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Review

Trace chromatographic analysis of dimethyl sulfoxide and related methylated sulfur compounds in natural waters

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Abstract

Dimethyl sulfoxide (DMSO) occurs in the environment as a result of a number of biogenic and anthropogenic production and emission processes. It is an environmentally significant compound because of its use as a substrate by bacteria and its potential role in the biogeochemical cycle of dimethyl sulfide (DMS), a climatically active trace gas. In this paper, current methods for DMSO determination at nanomolar levels in natural waters, all involving gas chromatography, are reviewed. Direct injection and separation of aqueous DMSO offers a simple and fast application, but exhibits limited sensitivity due to limitation on injection volumes. So far, most authors have preferred DMSO reduction and subsequent analysis of the evolved DMS by purge-and-trap preconcentration and flame photometric detection. Several reducing agents have been used, though some require cumbersome procedures or are very sensitive to operational conditions. The common algal component dimethylsulfoniopropionate (DMSP) acts as an interference in some reduction methods and, therefore, either DMSP elimination prior to DMSO analysis or correction a posteriori is required. DMSO can be analyzed along with DMS, methanethiol, dimethyl disulfide and DMSP in the same water sample, either sequentially or separately, so that comprehensive speciation of methylated sulfur is obtained. Owing to the biological activity of DMSO, appropriate water sampling and handling procedures must be applied. Acidification and freezing appear to be suitable for aqueous DMSO storage, although immediate analysis in the field is always preferable. Future directions of DMSO determination in aquatic environments are suggested. © 1998 Elsevier Science B.V. All rights reserved.

Keywords: Reviews; Water analysis; Environmental analysis; Dimethyl sulfoxide; Sulfur compounds, methylated; Dimethyl sulfide

Contents

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1. Introduction: DMSO in the environment

1.1. Sources and fate

Dimethyl sulfoxide (DMSO) is widely used as a solvent, lubricant, preservative and stabilizer in a number of industrial, agricultural and pharmaceutical applications (e.g., Refs. [1,2] and references therein). It is produced commercially by oxidation of dimethyl sulfide (DMS) with dinitrogen tetroxide [3]. It is a natural component in a wide range of beverages and foodstuffs (e.g., Refs. [4,5]) and, naturally, occurs in plants and animals (e.g., Ref. [6] and references therein), and has been found at nanomolar levels in marine, fresh and rain waters (see below).

Generation of DMSO by natural processes in aquatic environments is poorly understood. Enzymatic oxidation of DMS by bacteria is likely, since it has been observed with natural isolates. Most of the microorganisms capable of DMS-to-DMSO transformation are obligate anaerobes, including anoxyphototrophic bacteria (Refs. [7–9] and references therein). Only three aerobes, *Pseudomonas acidovorans*, *Nitrosomonas europaea* and *Nitrosococcus oceanus*, have been reported to do so [10,11]. However, actual DMSO production from DMS in oxygenic aquatic environments is still to be experimentally corroborated. Photic and aphotic chemical oxidation of DMS is another potential route for DMSO production [12,14]. It has been shown that photooxidation of aqueous DMS by visible light yields DMSO in the presence of photosensitizers [12]. However, results of recent field experiments indicate that DMSO, although produced in significant quantities by photooxidation within the oceanic photic zone, is only a minor DMS photoproduct [13]. Photochemical oxidation of DMS also occurs in the atmosphere [15], resulting DMSO may then be scavenged back to water bodies by wet deposition.

Loss processes for DMSO include oxidation, utilization by microorganisms and reduction to DMS. Oxidation to dimethyl sulfone ($DMSO_2$) takes place in the atmosphere [15,16], but in aqueous phase it proceeds too slowly to be significant [12]. A wide variety of microorganisms, including procaryotes and eucaryotes, aerobes and anaerobes, can reduce DMSO to DMS and/or utilize DMSO. Under anaerobic conditions, bacteria and yeasts have been observed to either reduce DMSO to DMS or to grow solely on DMSO [7,17]. Aerobic growth and reduction to DMS has been observed only in *Hyphomicrobia* and *Pseudomonas acidovorans* [7,10,17]. However, as for DMS-to-DMSO oxidation, the actual occurrence of biological DMSO-to-DMS conversion in oxic waters has not been demonstrated. DMSO can also be chemically reduced to DMS by sulfide [17].

1.2. Environmental significance

DMSO is relatively odorless and nontoxic at low concentrations [18] and, therefore, a pollutant of minor concern. As a natural substance, DMSO is one of the most abundant forms of methylated sulfur in marine and other natural waters, where it acts as a carbon and energy source for methylotrophs and for bacteria that can use it as an electron acceptor, respectively [17]. In particular, a great deal has been made of the implication of DMSO in the marine cycle of DMS. DMS is the most abundant volatile sulfur compound in seawater and a climatically active trace gas in the atmosphere. It is produced

from the algal compound dimethylsulfoniopropionate (DMSP) by coupled processes in the marine food web. DMS is supersaturated in most natural waters and, thus, there is a net flux of this compound into the atmosphere. In the air, DMS is oxidized to sulfate and sulfonate aerosols that play a key role in atmospheric acidity and the formation of cloud condensation nuclei over the oceans (e.g., Refs. [19,20]). Gaining knowledge of the factors that control DMS concentration in seawater and its emission to the atmosphere is of importance for climate studies and global change predictions. Since DMSO has been recognized as a potential source and sink for marine DMS, it has received increasing attention over the last few years.

