurities in the oxidant. In addition, effective removal of inorganic carbon present in the aqueous samples is required for the analysis of the target analyte DOC. Therefore, a procedural blank is employed to evaluate potential contamination: a ^{14}C-free PhA solution is subjected to the same experimental procedures as the unknown DOC samples.

DOC solutions were separately prepared by dissolving PhA (3.5 mg/50 mL) and IAEA-C8 (7.5 mg/50 mL) in UV treated Milli-Q water (18 MΩ·cm, total organic carbon < 3 ppb), followed by the addition of (8.3 mg/50 mL) calcium carbonate powder ($CaCO_3$, UNSW Internal Standard-4804). The mixtures were homogenized using an ultrasonic bath (20 min, 25 °C) to efficiently disperse the $CaCO_3$ powder (UNSW Internal Standard-4804) and simulate inorganic carbon in natural water samples. The bulk solutions are subsampled, 50 mL of the homogenized solution is transferred into a 100 mL crimp-sealed glass vial, followed by the addition of 1 mL of phosphoric acid (H_3PO_4, 85%, AR grade, Chem-supply Pty Ltd, Australia). The vial is crimp-sealed using a grey-butyl septa and aluminum crimp cap. All glassware is cleaned by combusted at 480°C for 4 hr, and grey-butyl septa are cleaned by immersion in 8 g/L potassium persulfate ($K_2S_2O_8$, ACS reagent, Sigma-Aldrich, Germany) solution at 60°C for 8 hr followed by a Milli-Q water rinse.

The synthetic DOC solution and phosphoric acid mixture is aggressively shaken for 1 min by hand and then left to react for over 12 hr, ensuring complete transformation of inorganic carbon (UNSW Internal Standard-4804) to CO_2. The headspace and the solution are purged with high-purity helium (He) gas (99.999%) for 20 min to effectively strip the synthetic solution and purge the headspace of the 100 mL vial. Following the helium purge, the sample is left undisturbed at room temperature for over 12 hr to allow dissolved gases to reach equilibrium, after which a second 20-min helium purge is conducted. The sample is then placed in an oven at 60°C overnight, ensuring that any residual inorganic

