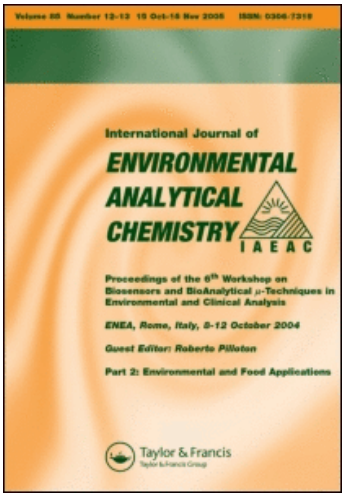

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A CONVENIENT DETERMINATION METHOD OF TOTAL SULFUR IN ENVIRONMENTAL AND BIOLOGICAL SAMPLES

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Oxidative digestion of a sample with H_2O_2 using a high-pressure double-vessel bomb was followed by ion-chromatographic determination of SO_4^{2-}, providing a convenient analytical method for total S in a variety of environmental samples. The reliability of this method was supported by the quantitative recoveries of sulfur from sediments spiked with various sulfur compounds (elemental S, FeS, FeS_2, L-cystine, and dibenzothiophene) as well as environmental certified reference samples, including 11 biological (plant: 6, animal: 5) and 3 non-biological (sediment: 2, vehicle exhaust dust: 1) samples. The method was applied to several samples (soils, sediments, humic and fulvic acids, aquatic bryophytes, and suspended marine particulates) from S-rich environments as a contrast to those from normal environments. Since the method allows simultaneous and labor-saving digestion of many samples, it may be useful for routine analysis of sulfur, despite the relatively long time (more than 6 h) is required.

KEY WORDS: Total sulfur, high-pressure bomb, wet digestion, ion chromatography, certified reference materials, environmental samples.

INTRODUCTION

Sulfur occurs in the environment mainly in the form of sulfate, elemental S, monosulfide, polysulfide (e.g. pyrite) and organic S, the latter including a variety of species, which are classified as ester sulfate or C-bonded S. Since the mobility (residence time) in the environment and the bioavailability of sulfur change depending on its form, knowledge of the natural abundance of S-species is essential for understanding S-dynamics. Therefore, several S-speciation techniques have been proposed, and applied successfully to some environmental samples[1–6].

Anthropogenic input of sulfur is still increasing in some areas, despite the fact it has decreased in others. In such areas, both excess and deficiency of sulfur are undesirable for the plant-soil ecosystem[3,7]. Therefore, rapid and extensive monitoring with respect to spatial and temporal changes in total S concentration is also important in many environments including water, sediment, soil and vegetation.

Many methods have been proposed for analysis of total S in environmental samples[2]. Most of them involve oxidative conversion of sulfur to sulfate by dry-ashing (biological samples), alkaline fusion (non-biological samples) or wet digestion (both types of sample), and subsequent determination of sulfate by gravimetric, turbidimetric, calorimetric, or ion-chromatographic methods. In recent years, a combination of wet digestion and ICP-AES[8] and an improved S-analyzer[9] have also become available.

However, in order to simplify the analytical procedure still further and to enable cross-checking of analytical values between several methods, another convenient and accurate method of total S analysis is necessary. In this study, oxidative digestion of a sample with H_2O_2 using a high-pressure double-vessel bomb[10] was combined with ion-chromatographic determination of SO_4^{2-}, providing a convenient method for analysis of total S in a variety of samples. The reliability of this method was supported by the recovery of sulfur from samples spiked with various sulfur compounds, and from environmental standard samples.