1.3. Field measurements in aquatic environments

A very limited number of DMSO measurements in natural waters have been made to date. The reason is that procedures with sufficient sensitivity and selectivity for trace DMSO analysis in complex mixtures were scarce until the 1990s. Table 1 shows available literature data for DMSO concentrations in marine, fresh and rain waters. Most of these data have been reported since 1992. DMSO occurred in all of the water bodies studied at concentrations lower than 200 n*M*, most commonly within the range 1–30 n*M*.

Table 1
DMSO concentrations in natural waters

	DMSO concentration (n*M*)	Ref.
Surface seawaters		
Coastal and open Pacific	19–181[a]	[72]
Open Pacific	4–20	[13]
Open Pacific	3.3	[60]
Coastal Pacific	6.3–124	[65]
Coastal Atlantic	6	[33]
Coastal Atlantic	1.2–13	[31]
Coastal Antarctic	<2.5–24	[36]
Arabian Sea	nd–18	[64]
North Sea	2.5–9.5	[64]
Mediterranean Sea	2.2–62	[62,63]
Salt lake waters	nd–180	[35]
	5–20[a]	[61]
Estuaries	5–86[a]	[72]
Freshwaters		
Rivers	<1–14	[72]
Lakes	<1–6	[72]
Meltwater ponds	1.9–185	[29]
Rainwaters		
Marine	5.9–8.3	[72]
Marine	<0.3–11	[16]
Marine	8.3–12	[33]
Marine	1.2–26	[31]
Continental	2.2–4.6	[16]

[a] Not corrected for DMSP interference.

2. Analysis of DMSO in water samples

2.1. General considerations

DMSO exhibits characteristic properties that have to be taken into consideration when designing a method for its analysis in aqueous systems (Table 2). A number of methods employing gas (GC), liquid (LC) and thin-layer chromatography (TLC) have been traditionally applied in the determination of DMSO in different matrices (Refs. [21,22] and references therein, [2,23–25]). However, only a few have been applied to aqueous solutions. Because of its high miscibility in water, separation of DMSO from the aqueous matrix constitutes the first difficulty. Extraction into organic solvents such as methanol or chloroform [5,25] does not ensure quantitative recovery [26]. Adsorption onto Tenax is more efficient, but thermal desorption from Tenax is not applicable because of the low volatility of DMSO [27]. Its use requires extraction with methanol and subsequent evaporation [16,27], increasing the complexity of both the analytical procedure and the instrumentation. Therefore, among methods employing chromatography, only (a) direct injection of the aqueous sample into a gas chromatograph and (b) reduction to DMS followed by purge, preconcentration and GC analysis of DMS offer quantitative yields, simplicity, and sensitivities close to those needed to address its distribution in natural waters. Thus, these two approaches will be evaluated hereafter.

2.2. Direct injection

Direct injection methods consist of injection of water volumes into a gas chromatograph and sub-

Table 2
Analytically relevant properties of DMSO and their methodological implications

Property	Effect	Implication
Relatively non-volatile (b.p. 189°C)	Liquid at ambient temperatures	Not purgeable Does not allow headspace sampling Easy to handle pure material
Very miscible with water	Low partitioning into the air phase	Not purgeable Does not allow headspace sampling Easy preparation of standard solutions
Excellent as solvent	Dissolves many substances	Readily contaminated by impurities
Highly polar	Readily sorbs to active surfaces e.g., glass	Requires deactivated glass surfaces for storage and handling
Aprotic	No proton-related speciation	No pH-dependent chemistry

sequent separation and detection of DMSO using either flame ionization detection (FID [23]) or flame photometric detection (FPD [26,28]). Direct injection with FID has been applied to biological fluids since the late 1960s [23], but detection limits were never lower than 200 μ*M* DMSO. Use of FPD improved sensitivity significantly, but not enough to ensure accuracy at n*M* levels [5]. With the use of microliter volumes injected into the gas chromatograph, and no preconcentration of the analyte prior to injection, de Mora and co-workers [26,29] reported on the need of sensitive FPD with a SF_6-doped hydrogen flame for DMSO detection in natural waters. DMSO is separated from $DMSO_2$ at 150°C with a PTFE column packed with 15% free fatty acid phase on 40–60 mesh Chromosorb T. When running saline water samples, a short guard column containing identical packing material is installed to prevent salts from depositing in the analytical column. Also, the hot injector liner (210°C) has to be cleaned of salt deposits with Milli-Q water after every 30 injections [29]. By these means, the authors reported detection limits as low as 0.06 n*M*, with a precision of 10% (relative standard deviation, R.S.D.). However, such a low detection limit seems hardly feasible with the use of microliter injection volumes. For example, at that concentration an injection volume of 10 μl would contain only a few fmol, which is far below the detection capabilities of current FPD systems.

2.3. Determination via reduction to DMS

Reduction of DMSO and subsequent gas-phase analysis of the DMS produced appears to be the technique of choice for most authors. Rather standard, although complex, methods have been reported for the determination of aqueous DMS (see below). Moreover, most researchers working on DMSO are also interested in DMS, so that sharing of instrumentation capable of determining both species constitutes an advantage.

