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A comparison of methods for the extraction of dissolved organic matter from freshwaters

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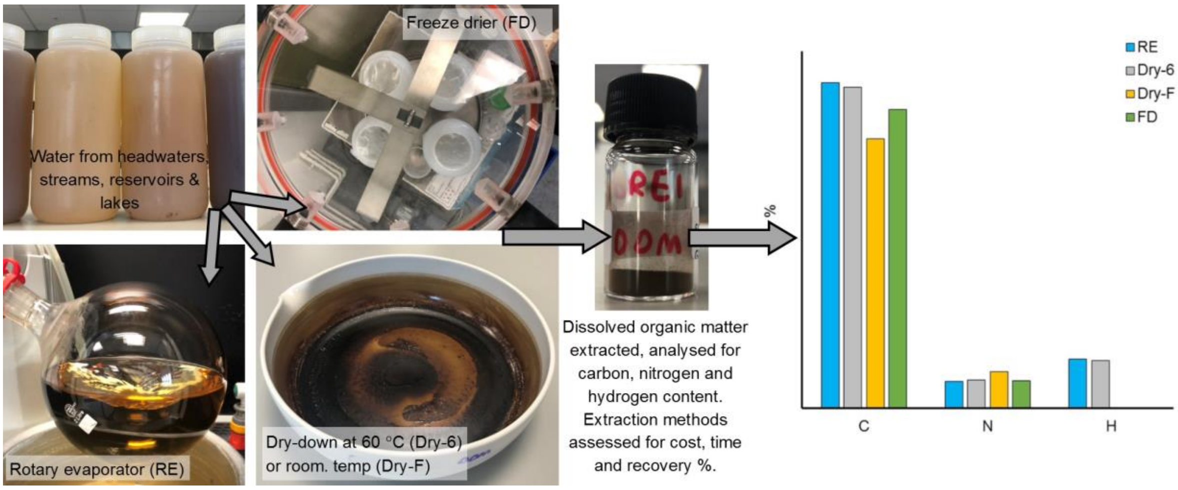
Highlights

- Methods of extracting dissolved organic matter from freshwaters vary in efficiency
- Dissolved organic matter was extracted from freshwaters in the UK
- Several methods were evaluated, and the time, cost and results of each assessed
- Rotary evaporation, freeze-drying and dry-down gave the most consistent results

Graphical Abstract



Dissolved organic matter extracted, analysed for carbon, nitrogen and hydrogen content. Extraction methods assessed for cost, time and recovery %.

Abstract

Studies of dissolved organic matter (DOM) composition have used several different methods to concentrate and extract the DOM from fresh water, but the impact of these methods on the composition of the DOM is relatively unknown, as very few studies use more than one method to compare results. The aim of this study was to use several methods, frequently used in the scientific literature, to concentrate and extract DOM from fresh water and compare the elemental and functional group composition of the extracted DOM. In addition, the cost, in terms of money, resources and time, were assessed for each method. The results showed that the elemental and functional group composition of the extracted DOM varied between methods significantly. The methods that yielded the most similar and reproducible DOM results were rotary evaporation, dry-down at 60°C and freeze-drying. Although each of these methods required a relatively expensive piece of laboratory equipment, this was a 'one-off' cost, and consumables and time per sample were relatively low. This study highlights the dangers of comparing DOM data from different studies when the DOM has been extracted via different methods. In future, it is recommended that studies of DOM composition report their methods of extraction clearly and consistently, ideally using one (or more) of the methods showing reliable results here.

KEYWORDS: elemental analysis, rotary evaporation, freeze drying, reverse osmosis, dialysis, dry-down

1. Introduction

Dissolved organic matter (DOM) is found in natural waters around the world, and plays an important role in the transport of nutrients, energy and carbon from terrestrial ecosystems to the atmosphere, aquatic and marine systems. Terrestrial inputs of carbon to inland waters are estimated to be 5.1 Pg C year^{-1} (Drake et al 2018), and processing of DOM in lakes and rivers releases 2.1 Pg C year^{-1} to the atmosphere as CO_2 (Raymond et al 2013). The composition of DOM controls the reactivity, and therefore the proportion of the matter that degrades and releases carbon dioxide (CO_2), or travels to the ocean (Bowen et al 2020). In order to better understand the link between DOM in fresh water and other factors such as degradability, catchment characteristics or water treatment efficiency, the DOM composition needs to be known. Water companies in the UK and Northern Europe need to know how the composition of DOM in their source waters impacts on the efficiency of drinking water treatment and the potential for the formation of harmful disinfection by-products (such as trihalomethanes; Kothawala et al 2017; Valdivia-Garcia et al 2019).

The DOM composition presented in existing literature on fresh waters may not be the ‘true’ composition of the ‘whole’ sample, as the composition may be altered by the method, and studies can only analyse the extractable fraction (Song et al 2018). With increased use of advanced molecular characterisation methods (e.g. Fourier transform ion cyclotron resonance mass spectrometry (FTICR-MS; Matilainen et al 2011)), it is important to be assured that the organic matter being characterised is not just a by-product of the concentration method or extraction method used.