EXPERIMENTAL

Analytical procedure[4,5]

A sample (3–10 mg of dried material) was placed in a Teflon Tuf-Tainer vial (volume: 7 ml) and 3 ml of 30% H_2O_2 was added as an oxidant. For a biological sample, 20 µl of 1 M H_3PO_4 (a stabilizer of H_2O_2) and 3–5 mg of S-free Fe_2O_3 (oxidation catalyzer) were also added to the above mixture. After the vial had been closed tightly and shaken vigorously to suspend the sample (and Fe_2O_3) in the solution, it was placed in the Teflon inner vessel (containing 1 ml of water) of a stainless steel high-pressure bomb (Figure 1). The bomb was heated for more than 6 h at 140–150°C in an electric oven (heating time was changed depending on decomposition of the sample). The digested solution (when it had leaked out of the vial, the water in the inner vessel was combined with it) was dried on a heating plate, and again dried after addition of 0.5 ml of 50% HF to decompose any silicate materials (HF treatment can be omitted for biological samples). The sulfate resulting from oxidative digestion of the sample was extracted from the residue with 5 ml of 0.1 M Na_2CO_3 (which can dissolve even slightly soluble sulfates such as $BaSO_4$, if the amounts are trace), and finally the extract was subjected to SO_4^{2-}-analysis by ion chromatography (Dionex, Column: HPIC-AS3, Eluent: 0.004 M $NaHCO_3$/0.0025 M

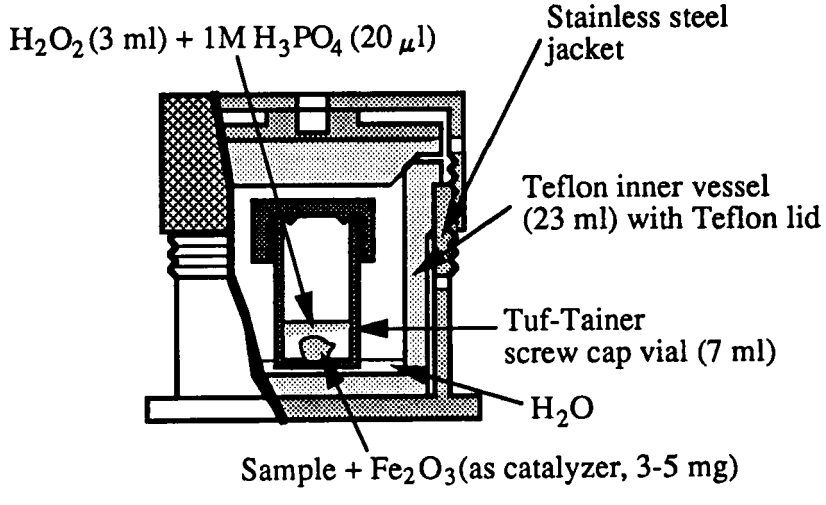

Figure 1 Double-vessel digestion bomb method for oxidation of samples with H_2O_2.

Na_2CO_3) after filtration (Chromatodisc, pore size: 0.45 μm) and appropriate dilution. S-free Fe_2O_3 was prepared from $FeC_2O_4 \cdot 2\ H_2O$ (98.5%) by heating at 650°C and subsequent thorough washing with 0.1 M Na_2CO_3.

X-ray fluorescence analysis of sulfur in biological samples

A sample (dried powder) was mixed well with a double weight of microcrystalline cellulose (Merck, for thin-layer chromatography) using an agate mortar. Three grams of this mixture was placed in a polyvinylchloride ring (4 mm thick, 35 mm i.d.) and pressed (28 metric ton, 30 s) to prepare a pellet. This pelleted sample was subjected to X-ray fluorescence analysis (spectrometer: Shimadzu VF-320A, wavelength dispersive type; X-ray tube: Rh-target, 40 V, 70 mA; spectro-crystal: Ge(111); detector: FPC). Sulfur determination was based on the XRF intensity of sulfur (Kα, 5.37 Å) normalized by that of rhodium (Lα, 4.67 Å) scattered from the sample (origin: Rh-target). Artificial standard samples (concentration range: 660 mg/kgS–5.34%S) were prepared from L-cystine (S: 26.69%) or brilliant green (S: 6.645%) after appropriate dilution with cellulose, and used for calibration.