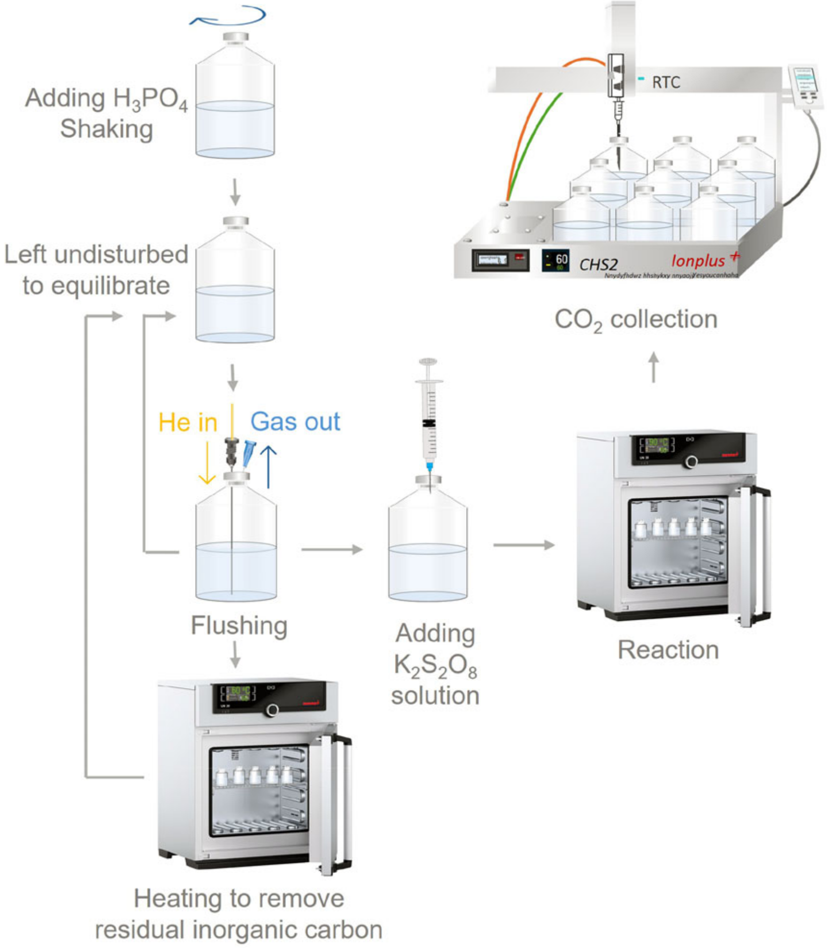

Figure 1. *Pretreatment steps for* ^{14}*C-DOC via wet oxidation method.*

carbon was fully converted to CO_2. The purge-equilibrium cycle was then repeated twice more to ensure the complete removal of inorganic carbon in the synthetic DOC solution.

The oxidizing reagent $K_2S_2O_8$ is prepared as a saturated solution (40 g/L) of $K_2S_2O_8$, prepared by dissolving 4 g of $K_2S_2O_8$ and 100 µL of H_3PO_4 in 100 mL Milli-Q water (Lang et al. 2013). The prepared oxidizing solution is sealed in 100 mL glass crimp vials and placed in an oven at 60°C for 8 hr for pre-oxidization. Any potential inorganic carbon contaminants are removed by purging the solution with high-purity helium gas prior to use a procedure which has been proven to effectively remove extraneous carbon contamination from the oxidant (Rauber et al. 2023).

After the final purge of inorganic carbon from the synthetic DOC solution, 10 mL of the pre-oxidized $K_2S_2O_8$ solution is immediately injected into the sample using a clean syringe (one 10-mL pre-wash with 40 g/L $K_2S_2O_8$ solution). To accelerate the oxidation process, the vial is shaken and placed in an oven at 90°C for 12 hr. The sample is taken out of the oven and allowed to cool to room temperature.

The automated Carbonate Handling System (CHS2, Ionplus) transfers the CO_2, produced from the oxidation of DOC and accumulated in the headspace of the 100 mL glass vial through a selectively permeable Nafion™ polymer membrane in a helium carrier gas. The Nafion™ polymer membrane transfers water molecules from one side of the membrane to the other by a first order kinetic reaction. The dry sample gas of CO_2 is transferred into the Automated Graphitization Equipment (AGE3, Ionplus) system with the helium carrier gas. The CO_2 purging time of the headspace is set to 360 s, with

Table 1. *Results of the tested standards (no chemical blank correction applied)*

Laboratory code	Standard	Material	Measured $F^{14}C$ ^{A}	Date ^{14}C yr BP ^{B}	Target size (µg C)
UNSW-3782	PhA	Phthalic anhydride	0.0015	52480	982
UNSW-3783	PhA	Phthalic anhydride	0.0017	51000	958
UNSW-3784	PhA	Phthalic anhydride	0.0014	52660	995
UNSW-3785	PhA	Phthalic anhydride & $CaCO_3$	0.0020	50100	743
UNSW-3786	PhA	Phthalic anhydride & $CaCO_3$	0.0018	50970	986
UNSW-3787	IAEA-C8	Oxalic acid & $CaCO_3$	0.1503	15223	979
UNSW-3788	IAEA-C8	Oxalic acid & $CaCO_3$	0.1505	15215	1000

$^{A}F^{14}C$ values have uncertainties of less than 0.0005 at the 1σ level. ^{B} There are several assumptions implicit in the citation of a conventional radiocarbon age (date), for example the Libby half-life for ^{14}C of 5568 years was used; 'before present' (BP) refers to 1950 for the reference year zero; and that 0.95 NBS Oxalic Acid provided the modern reference standard. Radiocarbon years BP (^{14}C yr BP) are the units to express the date.