2.3.1. Reducing agents

A suite of reagents have been used so far for reducing DMSO to DMS. They include chromium chloride ($CrCl_2$ [2]), stannous chloride ($SnCl_2$ [30]), titanium trichloride ($TiCl_3$ [31]), sodium borohydride ($NaBH_4$ [2,32,33]) and the enzyme DMSO reductase [34]. Table 3 shows a compilation of the reduction methods reported so far, and Table 4 displays their main analytical features.

As shown, $CrCl_2$ and $SnCl_2$, both already used in field work (Refs. [35,36], respectively), present no interferences. However, results are questionable because quantitative reduction yields have not been either obtained or tested at the nanomolar level. $CrCl_2$, $SnCl_2$ and $TiCl_3$ require cumbersome procedures for either preparation of reagents or reduction runs, including passage of the reagent through a reducing column and conservation in an inert atmos-

Table 3
Analytical methods for the determination of DMSO via reduction to DMS

Reducing agent	Ref.	Reaction conditions
$CrCl_2$/HCl	[2]	acid; 30 min reaction+purge
$SnCl_2$/HCl	[30]	strong acid; 30 min reaction at 100°C+cooling+purge
$TiCl_3$/HCl	[31]	strong acid; 2 h reaction at 50°C+cooling+NaOH+30 min+purge
$NaBH_4$/HCl	[2]	acid; 6 min reaction+purge
$NaBH_4$/HCl	[33]	addition of [$^2H_6$]DMSO internal standard; neutral; immediate purge while reacting
$NaBH_4$/HCl	[32]	slightly acid; 15 min reaction+HCl+purge
DMSO reductase/EDTA/FMN	[34]	semianaerobic; neutral; light; 20 min reaction and purge

phere [2], heating of the sample for reduction [30,31], or prevention/elimination of acidic fumes [30,31]. $NaBH_4$ is quite simple to use provided interferring DMSP is eliminated, although reaction yield is very sensitive to operational conditions if not well optimized. The first-developed method using borohydride, reported good recoveries and precision under optimal conditions [2], but has been rarely applied to fieldwork, probably because of the difficulty in controlling such conditions. To compensate for fluctuations in the efficiency of borohydride reduction, Ridgeway et al. [33] used isotope dilution GC–MS, where perdeuterated DMSO was added as an internal standard to aqueous samples and the DMSO content was determined by the ratio [$^2H_6$]DMS/DMS evolved after borohydride reduction. This method was sensitive and very precise. Its major drawback is the need for a mass spectrometer, which makes processing large numbers of samples and use in field work more difficult. Hence, it will not be further considered in this review. Simó et al. [32] reported a refinement of the former borohydride reduction method, where the order and amount of reagents were optimized and the interference from DMSP eliminated. Finally, the method using DMSO reductase offers accurate and precise results with the possibility of immediate analysis (no extra reaction time in addition to purge time) without any interference other than that of DMS [34]. Its major limitation, however, is the availability of this non-commercial enzyme. Advantages and drawbacks identified for the above methods are summarized in Table 5.

Thus, choice of a method for the reduction and determination of aqueous DMSO is up to the analyst and will depend upon laboratory setup, analysis immediacy and other analytes to be determined.

2.3.2. Instrumental and chromatographic requirements

After DMSO reduction, instrumentation for the determination of the evolved DMS is required. Although several instrumental configurations have

Table 4
Main analytical features of DMSO reduction methods

Reducing agent	Detection limit	Precision (as R.S.D.)	Mean red. yield	Mass range tested	Interf.	Ref.
$CrCl_2$/HCl	2.4 ng (0.3 n*M*)	5–20%	42%	2–2400 ng	none	[2]
$SnCl_2$/HCl	100 ng (128 n*M*)	10–25%	90–100%	1000–5000 ng	none	[30]
$TiCl_3$/HCl	78 pg (1 n*M*)	<10–30%, typically <10%	75–100%	0.08–2 ng	DMSP	[31]
$NaBH_4$/HCl	2.4 ng (0.3 n*M*)	<5–10%, typically <5%	quantitative (no data provided)	2–2400 ng	DMSP	[2]
$NaBH_4$/HCl	200 pg (0.05 n*M*)	6–20%, typically <10%	95%	3–500 ng	DMSP	[32]
DMSO reductase/ EDTA/FMN	1.25 ng (0.16 n*M*)	2%	99%	5–78 ng	none	[34]

Table 5
Advantages and drawbacks of the methods for the determination of DMSO via reduction to DMS

Reducing agent	Ref.	Advantages	Drawbacks
$CrCl_2$/HCl	[2]	no interferences	cumbersome preparation and handling of reagent low yield
$SnCl_2$/HCl	[30]	no interferences	low precision, not well tested for nanomolar levels heating required
$TiCl_3$/HCl	[31]	suitable for very small volumes	long procedure; heating required needs correction for DMSP efficiency dependent upon batch of reagent
$NaBH_4$/HCl	[2]	simple no need of instrumentation beyond that for DMS and DMSP	yield very dependent upon pH needs correction for DMSP
$NaBH_4$/HCl	[32]	simple no need of instrumentation beyond that for DMS and DMSP	needs correction for DMSP if not used sequentially
DMSO reductase/EDTA/FMN	[33]	no interferences fast: immediate results	obtention, storage and handling of enzyme neither chemical treatment nor storage of the sample prior to analysis are allowed

been reported for DMS analysis, they all exhibit common features. A general, basic configuration is shown in Fig. 1. Essentially, it consists of:

(a) Purge unit, made of deactivated glass and with an inlet and outlet for the inert sparging gas. The configuration of the purge flask, and the state and quality of the fritted glass through which the sparging gas is supplied, will both have an effect on purge efficiency [37]. Volumes of the purge unit usually range between 25 and 500 ml, allowing the sparging of slightly smaller sample volumes. For waters with very high DMSO concentrations, small sample aliquots can be brought to optimal purge volumes by dilution with Milli-Q water. Kiene and Gerard [31] have reported a purge unit that consists of a small vial connected to the sparging gas line by means of needles through the cap septum, thus allowing purging of volumes as small as 1 ml.

(b) Water elimination unit, where water vapor that saturates the sparge stream is eliminated without removing DMS. Traps filled with hygroscopic salts such as K_2CO_3 (e.g., Refs. [38–40]) are efficient in retaining water but not DMS only when kept in good conditions (not too wet). Alternatives include cold traps (−80 to −20°C, e.g., Refs. [2,39]) that require making and maintaining cold baths, and Nafion tubes

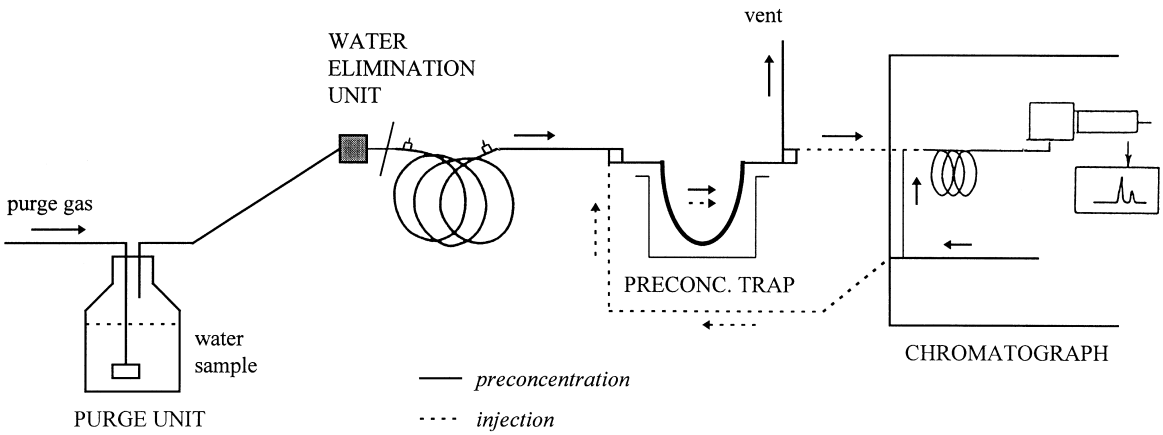

Fig. 1. Schematic diagram of the basic instrumental configuration for the analysis of DMS in water.

(e.g., Refs. [31,32,34,41,42]). In my opinion, Nafion tubes (now commercially available) are recommended because they provide a clean and efficient drying, seldom requiring maintenance.

(c) Preconcentration unit, where DMS is trapped and accumulates. Solid adsorbents (Tenax, molecular sieves) in glass or PTFE tubes have been used (e.g., Refs. [29,38]), but require some maintenance with the risk of efficiency losses. Cryogenic traps (−150 to −190°C) provide universal and efficient preconcentration ([43], e.g., Refs. [34,44–46]), though caution is needed. Cryotraps filled with solid adsorbents have also been reported [2,39,41,47].

These three units can be connected by PTFE tubing and operated separately from the chromatograph. However, the preconcentration unit must be connectable to the GC system, since injection is done directly from the trap by thermal desorption.

(d) Chromatographic unit, that is, a GC system provided with appropriate column and detector. PTFE columns packed with stationary phases especially created for volatile sulfur compounds (e.g., Carbopack BHT 100, Chromosil 310, Chromosil 330, Supelpack-S, Carbopack B/1.5% XE-60/1%, Porapak QS 80–100 mesh) are recommended. Among these, Chromosil 330 and Carbopack BHT 100 are preferred by most authors. Capillary columns such as SE-54, GS-Q, UCON 50 HB 5100, Pluronic 121 and SPB-1 offer better peak shapes for DMS, but they are much narrower than the trap lines, which may cause undesired pressure changes.

The GC temperature program must be chosen to provide good separation of DMS from other volatile sulfur species that also occur in natural waters, e.g., CS_2. For the packed columns mentioned above, isothermal or rated programs at temperatures of 30–80°C elute DMS within the first few minutes after injection. Capillary columns are usually operated with rated temperature programs between 30 and 200°C.