Some methods attempt to classify and quantify the DOM ‘in-situ’ - while it is in solution in natural water (Table 1). These methods include analysing its absorbance spectrum in the UV and visible wavelengths (e.g. Dobbs et al 1972; Peacock et al 2014), or using excitation emission fluorescence spectroscopy (e.g. Smart et al 1976; Chen et al 2003). Such approaches rely on ‘proxy’ measurements to characterise the coloured components of DOM - the organic matter is classified as ‘humic-like’, ‘fulvic-like’ or ‘more aromatic’ instead of quantifying the composition directly. Classifying and quantifying the DOM ‘in-situ’ is generally simple, relatively quick and inexpensive, and valuable information can be gathered, but depending on the wavelengths used, they are limited to the coloured portion of DOM (CDOM), and a proportion of DOM is ‘optically invisible’ to these techniques (Pereira et al 2014). The results are also influenced by other factors of the water chemistry, such as pH and ionic strength (Matilainen et al 2011). Both absorbance and fluorescence measurements were found to be sensitive to a range of environmental conditions (such as pH, ionic strength and metal ions), however this is likely related to the environmental conditions impacting the DOM structure, and the subsequent measured absorbance and fluorescence spectra (Spencer et al 2007).

In order to analyse and understand DOM more thoroughly, the DOM can be extracted from natural water, and analysed as a solid, or re-dissolved in higher concentrations than would naturally occur (e.g. Søndergaard et al 2003; Lv et al 2016; Whitty et al 2019). Studies often acknowledge the limitations of their extraction methods, and consequently refer to the DOM extracted fraction of material as a proportion of the whole, total DOM. For example, Dittmar et al (2008) refer to the DOM they extracted from seawater as “SPE-DOM”. Very few studies have used more than one method to extract the DOM, and so it is unknown how much the method of extraction impacts the DOM composition analysed in the study (Minor et al 2014; Tfaily et al 2012). Some researchers have used methods to concentrate the DOM before extraction, such as Koprivnjak et al (2009), who used reverse osmosis and electrodialysis, and Lankes et al (2008) who used rotary evaporation followed by freeze drying. Matilainen et al (2011) carried out a review into the methods for characterising natural organic matter (NOM – includes particulate and colloidal organic matter, not just the dissolved fraction), including assessing the methods for concentrating and extracting NOM from water. Their study focussed on methods used to extract a targeted fraction of the NOM, based on features such as molecular size and polarity, and discussed the pros and cons of each method. By targeting certain polarities and molecular sizes, the study acknowledged the methods included were not analysing the total portion of NOM, and so not reflecting ‘total’ NOM (Matilainen et al 2011). Whitty et al (2019) used ^{1}H-Nuclear Magnetic Resonance spectroscopy (NMR) to compare DOM extracted by freeze-drying with ‘whole water’, and discussed the pros and cons of other extraction methods. Despite these studies showing differences between NOM and DOM fractions extracted by different methods, there is very little evidence showing how the composition of the whole DOM composition is impacted by the extraction method. For the purposes and focus of this study, organic matter smaller than 0.7 µm is classed as DOM. A literature search was carried out to identify the most frequently used extraction and concentration methods (Table 1). Each method identified in the literature search was entered into Google Scholar (March 2020), along with "dissolved organic matter", "DOM", "water" and "surface OR inland OR fresh", and the number of results used as a proxy to reflect how popular/frequently used each method is. The most frequently used methods were found to be rotary evaporation, freeze drying, dry-down, solid phase extraction, reverse osmosis and dialysis. This is by no means a comprehensive study of all extraction methods, but includes the most common methods for extracting DOM, regardless of molecular size and polarity, from inland fresh waters. Resins, such as XAD-8, were not included in this study, as the literature search revealed several uncertainties, questions and criticisms of the reliability and extraction biases of this method, especially in regard to potential contamination from resin-bleeding, and chemical alteration of samples (e.g. Kitis et al 2001; Sleighter et al 2009). XAD-8 resins and the conditions needed to use them to extract DOM (such as pH changes) have been showed affect the characteristics and reactivity of the DOM fractions (Song et al 2009).

Table 1. The methods and techniques used to concentrate, extract and analyse DOM composition, and pros and cons of each method. Literature references listed include an early study using the technique and a recent example of use in a study on DOM composition. The number of results in a Google Scholar search (March 2020) containing the method and "dissolved organic matter", "DOM", "water" and "surface OR inland OR fresh", are shown in the final column.

Method	Pros	Cons	References	Google Scholar N
Absorbance	Easy and quick	Some DOM is colourless	Dobbs et al 1972 Peacock et al 2014	21300
Fluorescence	Proxy composition information Relatively quick	Some DOM does not fluoresce	Smart et al 1976 Chen et al 2003	21000
Rotary evaporation (RE)	Increased surface area for vaporization of the solution	Uses heat, which could alter the water chemistry	Craig et al 1950 Dean et al 2019	444
Solid phase extraction (SPE)	Relatively inexpensive, reliable	Extracted fraction depends on column, polarity, solvent	Thurman and Mills 1998 Lv et al 2016	3850
Dry-down (DRY)	Low-tech	Uses heat, which could alter the water chemistry At room temperature, microbial growth	Veitch 1904 Worrall et al 2018	101
Reverse osmosis (RO)	Concentrates DOM relatively quickly	Recovery percentage affected by pH of water	Hauck and Sourirajan 1969 Green et al 2015	3360
Freeze-dry (FD)	Results in a solid DOM without any other processing	Expensive equipment	Geiger and Cataldo 1969 Lankes et al 2008	127
Dialysis (DIA)	Resulting DOM contains fewer ‘impurities’ such as salts	Membrane size may exclude smaller DOM molecules	Tan 1977 Aristilde et al 2017	2380

The aim of this study was to compare different extraction methods used in the literature to quantify the impact on the elemental composition of the resulting DOM. While no extraction method can claim to be perfectly replicating the ‘natural’ composition of DOM as it would be in the water, this study aims to find the method that causes the least disruption to the composition, is economical (in terms of cost, resources and time) and is replicable and reliable.