a helium gas flow rate of 60 mL/min. The CO_2 is concentrated in a zeolite trap, which is heated to 420°C to release pure CO_2 into the graphitization reactor tube. The CO_2 sample is then reduced to graphite at 580°C with hydrogen on iron powder. Radiocarbon analysis of the graphite target is conducted using the Mini Carbon Dating System (MICADAS, Ionplus) at University of New South Wales in the Chronos Radiocarbon Laboratory (Turney et al. 2021) and the procedure is detailed elsewhere (Suter et al. 2010; Synal et al. 2007; Wacker et al. 2010). Transmission and fractionation are corrected and normalized to the NIST OxA2-SRM 4990C (OXAII) for the MICADAS AMS measurements, with radiocarbon results reported as the fraction of modern carbon ($F^{14}C$).

Results

The $F^{14}C$ value of PhA determined using the traditional high-temperature combustion method in the laboratory is 0.0015 ± 0.0006 (n = 626). The background value of PhA obtained using the wet oxidation method proposed in this study was 0.0015 ± 0.0002 (n = 3) (Table 1), and consistent with the expected value for traditional high-temperature combustion method. Thus providing, with confidence, the outlined wet oxidation method described above introduced negligible extraneous carbon contamination during this pre-treatment procedure.

In natural non-saline water samples, inorganic carbon is typically present. To evaluate whether inorganic carbon is effectively removed using this experimental method an additional 8.3 mg/50 mL of $CaCO_3$ powder (UNSW Internal Standard-4804, 0.1503 ± 0.0007 $F^{14}C$, 2553 ± 63 BP, n = 40) is added to each PhA sample. The $F^{14}C$ mean value of the PhA & $CaCO_3$ samples is 0.0018 ± 0.0001 (n = 2) (Table 1) and equivalent to a radiocarbon age of 50,500 BP. Again, the chemical blank of PhA verifies that modern CO_2 was not introduced by the method and the chemical blank of PhA and inorganic carbon show that the (1 mg C/50 mL) 2553 BP carbonate powder was stripped from solution before the DOC oxidation reaction. Further validation of the wet oxidation method was conducted using IAEA-C8 as an internal standard with added $CaCO_3$ powder. The $F^{14}C$ value of IAEA-C8 has been reported as 0.1504 ± 0.0017 (Clercq et al. 1997) and 0.1504 ± 0.0006 (n = 316) at Chronos Radiocarbon Laboratory. The weighted mean of the measured $F^{14}C$ values for IAEA-C8 processed with the wet oxidation method was 0.1503 ± 0.0001 (n = 2). This indicates that both the inorganic carbon present in the sample and that in the headspace of the crimp-sealed glass vials could be effectively removed, with oxidative reaction of the IAEA-C8 yielding an accurate value for the radiocarbon standard.