The detector must be selective for sulfur and sensitive enough. FPD is usually preferred because of its sensitivity and ''simplicity'', although it requires signal linearization. However, work with other detectors has also been reported. Hall electrolytic conductivity detection [27,46] gives a sensitive, linear response, but is rather complex and needs frequent maintenance for optimal work. Sulfur chemiluminescence detection [48] is also sensitive and linear, but sensitivity drifts with time. Finally, sulfur electron-capture detection [49] is extremely sensitive, but not very much suitable for field work because of signal variation under unstable conditions.

2.3.3. Interferences

Since DMSO determination relies on the conversion to DMS, removal of pre-existing DMS must be achieved. Therefore, the first step of the method should be a purge of volatiles efficient enough to ensure the quantitative elimination of DMS, while minimizing DMSO loss.

After purging, a number of sulfur compounds could still give rise to DMS upon reaction at the reduction step and thus interfere with DMSO analysis. This group of potential interferences includes dimethyl sulfone, sulfonium compounds such as DMSP, *S*-methylmethionine and *S*-adenosylmethionine, sulfur amino acids and derivatives such as cystine, cysteine, homocysteine, methionine and methionine sulfoxide, and other sulfur metabolites such as glutathione, thiamin and biotin. As was shown in Table 4, interference tests have been run by analysts while developing their reduction methods. No interference, among the substances tested, has been reported for $CrCl_2$ [2], $SnCl_2$ [30] and DMSO reductase [34]. Dimethylsulfoniopropionate (DMSP) is the only species, other than DMSO, that gives rise to DMS upon treatment with $NaBH_4$ [2,32,37] and $TiCl_3$ [31]. In both latter cases, DMS is generated by alkalinization during reduction, since DMSP cleaves into DMS and acrylate in alkaline media. Since DMSO occurs along with DMSP in most saline waters, either elimination of DMSP prior to DMSO analysis, or DMSP determination a posteriori and subsequent subtraction from DMSO results, must be performed.

Peaks other than DMS may also appear in the chromatogram of the volatiles produced from DMSO reduction. While reducing with borohydride and detecting with a single-flame FPD, Simó et al. [32] observed two important peaks eluting at shorter retention times than DMS. These were identified as boranes, evolved from the dissolution of borohydride. Chromatographic conditions could be applied to obtain baseline resolution of the interfering peaks

and DMS (Fig. 2). Boranes did not appear in dual-flame FPD when the same method was reproduced in another laboratory [37]. This is probably because of the higher sulfur specificity of the dual-flame detector, with less overlapping from other elements' chemiluminescence.

2.3.4. Blanks

Because of its ubiquitous occurrence, DMSO is often observed in blanks. Hence, considerable caution has to be taken with flask cleaning, sample collection and handling, and preparation of standards and reagents to keep procedural blanks as free of contamination as possible. Indeed, Kiene and Gerard [31] reported high and variable blanks associated with glassware and reagents. Washing all material with Milli-Q water, heating and using immediately upon cooling was the only way to keep glassware blanks ≤1 pmol DMSO (1 ml sample). Significant and variable blanks were also introduced by NaOH solution, if not prepared fresh, and by reducing reagent batches, thus prompting frequent blank runs and blank correction of analytical results [31]. Hatton et al. [34] observed concentrations as high as 15 n*M* DMSO in commercial analysis-grade water. Milli-Q water gave rise to erratic blanks, so that these authors used water obtained from reverse osmosis+distillation for routine analyses. Finally, Simó et al. [32] reported sporadically contaminated blanks, always ≤15 pmol DMSO (50 ml sample), when using fresh Milli-Q water and well-rinsed PTFE and glassware.

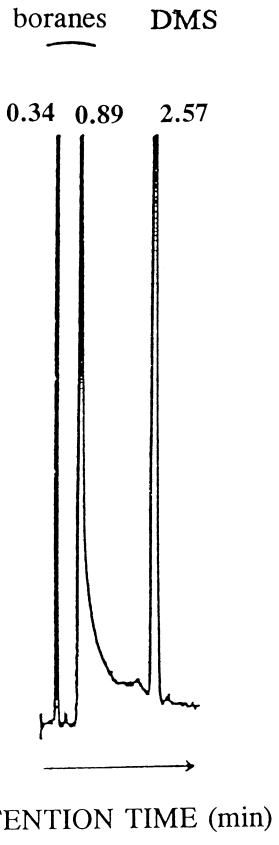

Fig. 2. Gas chromatogram of the products of DMSO analysis of a 50-ml seawater sample. Reduction of DMSO was performed by the $NaBH_4$/HCl method after removal of DMS and DMSP [32]. Column: Carbopack BHT 100 (Supelco) in PTFE tube. Carrier gas: N_2, 99.999%, 20 ml min^{-1}. Temperature: 1.5 min at 50°C, then to 100°C at 25°C min^{-1}. Detector: FPD system (Perkin-Elmer), with 95 ml min^{-1} of H_2, 99.999% and 170 ml min^{-1} of synthetic air.

Thus, as a general rule, Milli-Q-like water and well-cleaned PTFE and deactivated glassware are preferable. Water and reagent solutions should be used fresh. Frequent blank runs are to be performed.

2.3.5. Calibration techniques

Several techniques have been reported for producing calibration curves for DMSO quantification: (a) reduction of DMSO standard solutions, (b) hydrolysis of DMSP standard solutions, (c) sparging of DMS standard solutions and (d) injection of gaseous DMS standard mixtures.