Sulfur compounds

Pyrite (FeS_2) A naturally occurring crystal from the Kanahorizawa mine in Aomori Prefecture (volume: ca. 1 cm^3; $FeS_{1.804}$, according to S-analysis in this study) was pulverized in a Shatterbox grinder (Spex Ind.). After washing three times with $CHCl_3$, 1 M HCl (hot) and water successively, it was freeze-dried to obtain the purified pyrite ($FeS_{1.998}$). Since the powdered pyrite thus obtained was oxidized slowly in air, it was used as soon as possible after preparation.

Iron monosulfide (FeS) High-purity reagent (Rare Metallic, 99.9%) from a new bottle was used. Other chemicals used were of reagent grade.

RESULTS AND DISCUSSION

Recovery of sulfur from sediments spiked with sulfur compounds

Recovery of sulfur by the above procedure (H_2O_2-digestion/IC analysis) was investigated using sediments spiked with several sulfur compounds (Table 1). The sediments used were from Lake Biwa and Pond Sediment (NIES Certified Reference Material No. 2)[11] obtained from the National Institute for Environmental Studies. The former was collected by an Ekman dredge from the bottom surface of the southern part of Lake Biwa. It was mixed well just after sampling and then freeze-dried. The latter from NIES was used without further drying. Sulfur compounds used for spiking were FeS, FeS_2, elemental S, L-cystine and dibenzothiophene. Among these, FeS, FeS_2 and elemental S are the dominant forms of inorganic S in the environment. Cystine is a typical amino acid containing a disulfide linkage, and this group as well as a sulfhydryl group are ubiquitous not only in amino acids, peptides and proteins, but also in humic substances[6], which are major components of soil and sedimented organic matter. Organic S also

Table 1 Recovery of sulfur in sediment samples spiked with some typical S compounds.

Sample	*S added*		*S found*	*Recovery*
	Compound	*mg/kg*	*mg/kg*	*%*
L. Biwa sediment[#1]	–	–	724 (0.9, 6)[#3]	–
	L-cystine	267	987 (0.7, 4)	98.5
	FeS	365	1091 (1.5, 4)	101
	FeS_2	535	1261 (0.6, 6)	100
Pond sediment[#2]	–	–	2233 (1.7, 10)	–
(NIES, No. 2)	L-cystine	801	3042 (0.3, 4)	101
	FeS	365	2581 (0.3, 4)	95.4
	FeS_2	1069	3390 (1.5, 8)	108
	S^0	610	2863 (0.7, 6)	103
	Dibenzothiophene	2110	4406 (2.4, 4)	103

[#1]: A freeze-dried sample.
[#2]: The sample obtained from NIES was used without further drying.
[#3]: Average values (relative deviation [%], number of determinations).

occurs in environmental samples as many other forms of C-bonded S[2,3] such as $-SO_3H$ (sulfonic acid), $-SO_2$ (sulfone), -C = S (thioketone) and heterocyclic S, among which the latter may be the most resistant to oxidative digestion with H_2O_2. Therefore, dibenzothiophene was chosen as a representative of the compounds containing the above S-groups. In addition to C-bonded S, ester sulfate[2,3] is another important category of organic S in environmental samples. However, ester sulfate compounds were not included as spiking materials because they decompose easily, releasing SO_4^{2-}. As shown in Table 1, sulfur was recovered quantitatively (95.4–108%) from all the spike materials with relatively good precision (relative standard deviation: 0.3–2.4%). Therefore, the digestion procedure used in this study appears to be very effective for transforming most forms of sulfur in environmental samples to SO_4^{2-}.