2. Method

The DOM extracted from natural water using nine different methods was assessed. The time and costs of each method were recorded, and elemental and functional group composition of the DOM was analysed.

2.1. Sample collection

Water samples of between 5 and 20 L were taken from 14 fresh water bodies across the UK, including natural lakes, reservoirs and upland streams to ensure a variety of dissolved organic carbon (DOC) concentrations and DOM compositions (Table S1). The sites were located between 53.4 and 59.3 °N, and -3.9 and -0.7 °E. Samples of water from each water body were filtered (DI-rinsed 0.45 µm syringe filters) and analysed for DOC concentrations (Analytik Jena Multi NC2100 combustion analyser). The DOC concentrations ranged from 4.7 to 44.4 mg L^{-1}.

2.2. DOM extraction methods

All water for DOM extraction was filtered through 0.7 µm glass microfiber filters (VWR). This size of filter was used so as to include the colloidal fraction of organic matter, but exclude particulates. The filtered water was then split and subject to various methods to extract the DOM. The time taken, resources needed and mass of DOM collected via each method were recorded. Some methods were combined as the primary method concentrated rather than fully extracted the DOM and therefore did not result in a solid DOM sample.

2.2.1. Rotary Evaporation (RE)

One litre of filtered water was poured into an evaporating flask. The water bath temperature was 60°C, and cold tap water was used to cool the condenser, which was in a vacuum. The evaporating flask was topped up with filtered water as the 'clean' water in the sample evaporated and condensed in the receiving flask (Cranwell et al 2017). At most, 5 L of sample water was used. 'Clean' water from the receiving flask was routinely collected, filtered and analysed for DOC concentration. This clean water had a mean DOC concentration of 0.23 mg L^{-1} (range 0-0.96 mg L^{-1}; $n = 25$). A t-test showed there was significantly less DOC in the clean water than the initial water samples ($p < 0.001$). At most, less than 1 mg L^{-1} of DOC was discarded in the clean water.

The concentrated water remaining in the evaporating flask (less than 500 mL) was poured into a 1.1 L evaporating dish which was then put in an oven at 60 °C until the liquid had evaporated. The residue was then collected. This method was used on water from all 14 water bodies.

In order to analyse the reproducibility of the rotary evaporation method, water samples from a further 15 UK fresh waters were split into two and rotary evaporated, resulting in 15 pairs of DOM samples ($n = 30$). These samples were not included in the 'time, cost and recovery analysis', but were subject to elemental analysis (as in 2.3.).

2.2.2. Solid Phase Extraction (SPE)

Known volumes of up to 1 L of filtered water were poured through Bond Elut PPL 500 mg, 6 mL cartridges (Agilent) conditioned with 6 mL 100% methanol followed by 6 mL deionized water. This cartridge was chosen based on a search of literature using solid phase extraction, and found to be the type recommended for DOM extraction from natural water (Li et al 2019). The sample water was forced through the cartridge using a hand pump to create a small pressure gradient. The cartridge was then washed in 6 mL deionized water, air dried using the hand pump to pull air through cartridge. To elute the DOM sample, 12 mL of 100% methanol was used, which was then evaporated off at room temperature and the residue collected. This method was used on water from three sites.

2.2.3. Dry down (DRY-6, DRY-F)

The filtered water was poured into a 1.1 L evaporating dish and then kept either in a fume hood at room temperature (DRY-F) or in an oven at 60 °C (DRY-6) until all liquid had evaporated. The evaporating dish was chosen so as to have a large surface area to volume ratio. The evaporating dish was topped up routinely, until approximately 5 L of water had evaporated. The residue was then collected. Dry down in the oven (DRY-6) was used on water from 13 sites, and dry down in the fume hood (DRY-F) was used on water from six sites.

2.2.4. Reverse Osmosis (RO) followed by rotary evaporation (RE) or dry down (DRY-F)

Reverse osmosis removes ions and large particles from water under pressure, with the aim of providing cleaner water. Approximately 10 L of the 0.7 μm filtered water was pumped through a customised reverse osmosis unit. The unit contained a 5 μm polypropylene filter, a 1 μm polypropylene filter, and a carbon filter. The whole unit and filters were washed in deionised water before each sample was pumped through. The ‘dirty’ water was fed back into the unit and the ‘clean’ water collected separately, until the majority of the water was ‘clean’. Samples of this ‘clean’ water were collected, filtered and analysed for DOC concentration. The analysis of the clean water showed that it contained an average of 8 mg L^{-1} DOC. This was almost as high as the DOC concentration in some of the initial water samples, showing that a lot of the DOC in the original water was getting through the filters into the clean water fraction. The clean water fraction was discarded, and the final dirty water fraction, containing less DOM, was collected. The carbon filters were rinsed in DI water, and this was added to the dirty water (as it could have retained carbon molecules). Due to the high concentration of DOC in the clean water, this method was not continued.