(a) Because of the high miscibility of DMSO with water, preparation of aqueous standards by successive dilution of pure DMSO is easy. However, caution is needed with DMSO either occurring in freshly obtained water or evolved in aged solutions. Reduction of standard DMSO solutions in the same way as samples generates calibration curves that also serve for testing reduction yield, purge efficiency, precision, etc. (Fig. 3). Should consistent or systematic biases occur, they can be properly corrected. This is the ideal calibration technique for fast methods of DMSO analysis [34]. However, most of the methods take a rather long time to reduce and purge the sample and, therefore, frequent calibrations in this manner are not feasible in fieldwork.

(b) DMSP also permits easy preparation of aqueous standards. Alkaline hydrolysis to DMS is faster

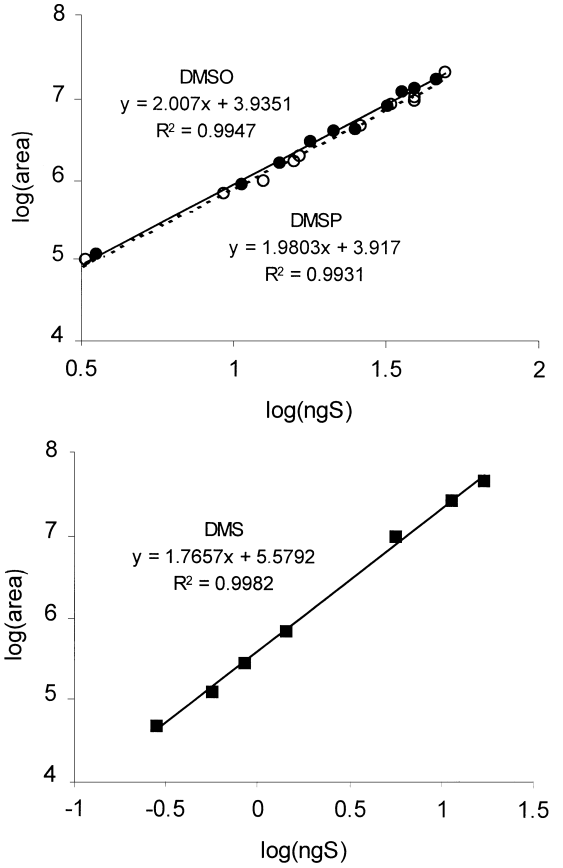

Fig. 3. Log(peak area) vs. log(mass) calibration curves produced with aqueous DMSO (top, filled circles, solid line), aqueous DMSP (top, open circles, dotted line) and gaseous DMS (bottom). DMSO and DMSP calibrations were performed with a dual-flame Aerograph FPD system at the University of East Anglia, while DMS calibration was carried out with a single-flame FPD system (Perkin-Elmer) at the CID-CSIC [37]. Results of linear regression analyses are displayed on the plots. Notice that the slopes of the log/log calibration lines at the UEA (top) are both almost equal to 2, whereas the DMS calibration at CID-CSIC (bottom) gave a slope significantly lower (see Section 2.3.5).

than most of the DMSO reduction reactions, thus providing faster generation of DMS calibration curves [50] (Fig. 3). Purge efficiency, line adsorptions and chromatographic behaviour are also tested, yet no information on reduction yield and variability is provided.

(c) This technique presents the same advantages as that with DMSP. Nonetheless, because of the low aqueous solubility of DMS and its volatile nature, preparation of accurate aqueous standard solutions first requires dissolution of cold DMS in ethylene glycol prior to formulating successive dilutions [40].

(d) Permeation tubes provide a source of gaseous DMS which can be easily sampled with a gas-tight syringe after dilution in an inert gas stream, and readily injected into the chromatographic column [32,44]. A whole calibration curve can be produced within 10 min (Fig. 3). This is the fastest way of calibrating the chromatographic system, although the reaction and purging steps are not tested. This is a very useful technique for frequent calibrations during fieldwork, but does not exempt the analyst from running periodic DMSO calibrations.

Since the FPD is the most commonly used detection method for DMS/DMSO analysis, some comments on its calibration are warranted. FPD generates a signal that is nonlinear with respect to mass, because the detector's response is proportional to the n power of sulfur amount. n is ideally equal to 2, but usually in the range $1.5<n<2.5$ [51]. Many authors linearize the FPD response by means of a linear regression between the square root of the peak area and the mass of sulfur. However, this linearization should be applied only when $n=2$. A more general linearization is obtained using log(area) vs. log(mass), where the slope equals n (e.g., Ref. [44]). Analysts should start with this logarithmic linearization to find out whether or not n approaches 2 and, hence, whether the square root linearization can be applied or the log/log technique is optimal. A further caution is required when working with very high DMSO concentrations. It has been reported that the slope of the linearized FPD plot may change at high masses [52]. Hence, identification of several linear segments along the calibration line may be required to avoid making large errors from extrapolation.

3. Methylated sulfur speciation

In aquatic ecosystems, methylated sulfur follows a rather complex cycle that involves DMSO and DMS, in addition to a series of volatile and non-volatile species, namely methanethiol (MeSH), dimethyl disulfide (DMDS) and DMSP [9,53,54]. Determination of all of these is necessary for a full understanding of processes undergone by sulfur in water

bodies. Such a determination can be achieved by means of individual or combined procedures using the same instrumentation employed for DMSO and DMS analyses. A summary of these compatible analytical methodologies for individual methylated sulfur compounds is provided below.

