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Simple, quantitative method for low molecular weight dissolved organic matter extracted from natural waters based upon high performance counter-current chromatography

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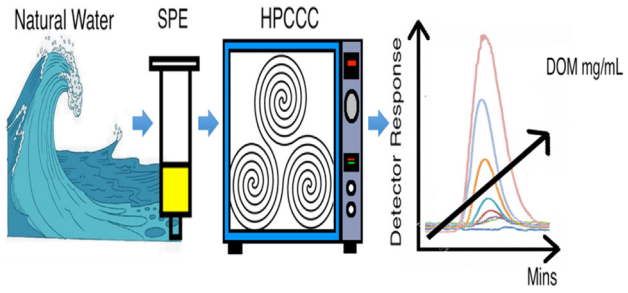
HIGHLIGHTS

- New chromatographic method for quantification of extracted dissolved organic matter.
- First application of reversed-phase HPCCC to marine dissolved organic matter.
- Method requires extraction of only small volumes of natural waters.
- Quantitation based upon both UV absorbance and evaporative light scattering detection.

GRAPHICAL ABSTRACT



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ABSTRACT

A simple, high-performance counter-current chromatography method with sequential UV absorbance (254 nm) and evaporative light scattering detection (ELSD) was developed for the quantification of pre-extracted low molecular weight dissolved organic matter (DOM) extracted from natural waters. The method requires solid-phase extraction (SPE) extraction of only small volumes of water samples, here using poly(styrenedivinylbenzene)-based extraction cartridges (Varian PPL). The extracted and concentrated DOM was quantified using reversed-phase high-performance counter-current chromatography (HPCCC), with a water/methanol (5:5) mobile phase and hexane/ethyl acetate (3:7) stationary phase. The critical chromatographic parameters were optimised, applying a revolution speed of 1900 rpm and a flow-rate of 1 mL min^{-1}. Under these conditions, 50 μL of extracted DOM solution could be injected and quantified using calibration against a reference natural dissolved material (Suwannee River), based upon UV absorbance at 254 nm and ELSD detection. Both detection methods provided excellent linearity ($R^2 > 0.995$) for DOM across the concentration ranges of interest, with limits of detection of 4 μg ml^{-1} and 7 μg ml^{-1} for ELSD and UV absorbance, respectively. The method was validated for peak area precision (<5%), and accuracy and recovery based upon spiking seawater samples prior to extraction, together with DOM solutions post-extraction (>95% recovery). The developed method was applied to the determination of the concentration of DOM in seawater, based upon initial sample volumes as small as 20 mL.

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1. Introduction

Dissolved organic matter (DOM) in natural waters (seawater and freshwater) represents a complex and heterogeneous mixture of organic compounds, with a wide range of polarity and chemical complexity [1–5]. This complex organic matrix can be defined practically as all organic-based materials that pass through a 0.20 μm pore size filter, which includes, for example, carbohydrates, amino acids, lipid-like material, carboxyl-rich alicyclic molecules or CRAM [2–4,6,7], and all manner of other complex terrigenous and anthropogenic compounds. The important role of DOM within both freshwater and marine biochemical processes is a subject of much investigation, including its function as a source of carbon and other essential nutrients [8]. DOM also represents one of the Earth's largest carbon reservoirs, comparable to the amount of carbon present within the atmosphere [9]. There is little doubt therefore of the importance of understanding the nature, source and distribution of this material, particularly when striving to understand and predict global carbon cycles.

Analytical approaches to DOM characterisation and quantification have been the subject of a number of reviews [10–13]. Within these, reference to the development of quantitative methods for freshwater and seawater DOM can be found, although the majority of these methods are based upon traditional physical and spectroscopic (including florescence) based methods. Currently, most of the measurements of DOM are derived from the determination of dissolved organic carbon (DOC) and the total (i.e., dissolved and suspended) organic carbon (TOC), obtained using high temperature catalytic (HTC) oxidation of total organic carbon to CO_2, which is later analysed by infrared absorbance detection [14,15]. Stubbins and Dittmar have taken this technology further by developing a low volume method for the quantification of DOC and dissolved nitrogen, using a modified Shimadzu HTC TOC analyser [16]. The method could be applied to DOC quantification in aqueous samples of a volume less than 600 μL, with a LOD of 3.4 μM quoted for DOC deep seawater consensus reference material.