Once the volume of ‘dirty’ water was approx. 2 L, this was collected, then split, with 1 L further evaporated in the rotary evaporator (RO-RE; as in 2.2.1.) and 1 L evaporating to dryness in an evaporating dish at room temperature (RO-DF; as in 2.2.3.). Both reverse osmosis methods were used on water from two sites.

2.2.5. Freeze Drier (FD)

A known volume of filtered water was placed in the freeze drier at -50 °C until the water had sublimated, and the remaining solid material was collected. This method was used on water from five sites.

2.2.6. Dialysis (DIA) followed by FD or DRY-F

A total of 200 mL of filtered sample water was poured into regenerated natural cellulose membrane dialysis tubing (10 kDa MWCO (molecular-weight cut-off), 29 mm diameter, Spectrum Labs), clipped at the bottom and top. The dialysis tubing was then put into a beaker of deionised water. The deionised water was replaced regularly. The water was split into two

100 mL volumes, one of which was put in the freeze drier (DIA-FD; as in 2.2.5.), while one was left to evaporate at room temperature (DIA-FH; as in 2.2.3.). Both dialysis methods were only used on water from one site.

2.3. DOM analysis

The extracted DOM was weighed, then analysed by elemental analysis to measure the content of carbon, nitrogen and hydrogen in the samples. The samples were treated with hydrochloric acid to remove inorganic carbonates, then analysed again for the organic carbon proportion. A sub-set of samples (n = 23) were also analysed by solid state ^{13}C nuclear magnetic resonance (NMR) to look for differences in functional group distribution. The method of NMR used means that directly quantifying the results was not possible; however, they can be compared to each other, and expressed relative to the amount of total C in the DOM sample (Hockaday et al 2009). The 23 samples analysed by NMR included DOM extracted from nine sites (sites 1-9), by six different methods (both dry down methods, freeze drying, rotary evaporation, and both reverse osmosis methods).

Due to various time, equipment and water constraints, not all extraction methods were used on water from all sites. One method, rotary evaporation (RE), was carried out on water from all sites (n = 14), so the composition data were analysed relative to the RE extracted DOM sample. This does not mean that the composition of the RE extracted DOM was presumed to be the ‘true’ DOM composition, only that this method was applied to water from all 14 sites.

2.4. Time, cost and recovery

In order to evaluate each method, the following were recorded, and ranked from ‘best’ to ‘worst’:

- The time taken from the start of sample processing to collecting the final DOM sample, and the **rate of processing** (litres per hour). Methods were considered ‘better’ if they had a relatively fast processing rate, or took a shorter amount of total time.
- The **recovery percentage**, relative to the DOC concentration of the original water sample. Recovery percentages of over 100% were possible, as the water used for DOM extraction was filtered through a larger filter size than the water analysed for DOC concentration (0.7 vs 0.45 µm filter) in order to include colloidal organic matter. Ideally, the carbon content of the DOM would be similar to the carbon concentration in the original water, resulting in a recovery percentage close to 100%. Rank scores were allocated based on how far from 100% the mean values fell. The recovery percentage for each sample was compared to various water chemistry variables, to investigate which properties of the water or DOM influenced the recovery percentage of the DRY-6, DRY-F, FD and RE extraction methods (where $n > 2$). The DOM samples extracted by RE and DRY-6 methods (where $n > 10$) were also analysed grouped by water body type (e.g. headwaters and streams). Variables used to calculate the recovery percentage (DOC concentration and carbon content of the DOM) were not included in this analysis.
- The approximate **cost of consumables per sample**. Lower costs per sample were considered ‘better’.
- If the method required any larger laboratory equipment (lab oven, rotary evaporator, freeze drier), these were not included in the cost per sample, but were considered when ranking the total cost per method. The ‘**large equipment score**’ was the cost of the large equipment divided by 1000, to give a number between 0 and 10.

Certain costs were not included, such as electricity and tap water, but these were reflected in the total time taken. Costs of collection and analysis of samples was not included as this will be the same for all samples. The three criteria (rate of processing, recovery percentage and cost per sample) were ranked, and added to the large equipment score. Each method was allocated a score based on the sum of these.

3. Results

A total of 47 DOM samples were successfully extracted by all methods (Tables 2 and 3). The additional 15 pairs of rotary evaporated samples were analysed separately (n = 30).

Table 2. The mean average total carbon, nitrogen and hydrogen, and organic carbon, relative to the average content of the RE DOM. The closer the value to 1, the more similar the DOM composition is to the RE DOM composition. Values in brackets are the standard errors.