MeSH is a volatile species that accompanies DMS in the first steps of the analytical procedure. Special caution has to be applied to sample collection and handling because MeSH is more polar, soluble and reactive than DMS and contact with metal surfaces and gas-flowing through wet lines may cause significant MeSH losses. It is readily sparged along with DMS. Nafion tubes for drying [44,54] and preconcentration by cryogenic trapping are recommended [35,44–46,54], since solid and cold driers may cause MeSH loss if wetted (e.g., Ref. [45]) and solid adsorbents that are efficient for DMS may not be so for MeSH. Most chromatographic columns described above, when programmed at moderate temperatures, permit good separation of MeSH from DMS and other sulfur volatiles [35,38,44–46,54,55]. Calibration is performed with permeation tubes.

DMDS is less volatile than MeSH and DMS, but is usually purged along with these compounds. In most common columns, DMDS elutes well after DMS, and a temperature program up to $\geq$100°C is usually required to obtain good peak shape [44,45]. DMDS is not as surface reactive as MeSH. However, DMDS results can be compromised by the fact that it is the oxidated form of MeSH. Therefore, caution in sample handling must be taken to prevent artifactual production from MeSH.

Dissolved DMSP is determined as DMS, after cold hydrolysis in alkaline conditions (pH~13) [56,57]. At room temperature, alkali quantitatively cleaves DMSP into DMS and acrylic acid. Then, DMS is stripped from the solution and measured as described above. Reaction time does not appear to be critical to hydrolysis yield. Some authors recommend a minimum of 6 h to ensure quantitative reaction at pH 13 (e.g., Refs. [32,57]). Others express the feasibility of leaving the vials reacting over days and weeks (e.g., Ref. [58]). In contrast, some authors report immediate conversion in the purge flask with a large excess of NaOH [50,59].

Particulate DMSP is determined in the same way on the filter used for sample filtration. In this case, a hydrolysis time of several hours is strongly recommended. Bates et al. [60] mentioned variable accuracy losses in DMSP determination due to filtration. They claimed that partitioning between dissolved and particulate DMSP can be significantly affected by filtering pressure.

Combination of these procedures for DMS, MeSH, DMDS, DMSP and DMSO analyses, either separately or sequentially as illustrated in Fig. 4, should allow a full description of the methylated sulfur speciation. To date, however, the simultaneous determination of DMSO, DMSP and volatile species in natural waters has been performed in very few cases [28,34,35,52,57,60–65].

4. Sample collection, handling and storage

In addition to exercising caution with hardware and procedures to get null blanks (see above), further consideration of sample collection, handling and storage is merited.

First, surfaces in contact with sample solutions should be PTFE or glass, deactivated by silanization to prevent adsorption onto polar sites. Also, since DMSO occurs in close association with microbiota, potentially being an exudate or an intracellular metabolite [66], shaking of the water during sampling and handling should be avoided and filtration should be done gently. Furthermore, sample transport and storage in dark flasks will prevent photochemical formation of DMSO. If DMS is to be determined in the sample before DMSO analysis, collection and handling procedures for volatile reduced compounds must be applied. These include eliminating headspace following sampling and minimizing aeration during sample transference with syringes through septa or by pumping through tubes and valves. Owing to the good microbial substrate properties of DMSO, decreases in DMSO concentrations in stored waters can be anticipated unless biological activity is somehow prevented. Indeed, DMSO losses from untreated samples occur over several days [2,31,67]. Hence, analysis in the field, immediately after sample collection, is preferable. Nevertheless, Andreae [2] reported no changes in DMSO concentrations over several weeks after purge of DMS, acidification of the sample and storage at 4°C. Likewise, Kiene