Analysis of sulfur in environmental standard samples

In order to check the precision throughout the analytical procedure, some environmental standard samples, to which certified or reference values had been given, were analysed (Table 2). They included 11 biological (plant: 6, animal: 5) and 3 non-biological (sediment: 2, vehicle exhaust dust: 1) samples, obtained from NIST (USA), NRCC (Canada), BCR (European Community), and NIES (Japan). Analysis was performed with intact samples (without pre-drying), except for Lobster Tomalley (NRCC, TORT-1), which was analysed in a dry state. Correction for water content was made by measuring moisture content separately in another aliquot of the same sample.

X ray fluorescence analysis is useful technique for sulfur determination, especially in biological samples, because of the low concentration of heavy metals and relatively constant composition of the matrix. Therefore, the NIES biological samples, available in large quantities (XRF-analysis required more than 1 g of the sample for each determination), were also analysed for sulfur by XRF (see Table 2). All of the results, except for Pepperbush (NIES, No. 1)[12], whose relatively high concentrations of Mn (2030 mg/kg), Fe (205 mg/kg), Zn (340 mg/kg) and Ba (165 mg/kg) interfered with the

Table 2 Analysis of total sulfur in environmental certified reference materials

Sample	*Water[#1] content (%)*	*S, mg/kg or %*			*Certified or reference values*
		Observed conc.	*After correction for w.c.*	*XPF*	
Biological standard					
Pepperbush (NIES, No.1)	7.8	3120 ± 60(3)[mg/kg]	3380 ± 60(3)	–	–
Chlorella (NIES, No. 3)	5.2	6050 ± 160(4)[mg/kg]	6390 ± 170(4)	6970 ± 30(3)	6900 ± 100(5)[#2]
Tea leaves (NIES, No. 7)	4.1	2020 ± 40(5)[mg/kg]	2100 ± 40(5)	2170 ± 20(3)	–
Sargasso (NIES, No. 9)	6.2	1.09 ± 0.01(5)[%]	1.17 ± 0.01(5)	1.03 ± 0.01(3)	1.13[#3]
Orchard leaves (NIST, SRM1571)	6.0	1750 ± 40(4)[mg/kg]	1860 ± 40(4)	–	1860 ± 90(4)[#2] 2300 ± 100(4)[#4] 2150 ± 380(7)[#5] 2120 ± 50(2)[#6] 1700 ± 200(1)[#7]
Citrus leaves (NIST, SRM1572)	6.5	3870 ± 30(4)[mg/kg]	4140 ± 30(4)	–	4070 ± 90[#8]
Bovine liver (NIST, SRM1577a)	2.8	7740 ± 70(3)[mg/kg]	7960 ± 70(3)	–	0.78[#8]
Lobster Tomalley (NRCC, TORT-1)	–	–	1.24 ± 0.01(5)[%,#10]	–	1.22 ± 0.10[#8]
Fish tissue (NIES, No. 11)	2.3	8500 ± 80(5)[mg/kg]	8700 ± 80(5)	9050 ± 230(3)	–
Human hair (BCR, CRM397)	3.8	4.31 ± 0.08(5)[%]	4.59 ± 0.08(5)	–	–
Human hair (NIES, No. 13)	6.2	4.54 ± 0.06(6)[%]	4.84 ± 0.07(6)	4.74 ± 0.05(3)	4.86 ± 0.04[#9] 4.9[#3] 5.3 ± 0.5(6)[#9]
Non-biological standard					
Pond sediment (NIES, No. 2)	11.9	2230 ± 40(10)[mg/kg]	2530 ± 40(10)	–	2300[#3]
Marine sediment (NIES, No. 12)	5.4	1.99 ± 0.03(5)[%]	2.10 ± 0.03(5)	–	2.00 ± 0.02[#3]
Vehicle exhaust particulates (NIES, No. 8)	2.9	2.77 ± 0.04(5)[%]	2.85 ± 0.04(5)	–	2.1[#3] 5.0[#3]

( ): number of determinations.
[#1]dried at 85°C for 4 h (biological standards) or at 105°C for 4 h (non-biological standards); Values show averages of 3-5determinations.
[#2]M. Morita, *et al.*, *Anal. Chim. Acta*, 166, 283 (1984).
[#3]K. Okamoto, pers. commu.
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[#8]Certified values.
[#9]J. Yoshinaga, pers. commu.
[#10]dried materials were analysed.

analysis, agreed with the values obtained by H_2O_2-digestion/IC analysis, thus supporting the reliability of the wet digestion method.