Method	N	Total C	Total N	Total H	Organic C
dry down at 60 °C	13	0.99	1.07	0.97	0.95
(DRY-6)		(0.02)	(0.03)	(0.02)	(0.03)
dry down at room. temp.	6	0.83	1.51	1.08	0.81
(DRY-F)		(0.04)	(0.21)		(0.06)
reverse osmosis-rotary evaporation	2	0.46	0.14	0.56	0.17
(RO-RE)		(0.03)	(0.08)	(0.03)	(0.13)
reverse osmosis-dry down at room. temp.	2	0.49	0.23	0.51	0.12
(RO-DF)		(0.07)	(0.12)	(0.06)	(0.09)
freeze drier	5	1.05	1.02	-	1.04
(FD)		(0.09)	(0.02)		(0.03)
dialysis-freeze drier	1	2.06	1.55	-	-
(DIAFD)					
dialysis-dry down at room. temp.	1	1.04	2.59	-	-
(DIAFH)					

3.1. Elemental and functional group composition

The results show that the most significant differences in DOM compositions were between the sites, rather than the method of extraction, and so all further analysis of elemental data was carried out on the composition relative to the 14 rotary evaporated (RE) DOM composition for each site (Table 2). The RE DOM composition was used as this method was carried out on water from all sites. The closer the value to 1, the more similar the extracted DOM composition is to the RE DOM composition. The solid phase extraction (SPE) method resulted in DOM samples that were too small to analyse further, so it was not continued.

The average elemental composition of the dry down at 60 °C **(DRY-6)** DOM was between 0.95 and 1.07 of the RE DOM values. There was not enough H data for the DOM extracted by freeze drier (**FD)**, but for total and organic C, and total N, the average results across sites were 1.05, 1.04 and 1.02 of the RE DOM values, respectively. A one-way ANOVA on extraction method (RE, DRY-6 and FD) showed no significant differences ($p > 0.05$) in the raw composition data (total C, N, H, and organic C) between the three methods. These data showed that despite the differences in methods, involving changing the temperature and pressure of the samples, the DOM extracted was similar in elemental composition.

The reverse osmosis (**RO-RE** and **RO-DF)**, dry down at room temperature (**DRY-F)** and dialysis (**DIAFH** and **DIAFD**) methods resulted in samples with very different composition data, varying between 0.12 and 2.59 of the RE data. It was not surprising that the dialysis method DOM was different to the other methods, as the size of membrane used allows smaller molecules to pass through it, retaining larger molecules. This resulted in the loss of very low molecular weight DOM, resulting in different DOM composition. From the DOC concentration of the ‘clean’ fraction of the water disposed of by the reverse osmosis (average

8 mg L^{-1}), it was clearly not extracting all of the DOM in the water, and so the elemental analysis of the resulting DOM would be different to that extracted by other methods. The DRY-F method took an average of 392 hours per sample (more than 16 days) to evaporate less than 2 L of water. During this time, the water was warm and light; any microbes smaller than 0.7 µm would have remained after filtration and could have degraded the DOM, releasing CO_2. This would have led to a preferential loss of the more reactive carbon, changing the DOM composition and DOC concentration, as reflected in the lower recovery of DOM (69%).

The additional 15 pairs of rotary evaporated samples had incredibly similar results – paired t-tests showed there were no significant differences in the total C ($p = 0.46$), N ($p = 0.34$), or H ($p = 0.41$) between the replicates ($n = 30$).

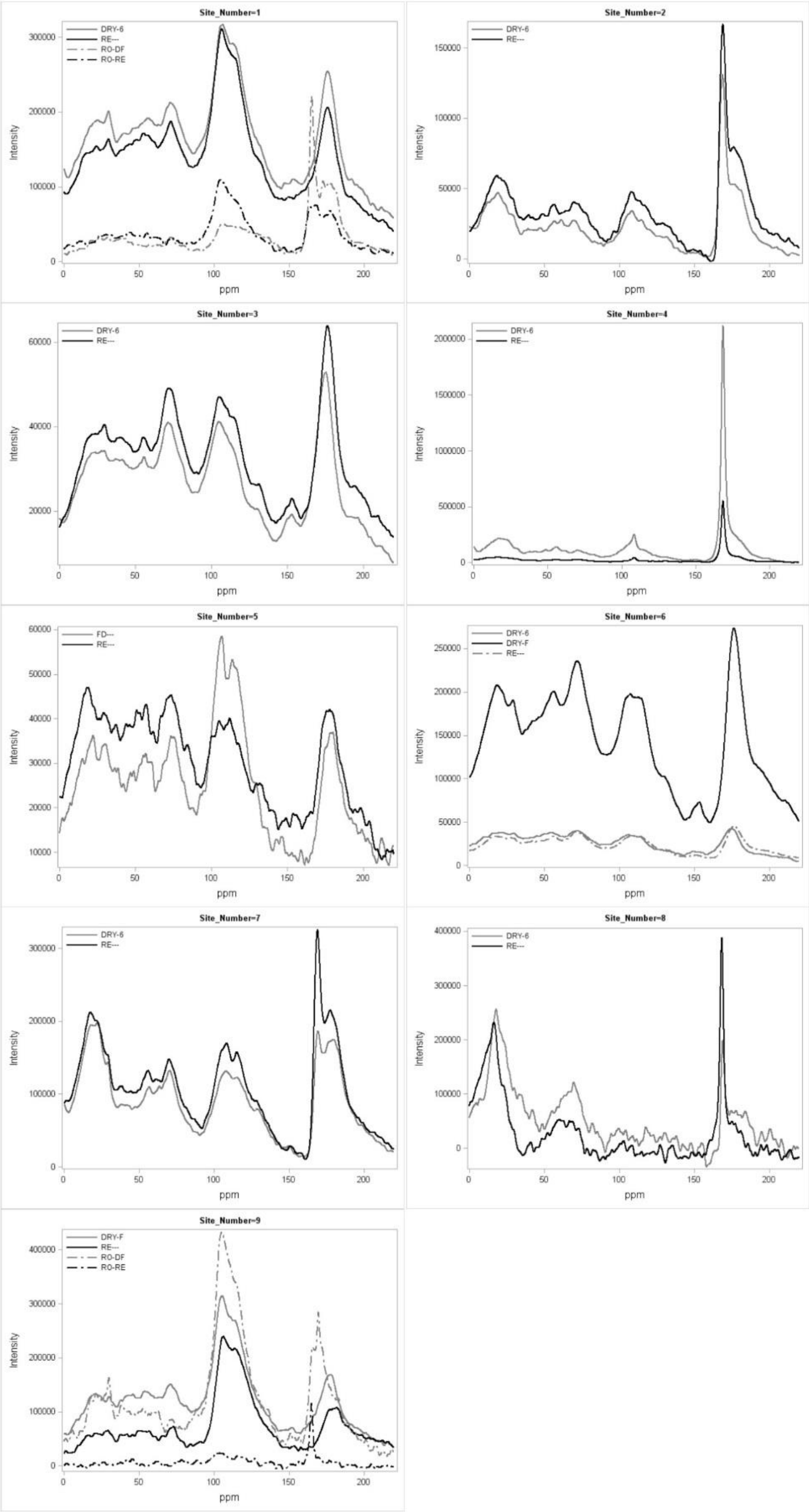
The ^{13}C-NMR was carried out on 23 samples from nine of the 14 field sites (Figure 1). Similar samples will have similar spectra – the lines will be close together and follow a similar pattern. If the samples have different functional group compositions, the lines will be different.