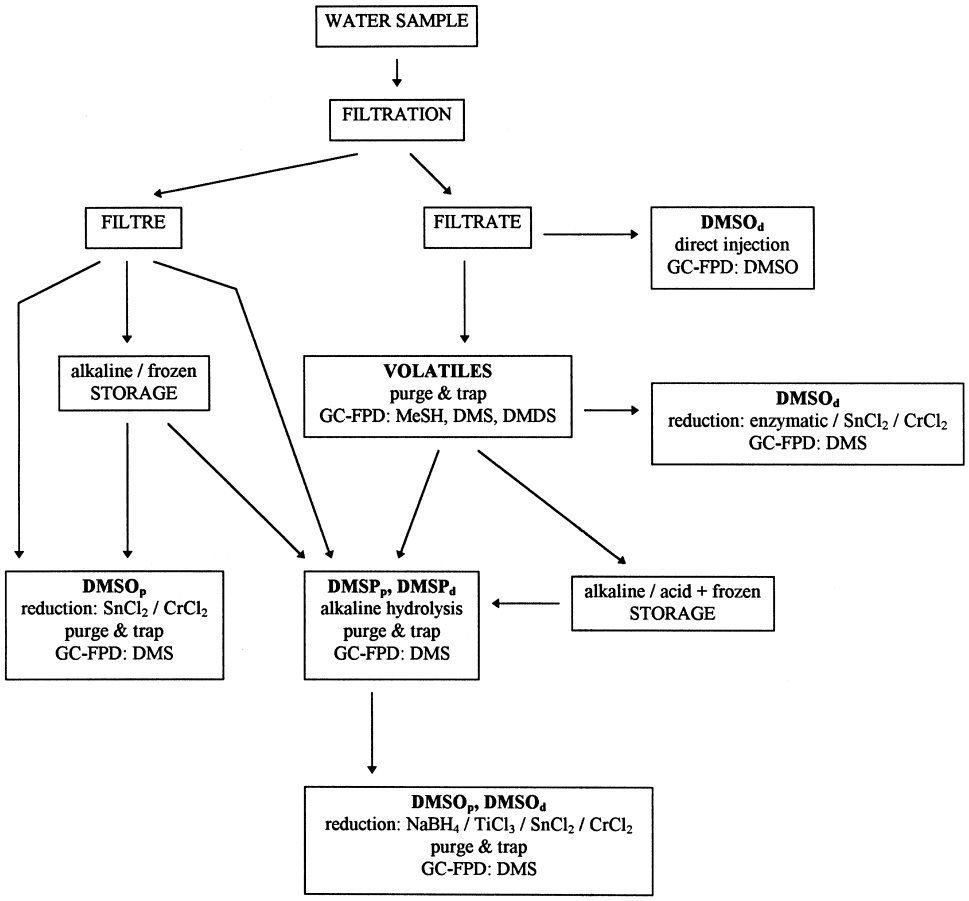

Fig. 4. Diagram of a general analytical procedure for the determination of DMSO and other dimethylated sulfur species in natural waters.

and Gerard [31] and Simó et al. [32] observed no changes, beyond the bounds of analytical precision, over weeks after either acidification, freezing at −20°C or both (Table 6). Other authors [34,64] have kept untreated samples in the dark at the in situ temperature for up to 8 h until analysis. Loss tests should be performed before doing so, since significant changes in DMSO concentration within few hours have recently been observed in short-term, dark incubations of whole seawaters [67].

Similar results to those in Table 6 have been obtained for the storage of DMSP, a compound whose simultaneous determination with DMSO is commonly attempted. Conversely, as much as 50%

Table 6
Storage tests for filtrated, aqueous DMSO samples

DMSOaq	Concentration (n*M*)	Storage time	Concentration change upon storage by			
			+HCl/4°C	−20°C	+HCl/−20°C	Ref.
DI water	10–12	3 weeks	+6.7%	+2.5%		[31]
Seawater	11–12	3 weeks	+15.4%	−2.7%		[31]
DI water	4	2 weeks			+9%	[32]
Seawater	7	2 weeks			+12%	[32]

of the DMS present may be lost during storage and/or handling of frozen samples [32]. The same effect is to be expected for the other volatile species: MeSH and, to lesser extent, DMDS. Therefore, sample storage by acidification and freezing appears appropriate only for the measurement of DMSO and DMSP, that is, after field sparging and determination of DMS and MeSH (+DMDS).

Dissolved and particulate DMSP can also be stored with NaOH (pH≥13), in well crimped vials in the dark, over periods of weeks and months [58].

5. Future directions

Interest in DMSO determination is anticipated to increase in the next few years [64]. I have identified several aspects for which further research is warranted:

Comparison and intercalibration of methods is needed. Especially, corroboration of indirect (reduction) techniques with direct (direct injection, mass spectrometry) methods is essential.

Automation would be a great advance, since the study of natural substances in water bodies requires as many measurements in short times as possible to get an interpretable picture of ongoing processes. In-flow reaction techniques are a possible avenue. Automated analyzers are currently operating for DMS [68] and under development for DMSP [69].

Development of direct methods for DMSO determination deserves further attention, since these methods should allow fast analyses and the easiest way toward automation. GC injection approaches permitting introduction of relatively large volumes have been reported recently (e.g., splitless precolumns). This should improve the sensitivity of the direct injection method by factors of 10 to 100. However, the applicability of these injection techniques, designed for organic solvents, to natural, aqueous matrices is not obvious. It seems more feasible their use in solid-phase extraction (SPE) methods. As commented above, DMSO is well retained by solid absorbents, from where it is recovered by elution with polar organic solvents. Large-volume injection techniques would exempt from solvent evaporation prior to GC. Also, solid-phase microextraction (SPME) with newly developed polar column coatings such as Carbowax appears a promising technique for DMSO preconcentration from water samples. The applicability of SPME to field sampling, as well as the automation of SPME–GC methods, have been already reported [70]. Finally, mass spectrometry (MS) with direct sample introduction should permit very sensitive, real-time measurements. This has been achieved for atmospheric DMSO [71] using atmospheric pressure chemical ionization MS operated in the selected ion monitoring mode. Application of MS to aqueous DMSO should face the challenge of water removal. Water elimination techniques for flow analysis systems (e.g., pervaporation interfaces) are currently under development.

The above reviewed methods have been applied to the determination of dissolved DMSO in natural waters. As a biologically active substance, it has been anticipated that DMSO should also occur in the particulate phase of water bodies. Only recently, has this been confirmed by borohydride reduction runs of seawater-derived particulates [66]. Testing the applicability of each method to particulate DMSO analysis will be an urgent need in the very near future.

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