Although not standard materials, the analytical results for three marine sediments (provided by T. Masuzawa) containing quite different concentrations of sulfur (840 mg/kg (CV: 0.9%, number of determinations: 5), 1320 mg/kg (6.0, 6) and 3400 mg/kg (2.9, 6)) also agreed with those obtained by aqua regia digestion/colorimetric analysis (830, 1280, and 3980 mg/kg; Masuzawa, pers. commu.) and those determined using an elemental analyzer (830 (5.2, 4), 1200 (9.9, 5), and 3400 mg/kg (5.3, 5); Carlo Erba, Model 1106). In addition, the results for three humic acids extracted from the Lake Biwa sediments (9760 (0.7, 4), 9950 (1.3, 5), and 10030 mg/kg (2.7, 5)) were in fair agreement with those obtained using the elemental analyzer (1.07, 1.05, and 1.09%). When samples being rich in lipid or wax (e.g. some animal samples, plant cuticle wax) were analysed, the recoveries were sometimes depressed, probably due to poor contact of the oxidant (aqueous H_2O_2) to the hydophobic surface of the sample. Even in such cases, satisfactory results were obtained by addition of a small amount (ca. 1 mg) of non-ionic surfactant (e.g. Tween 40) to the digestion mixture.

Analysis of several samples from S-rich and reference environments

Sulfur concentrations were analysed in several samples collected from S-rich and normal (reference) environments (Table 3). Acid surface (0–15 cm) soils ($pH_{(H_2O)}$ = 2.81–3.93, $pH_{(KCl)}$ = 2.23–3.68), which had been exposed to S-containing gas (mainly H_2S), were collected from 13 representative volcanic regions of Japan[4]. These soils contained considerably higher concentrations of sulfur than reference soils ($pH_{(H_2O)}$ = 4.10–4.74, $pH_{(KCl)}$ = 3.54–4.50). Since the major portion (≧ ca. 80%) of sulfur is organic even in volcanic acid soils[4], the excess sulfur in acid soils may be mainly responsible for S-rich organic matter. This is also supported by the higher concentrations of sulfur in humic acids extracted from the above acid soils relative to those from reference soils. The excess organic S probably occurred as C-bonded S[5,6].

In the southern part of Lake Biwa, the sulfur concentration in the lower (5–10 cm depth) reduced sediment was about twice that in the surface (0–5 cm depth) oxidized sediment. Since Eh decreased to ca. 100 mV below a depth of 5 cm[13], H_2S, resulting from the decomposition of S-containing organic matter (e.g. amino acids, proteins), and to a lesser extent from sulfate reduction, may have been partly immobilized in the lower sediment. Therefore, most of the sulfur enriching the lower sediment was inorganic (Takamatsu, unpublished). However, accumulation of organic S was also possible, as expected from the occurrence of S-rich humic and fulvic acids in the lower sediment (see Table 3). Direct incorporation of H_2S into organic matter may be responsible for this phenomenons[14,15].

Two aquatic bryophytes (*Jungermannia vulcanicola* Steph. and *Scapania undulata* (L.) Dum.) were collected from the Kashiranashi stream, located in the Shimokita Peninsula, Aomori Prefecture. The water of the stream is acidic and rich in SO_4^{2-} (upstream: pH = 4.2–4.3, SO_4^{2-} = 60–61 mg/l; mid- and downstream: pH = 4.2–4.6, SO_4^{2-} = 56–59 mg/l) throughout its course (ca. 800 m in length), due to inflow of cold acid springs of volcanic origin[16,17]. In spite of the higher concentration of SO_4^{2-} relative to that in the surrounding neutral streams (average for 13 streams: 5.5 ± 2.3 mg/l), the sulfur concentration in the bryophytes was within the normal range (cf. 1000–9000 mg/kg[18]; 1860–4140 mg/kg, Table 2). However, *J. vulcanicola* from the upstream region

Table 3 Total S in the samples from S-rich and normal environments.