As with the elemental composition data, these data showed similar results for the **RE** and **DRY-6** methods, and more variable results for the **RO-RE** and **RO-DF** samples. In the water from Site 1, the RO-RE and RO-DF methods have very low intensities at the majority of ppms, whereas the DRY-6 and RE samples have very similar spectra. Peaks in the lower range ppms (below 100) reflect functional groups such as C-alkyls, N- alkyls and O-alkyls, and these seem to be present in the DRY-6 and RE samples, but much lower or even missing from the RO-DF and RO-RE samples.

The RE and DRY-6 methods also had very similar results in water from study sites 2, 3, 6, 7, 8 and 9. The DOM from Site 4 are the only samples where the DRY-6 and RE samples have different intensity results, however the majority of the peaks are at the same ppm locations.

The DOM from water from Site 5 was extracted by FD and RE, and both spectra show similar patterns, but much lower intensities than in samples from other sites. The four samples from Site 9 show similar spectra to Site 1, although the RO-DF sample has the highest intensities and RO-RE has the lowest – missing several of the peaks present in the other samples.

Figure 1. The NMR spectra for each sample (n = 23, from 9 sites). The ’intensity’ (y-axis) is relative to the total C in each sample. Similar samples will have similar spectra – the lines will be close together and follow a similar pattern. If the samples have different functional group compositions, the lines will be different.


Site_Number=1
DRY-6
RE---
RO-DF
RO-RE
Intensity
300000
200000
100000
0
ppm
0
50
100
150
200
Site_Number=2
DRY-6
RE---
150000
100000
50000
Site_Number=3
DRY-6
RE---
60000
40000
20000
Site_Number=4
DRY-6
RE---
2000000
1500000
1000000
500000
Site_Number=5
FD---
RE---
60000
50000
40000
30000
20000
10000
Site_Number=6
DRY-6
DRY-F
RE---
250000
200000
150000
100000
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Site_Number=7
DRY-6
RE---
300000
200000
100000
Site_Number=8
DRY-6
RE---
400000
300000
200000
100000
Site_Number=9
DRY-F
RE---
RO-DF
RO-RE
400000
300000
200000
100000

3.2. Time, cost and recovery

The results of this analysis show that the methods ranked as ‘best’ with the lowest overall scores were the rotary evaporation and dry-down methods (Table 3). The **SPE** method resulted in DOM samples that were too small to analyse further, so it was not continued. It was also relatively expensive per sample. The resulting overall score was 17, and therefore it was ranked fourth.

Table 3. The rate (mL hr-1), recovery percentage (± standard error) and cost per sample (GBP and USD) for each method. The overall score is the sum of the ranks of rate, recovery percentage and cost per sample, and large equipment score (lower number = better method). The notation ‘n.a.’ = no additional equipment and/or costs. Rank and score values are in italics.