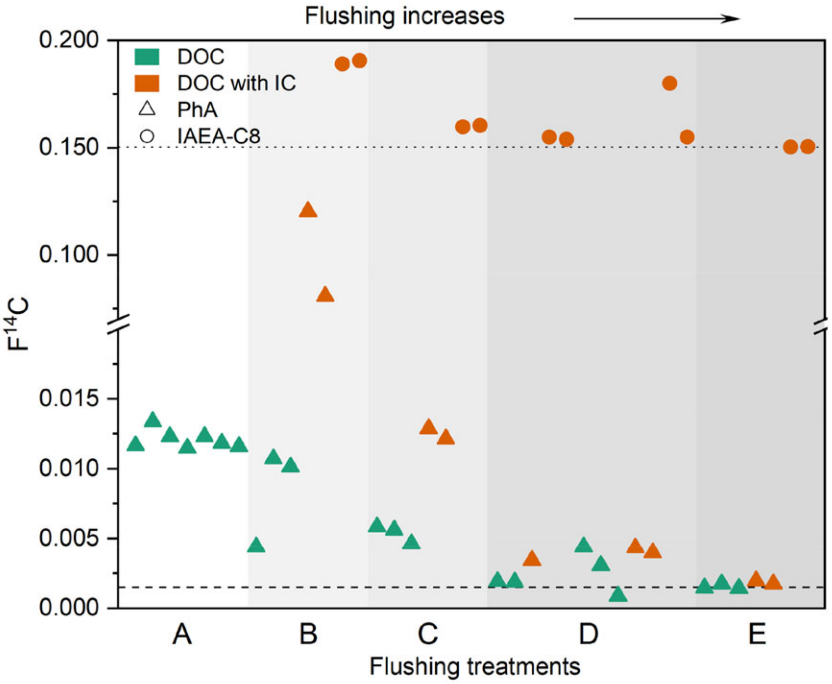

Figure 2. *Radiocarbon measurements of phthalic anhydride (PhA, triangle) and International Atomic Energy Agency Oxalic Acid standard (circle) obtained from wet oxidation method with different flushing strategies. A, headspace flushing; B, headspace and solution flushing once for 10 min, C, headspace and solution flushing once for 20 min, D, headspace and solution flushing 3 times for 20 min every time; E, headspace and solution flushing 4 times for 20 min every time. DOC samples without IC are colored in red and with IC are colored in blue. Dotted line and dashed line represent the expected* $F^{14}C$ *value for IAEA-C8 and PhA, respectively. All* $F^{14}C$ *values have uncertainties of less than 0.0008 at the 1σ level.*

Discussion

Effective removal of extraneous carbon is crucial for obtaining accurate radiocarbon values. Based on the measured inorganic carbon content in the samples, it is possible to calculate the theoretical amount of H_3PO_4 required to completely convert inorganic carbon to CO_2. Consequently, adding excess H_3PO_4 to the samples and purging with CO_2-free gas is a reasonable approach to effectively remove both the inorganic carbon present in the sample and that in the headspace of the vial. However, experimental results indicate that multiple stages of helium purging are necessary to achieve effective removal of extraneous inorganic carbon in the matrix to allow for the accurate measure of the target analyte DOC (Figure 2).

Different sample purging strategies were applied to evaluate their effects on radiocarbon analysis of DOC (Figure 2). When only the headspace of the sample was purged, the $F^{14}C$ value of PhA without added inorganic carbon was 0.0121 ± 0.0006 (n = 7), indicating that extraneous carbon contamination was not effectively removed. When a single purging step was applied to the entire sample, including both the headspace and liquid phase, the background value improved to some extent, yet trace amounts of extraneous carbon remained (Figure 2, B, C). When the number of purging cycles was increased to three (Figure 2, D), with equilibration periods between each purging step to allow for gas-liquid equilibrium, the $F^{14}C$ value of PhA without inorganic carbon was 0.0024 ± 0.0012 (n = 5), while that of PhA with inorganic carbon was 0.0039 ± 0.0004 (n = 3). Correspondingly, the $F^{14}C$ value of IAEA-C8 with inorganic carbon was also higher than expected values (Figure 2, D). This suggests that three purging cycles are still insufficient to completely remove extraneous carbon.

When the number of purging cycles was further increased to four (Figure 2, E), both PhA with and without added inorganic carbon reached $F^{14}C$ values comparable to expected $F^{14}C$ value of PhA via the traditional high-temperature combustion method (0.0015 ± 0.0006, $n = 626$). Recent studies have reported DOC radiocarbon analysis methods using 12 mL Exetainer vials, where a single purging step was sufficient to effectively control extraneous carbon contamination (Lang et al. 2016; McIntyre et al. 2016; Strähl et al. 2024). However, based on the comparison of purging strategies in this study, a multi-stage approach (four purging-equilibration cycles) is necessary for effective removal of extraneous carbon from DOC samples of tens of milliliters in volume.