Sample	*Number (n) of samples analysed*	*S, mg/kg* Av. (min. – max.)	*Remarks*
Soil, acidic	13	2190 (900–4100)	from 13 volcanic acidic regions (soil $pH_{(H_2O)}$ = 2.81–3.93)
Soil, normal	9	1140 (600–1700)	from 9 normal regions (pH = 4.10–4.74)
Humic acid, acidic	13	5640 (3000–9800)	from the above volcanic soils
Humic acid, normal	9	3390 (3000–4100)	from the above normal soils
Sediment, oxidized	3 (n of subsamples)	710 (600–860)	oxidized sediment (depth: 0–5 cm) from Lake Biwa (southern part)
Sediment, reduced	3 (n of subsamples)	1320 (1250–1380)	reduced sediment (5–10 cm) from Lake Biwa (southern part)
Humic acid, oxidized	1	8390	from the above oxidized sediment
Humic acid, reduced	1	1.06%	from the above reduced sediment
Fulvic acid, oxidized	1	8050	from the above oxidized sediment
Fulvic acid, reduced	1	1.21%	from the above reduced sediment
Jungermannia vulcanicola, upstream	4	3820 (3310–4350)	from upstream, Kashiranashi Stream (pH = 4.2–4.3, SO_4^{2-} = 60–61 mg/l)
Jungermannia vulcanicola, downstream	6	3400 (2610–4270)	from downstream, Kashiranashi Stream (pH = 4.2–4.6, SO_4^{2-} = 56–59 mg/l)
Scapania undulata, upstream	5	2670 (2570–2790)	fromupstream, Kashiranashi Stream
Scapania undulata, downstream	4	2690 (2550–2770)	from downstream, Kashiranashi Stream
Marine suspended particulates, anoxic	11	5.07 (2.16–13.2) %	by filtration (Ultipor N_{66}, 0.45 μm) of anoxic sea water from Tokyo Bay
Marine suspended particulates, oxic	2	4380 (3900–8700)	by filtration of oxic sea water from Tokyo Bay

Values are based on dried materials.

contained slightly higher concentrations of sulfur than that from the mid- and downstream regions, resulting mainly from accumulation of HgS particles in the cell walls. Although *S. undulata* also accumulates HgS, no clear difference in sulfur concentration was evident between the samples from upstream and those from mid- and downstream, probably due to its low ability for HgS accumulation[16].

Marine suspended particulates were collected from oxic and anoxic (so-called "AOSHIO") surface waters of Tokyo Bay by filtration (Ultipor N_{66}, 0.45 μm). Sulfur in the samples from the oxic water showed a similar concentration to that in marine phytoplankton (0.3–0.6%[18]), and occurred mainly as organic species (Takamatsu, unpublished). On the other hand, the samples from the anoxic water contained a large quantity of sulfur, the majority (ca. 60% on average) being elemental S (Takamatsu, unpublished). In the anoxic water, colloidal particles of elemental S may have occurred as a result of mixing of upwelling hypolimnetic water rich in H_2S with the oxic surface water.

The analytical procedure for sulfur described here is convenient and applicable to a variety of environmental and biological samples. The limit of determination, although varying with the amount of sample used and the sensitivity of IC, was sufficiently low (ca. 20 mg/kgS) under the conditions employed. Although the time required for digestion is relatively long (mole than 6 h), easy operating and contamination-free digestion of many samples is possible by the use of high-pressure digestion bombs, and thus this method may also be useful for routine analysis of sulfur.

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