Recently Sandron *et al.*, introduced the use of high-performance counter-current chromatography (HPCCC) for the partial fractionation of DOM, using normal phase conditions, and they applied this separation technique to DOM pre-extracted from a freshwater source [17]. HPCCC is a chromatographic technique based upon the partitioning of material between two immiscible solvent systems, and it has gained particular application in the fractionation and isolation of natural and synthetic products [18,19]. Due to the principle of distribution of all species between two immiscible solvent systems, this liquid–liquid phase chromatographic method offers the following advantages: (1) no irreversible adsorption and therefore complete recovery of the chromatographed sample, (2) simple technology (low-pressure method, readily scalable), and (3) low cost of operation (tubing in place of traditional chromatographic columns). However, the most important of these features is the potential to completely recover all injected sample materials, as the stationary phase itself can also be eluted from the column tubing at the end of each run and collected for further analysis. With previous traditional liquid chromatography approaches to DOM separation and isolation, such as size exclusion chromatography (SEC) for example, the complexity and diversity of the material inevitably results in irreversible adsorption of some components, leading to semi-permanent column contamination and unresolved and excessively broad eluting 'humps' in the place of quantifiable peaks [20–23].

In the previous work using HPCCC for DOM fractionation, the application of normal phase HPCCC saw a considerable proportion of the extracted DOM sample elute unretained from the column [17]. Therefore, the method was not suitable for any quantitative applications. In reversed-phase HPCCC, wherein the less polar organic phase is retained within the column (acting as the hydrophobic stationary phase), this formally unretained DOM should now exhibit retention, and thus permit quantification against similar standard materials. Reversed-phase HPCCC uses water (or aqueous buffer) as the mobile phase in combination with less polar solvents such as acetonitrile (MeCN) or methanol (MeOH), whose concentration can be adjusted to manipulate retention. Reverse-phase chromatography also has the advantage of being able to use pH selectivity to improve separations.

Therefore, within the following paper, the advantages of reversed-phase HPCCC as a technique for the quantification of pre-extracted DOM have been explored. A reversed-phase HPCCC method was developed and applied to coastal seawater sourced DOM quantification, obtained using a standard solid phase extraction (SPE) procedure, with water sample collection volumes of less than a litre. Given the heterogeneous nature of DOM, both UV absorbance and evaporative light scattering detection (ELSD) were used in tandem following the HPCCC separation. The potential of the simple approach to routine quantification of extracted DOM from large numbers of water samples, without the need for large volume sampling, is presented.

2. Materials and method development

2.1. Solvents and reagents

MeOH, ethyl acetate and HCl were obtained from Sigma–Aldrich (Sydney, Australia). Hexane was purchased from Emsure (Merck, Kilsylth, Victoria, Australia). Deionised water was obtained using a Milli-Q system (Millipore, Melbourne, Australia). The dye, Acid Red 18 ($C_{20}H_{11}N_2O_{10}S_3Na_3$), was obtained from Sigma–Aldrich (Sydney, Australia) and used as received as a void volume marker.

2.2. Standards and seawater DOM

Suwannee River natural organic matter (SR-NOM), identified as 2R101N and isolated in 2012, was obtained from the International Humic Substances Society (IHSS). SR-NOM is commonly used as a standard reference material in DOM studies, as the source and composition of this material has been well described previously [24,25]. Coastal seawater samples were collected from Kingston, Tasmania, Australia (42°58′37″S 147°18′30″E).

2.3. Preparation of standard solutions

A standard stock solution of SR-NOM (5.000 mg mL^{-1}) was prepared in a solution of MeOH and water (5:5). Working standard solutions ($n = 10$) were prepared by diluting the stock solution with 50% MeOH over the range 2.525–0.005 mg mL^{-1}. The standard stock and working solutions were all prepared shortly before use and the stock standard materials and extracted dried DOM stored in air tight containers at −80 °C at all other times.

2.4. Preparation of sample solutions

DOM was isolated from the seawater source as described by Dittmar *et al.* [6] and Green *et al.* [24]. Briefly, the water was filtered through glass microfiber Whatman GF/F filters (0.20 μm pore size; Fisher Scientific, Australia) and acidified to pH 2 using HCl (32%). These steps were performed immediately after sampling. Different volumes of seawater were then passed through prewashed Varian PPL cartridges (Varian, Stockport, UK) containing 1 g of poly(-styrene divinylbenzene) (PS-DVB) particles in 10 mL volume polypropylene columns, and the retained DOM eluted using one