This multi-step purging process increases the sample preparation time of DOC analysis, however a prerequisite for consistent backgrounds and accurate results. Additional methods and types of sparging apparatus should be investigated to reduce the preparation time of this critical step. Many previous studies have generally reached a consensus that $F^{14}C$ results are dependent on the carbon content of the sample (Lang et al. 2016; Leonard et al. 2013; Strähl et al. 2024; Xu et al. 2021; Ziolkowski and Druffel 2009), especially for low concentration DOC sample, where the uncertainty in $F^{14}C$ values increases significantly.

The method described here outlines an effective procedure for a known concentration of DOC, the effective concentration of DOC solutions of PhA and IAEA-C8 are both 3.3 mmol/L (2 mg C in 50 mL). With the oxidation of DOC by $K_2S_2O_8$ and recovery of CO_2 sample from the headspace at 100% efficiency, these concentrations of DOC in the PhA and C8 solutions yield 2000 μg C for the graphite target and AMS radiocarbon analysis. Results indicate a recovery rate of DOC in this improved wet oxidation method was $58.4\% \pm 7.4\%$ ($n = 6$), which may be due to the residual CO_2 remaining in gas-liquid equilibrium (Molnár et al. 2013). Molnár et al. (2022) suggested that by performing timed repeats of CO_2 collection from the headspace, the recovery rate could be increased to 75%–80%. In addition, methods and types of sparging apparatus could be investigated to improve the headspace recovery. Methods which sparge the liquid sample and collect the headspace gas simultaneously may may enhance CO_2 capture efficiency, thereby effectively increasing the DOC recovery rate in the wet oxidation method. It is important be noted that incomplete CO_2 recovery from the headspace may cause kinetic fractionation of ^{13}C, whereas ^{14}C is largely unaffected as the values are normalized to a standard $\delta^{13}C$ values during data processing (Molnár et al. 2013; Takahashi et al. 2021; Xu et al. 2021). Consequently, the method is appropriate for ^{14}C-DOC analysis, but not for $\delta^{13}C$ studies. Moreover, due to the incomplete recovery of CO_2, carbon isotope measurements alone cannot determine whether all DOC has been fully oxidized. To improve the recovery, we recommend that the oxidation efficiency of DOC could also be assessed via measuring residual DOC concentrations in the post-reaction solution in future studies.

The method indicated that a concentration of 20 mg C/L DOC (1 mg C/50 mL) is required for sufficient CO_2 to yield a 500 μg C for a graphite target sufficient for high-resolution radiocarbon analysis. Increasing the sample volume is an effective approach to obtaining sufficient carbon for reliable radiocarbon values in lower concentration DOC samples. In natural freshwater samples, DOC concentrations vary from 0.002 mg C/L to 1041 mg C/L (Marwick et al. 2015; McDonough et al. 2020). A lower recovery rate requires larger sample volumes for low-DOC-content samples, thereby increasing the difficulty of radiocarbon analysis for DOC. This improved wet oxidation method could be coupled with existing rotary evaporation techniques to improve the range of DOC concentrations measurable by AMS.

Conclusion

This study presents an improved wet oxidation protocol for DOC radiocarbon analysis, demonstrating low procedural blanks, effective removal of extraneous carbon, and reliable radiocarbon measurements comparable to conventional high-temperature combustion techniques. The implementation of multiple purging-equilibration cycles was found to be essential for eliminating inorganic carbon contamination, particularly for larger sample volumes. Future experimental optimizations should focus on enhancing DOC recovery rates and combining rotary-evaporation preconcentration techniques to investigating the influence of varying DOC concentrations on $F^{14}C$ measurement results, aiming to reduce sample size requirements for the analytical method and the dynamic range of DOC concentrations measurable in natural sample.

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