Method	Rate (mL hr^{-1}) and *rank*	Recovery (% ± SE) and *rank*	Cost per sample (£, $) and *rank*	Large equipment cost (£, $) and *score*	Overall score and *rank*
RE	897.44	100 (11)	9.43, 12.39	2000, 2630	10
	1	*1*	*6*	*2*	*1*
DRY-6	39.94	93 (8)	2.21, 2.90	2000, 2630	10.5
	2	*2*	*2.5*	*2*	*2*
DRY-F	4.59	69 (10)	2.21, 2.90	n.a.	12.5
	6	*4*	*2.5*	*0*	*3*
SPE	503.33	-	102.67, 134.91	n.a.	17
	2	*8*	*7*	*0*	*4*
RO-RE	100.40	158 (87)	384.50, 505.25	2000, 2630	18.5
	3	*5*	*8.5*	*2*	*5*
FD	5.36	89 (6)	n.a.	10000, 13140	19
	5	*3*	*1*	*10*	*6*
DIA-FH	0.46	37	8.79, 11.55	n.a.	20.5

	9	*7*	*4.5*	*0*	*7*
RO-DF	2.92	159 (75)	384.50, 505.25	n.a.	21.5
	7	*6*	*8.5*	*0*	*8*
DIA-FD	1.39	237	8.79, 11.55	10000, 13140	31.5
	8	*9*	*4.5*	*10*	*9*

The **RE method** had a low cost per sample and the recovery percentage of DOM was high (100 ± 11%). Water samples with a low starting DOC concentration (less than 10 mg L^{-1}) had a much more variable recovery percentage (range 36 to 183%), whereas water samples with higher starting DOC concentration (above 10 mg L^{-1}) had a lower recovery percentage range (64 to 147%). The recovery percentage was positively correlated with the pH of the source water ($p = 0.05$, $R^2 = 0.28$, $n = 14$); DOM samples with lower recovery percentages were from water sources with lower pH values. The relationships between pH and recovery percentage were stronger when the DOM samples were grouped according to the type of water body sampled. The pH of the source water explained the majority of the variation in the DOM recovery percentages from headwaters and streams samples ($p < 0.01$, $R^2 = 0.97$, $n = 6$). The method overall score was 10; this was the lowest overall score of all the methods and so this method was ranked first.

DRY-6 and **DRY-F** were relatively simple, requiring the least equipment of all methods tested; therefore, the cost was very low per sample. The recovery rate of DRY-6 was 93%, whereas the recovery rate from DRY-F was 69%. The recovery percentage of DOM extracted by the DRY-F method was negatively correlated with the pH of the source water ($p = 0.04$, $R^2 = 0.71$, $n = 6$); DOM samples with lower recovery percentages were from water sources with higher pH values. There was no significant correlation between the DOM recovery percentage and water pH in samples collected by the DRY-6 method ($p = 0.47$, $n = 13$). When grouped by water body type, the samples from headwaters and streams had a significant negative relationship ($p < 0.01$, $R^2 = 0.89$, $n = 6$). The recovery percentage of DOM extracted by the DRY-6 method was weakly positively correlated with the nutrient concentrations (nitrate and nitrite, total nitrogen (N) and total phosphorous(P)) of the source water; however the majority of values were very close to or at the detection limit of the analysers. It is possible that DOM samples with lower recovery percentages were from water sources with lower nitrate, nitrite, total N and total P. Their respective overall scores were 10.5 (DRY-6) and 12.5 (DRY-F), making them ranked second and third.

The **FD method** was also very simple; however, the freeze-drier was the most expensive single piece of equipment used. This was the only method that directly resulted in solid DOM samples without having to evaporate off the remaining water (either at room temperature or at 60 °C in an oven). The recovery rate of this method was 89%, with the smallest range of any method tested in the experiment (74 to 112%). There were positive correlations between the absorbance at eight UV and visible light wavelengths (665, 470, 465, 436, 400, 360, 265 and 254 nm) and the recovery percentage of the five DOM samples extracted by FD. Water samples with higher absorbance values had higher DOM recovery percentages. The recovery percentage was weakly positively correlated with the nutrient concentrations (nitrate and total P) of the source water; however the majority of values were very close to or at the detection limit of the analysers. It is possible that DOM samples with lower recovery percentages were from water sources with lower nitrate and total P. The FD method overall score was 19, and it was ranked sixth, increased by the cost of the large equipment needed.

RO-RE and **RO-DF** had very similar results to each other; however, the RO equipment was relatively expensive per sample. The RO methods were carried out on water from two sites: the site with lower DOC concentration (8.6 mg L^{-1}) resulted in very high recovery percentages (234% RO-DF; 245% RO-RE), whereas the site with higher DOC (21.1 mg L^{-1})

resulted in lower recovery percentage (84% RO-DF; 71% RO-RE). Their respective overall scores were 18 (RO-RE) and 22 (RO-DF), ranked fifth and eighth overall.

The **DIA-FD** and **DIA-FH** methods were limited by the volume of water that could be dialysed at each time, resulting in small DOM samples. The dialysis tubing is relatively expensive and to extract a larger DOM sample would have increased the cost of this method. The water sample that was subject to dialysis had a starting DOC concentration of 44.4 mg L^{-1} (the highest of all the samples used in this study), but the DIA-FH had one of the lowest (and therefore worst) recovery percentages (37%), showing a lot of the carbon measured in the DOC concentration was not extracted and present in the DOM. The overall scores for the two methods using dialysis were 20.5 (DIA-FH) and 31.5 (DIA-FD), ranked seventh and ninth overall.

4. Discussion

The DOM extracted from natural water using nine commonly used methods was assessed. The time and costs of each method were recorded, and elemental and functional group composition of the DOM was analysed. The results showed that the most reliable and similar composition results were obtained from the rotary evaporator (RE), freeze dryer (FD) and dry down at 60 °C (DRY-6) methods (Table 2; Figure 1). The consistent DOM composition and NMR results across these methods suggest they extracted a 'real' DOM composition, or that all three methods altered the elemental and functional group composition in the same way. The rotary evaporator, freeze dryer and dry down at 60 °C methods also had relatively fast rates and low costs per sample (Table 3). The scoring system resulted in a higher score (therefore a 'worse' method) for methods that included a freeze dryer, due to the large equipment cost (~£10,000, Table 3). Calculating the overall score without the large equipment cost, the lowest scores (and therefore 'best' methods) are RE, DRY-6 and FD (scores are 8, 8.5 and 9), the same three methods as found to be the most reliable and consistent in the elemental analysis. The high cost of the freeze drier is a one-off cost, and could be offset by the practically zero consumable costs per sample.

Thacker et al (2005; 2008) used rotary evaporation to extract DOM from water. They used a water bath at 45 °C, but the temperature of the evaporating sample was measured as 20 °C. They were confident that the temperatures involved would not result in losses of DOM, and report extracted recoveries of between 70 and 91% (2005 study) and 93 to 107% (2008 study). The slightly higher temperature water bath used in the current study (60 °C) would likely result in a 35-40 °C evaporating temperature for the sample; the high recovery rates indicate that there was hardly any loss of volatile carbon compounds at these temperatures.

The only difference between the RE and DRY-6 methods in this study was the vacuum in the rotary evaporator; in both methods the water was heated to 60 °C. The elemental and functional group compositions were most similar between these two methods.

The water chemistry of the source water influenced the recovery percentages. The pH of the source water was found to impact the recovery percentage of the DOM samples extracted by both RE and DRY-F, but not DRY-6 or FD. Higher water pH increases the solubility of DOM; it is possible that this impacts the recovery percentage during rotary evaporation and dry down. Higher absorbance values in water may lead to higher recovery percentages of DOM extracted by freeze drying, and lower nutrients (especially nitrate and total phosphorous) may lead to lower recovery percentages of DOM extracted by freeze drying and dry down at 60 °C (although more work needs to be done to investigate this further).

High absorbance values in water often indicate high DOC concentrations, especially in waters draining catchments dominated by peat soils (Wallage and Holden 2010), and so it is likely that the correlation between absorbance and recovery percentage is controlled by high DOC concentrations in samples with high absorbance values. In future studies, if DOM is to be extracted using these methods from source waters with a wide range of pH and absorbance values, then the recovery percentage will likely be affected.

Søndergaard et al (2003) used freeze-drying to extract DOM from estuary water and found a 97% recovery. They compared the freeze-dried DOM to ‘fresh’ and found no differences in DOC bioavailability, suggesting the freeze-drying process did not alter the DOM significantly. The freeze-dried DOM allowed them to artificially manipulate the DOM and DOC concentration without adding water (and therefore changing the water chemistry of their samples), another benefit of extraction. Whitty et al (2019) compared DOM extracted by freeze-drying with ‘whole water’ from two water sources, and showed a 16% and 26% total change in relative intensities across six integrated regions of ^{1}H-NMR spectra. The freeze-dried samples lost oxygenated functional groups – these differences were attributed to changes in the DOM composition during the freeze drying process, suggesting structural changes do take place in the freeze-drying samples (Whitty et al 2019).

Kitis et al (2001) investigated the impact of reverse osmosis on DOM reactivity. They found minimal loss of DOM (94-98% recovery) in water with DOC concentrations ranging from 2 to 25 mg L^{-1}, similar to this study. This shows that reverse osmosis can be used to recover DOM, however the method used in Kitis et al (2001) was much more complicated, requiring customised equipment, and therefore likely to be more costly in money and time than the method used here.

The aim of this study was not to question the reliability of DOM composition data extracted by different and varied methods used in past studies that have provided interesting and valuable results, but to highlight the risks associated with comparing across studies. If the aim of a new study is to compare DOM to previously published research, it is recommended that the exact same method of DOM extraction be used. This also highlights the importance of systematic and thorough reporting of the extraction methods used in studies of DOM composition. In future studies of DOM extraction, especially from fresh waters, the methods recommended are: rotary evaporation at 60 °C, freeze drying, and dry-down at 60 °C, as these have provided the most reliable and consistent results.

5. Conclusion

DOM was shown to be highly variable in elemental and functional group composition using samples from 14 water bodies across the UK. The results showed significant differences in composition between DOM extracted via different, commonly used methods. The DOM compositions were most consistent and reliable when extracted using the rotary evaporating, freeze drying and dry-down at 60 °C methods. Using a rank and scoring system based on time, cost, resources and recovery percentages, the ideal methods were identified as rotary evaporation and dry-down at 60 °C. Freeze drying, despite having the greatest large-equipment cost, is also a recommended method, as it had the lowest cost per sample and a high recovery percentage.

The results show that composition data of DOM extracted by different methods are not comparable. It is recommended that future studies of DOM composition use one of the methods suggested, systematically report the method used and the recovery percentage, and if necessary, extract DOM by more than one method for comparison. If comparing DOM compositions from earlier literature, it is recommended to follow the method of extraction used in the original work, to ensure any differences are not due to the extraction method.

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