Vol. 286, No. 3, February 28, 1994
Completing Volume 286

ISSN 0003

Special Issue
PAPERS PRESENTED AT THE WORKSHOP ON THE STATE OF METHOD DEVELOPMENT AND IMPROVEMENT OF ENVIRONMENTAL ANALYSIS WITHIN THE BCR PROGRAMME
BRUSSELS, BELGIUM, FEBRUARY 17–19, 1993
(Guest Editor: Ph. Quevauviller)

ANALYTICA CHIMICA ACTA

An international journal devoted to all branches of analytical chemistry

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Publication. *Analytica Chimica Acta* appears in 16 volumes in 1994 (Vols. 281–296). *Vibrational Spectroscopy* appears in 2 volumes in 1994 (Vols. 6 and 7). Subscriptions are accepted on a prepaid basis only, unless different terms have been previously agreed upon. It is possible to order a combined subscription (*Anal. Chim. Acta* **and** *Vib. Spectrosc.*).
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ANALYTICA CHIMICA ACTA

An international journal devoted to all branches of analytical chemistry

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Papers presented at the Workshop on the State of Method Development and Improvement of Environmental Analysis with the BCR Programme, Brussels, Belgium, February 17–19, 1993

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SPECIAL ISSUE

PAPERS PRESENTED AT THE WORKSHOP ON THE STATE OF METHOD DEVELOPMENT AND IMPROVEMENT OF ENVIRONMENTAL ANALYSIS WITHIN THE BCR PROGRAMME BRUSSELS, BELGIUM, FEBRUARY 17–19, 1993

Analytica Chimica Acta, 286 (1994) 271
Elsevier Science B.V., Amsterdam

PREFACE

The international demand for environmental analysis has increased significantly in recent years in response to more stringent legislation and greater public awareness. This has presented the analytical chemistry community with a number of challenges including the need for lower limits of detection, the analysis of complex sample matrices and speciation/fractionation studies of labile samples. In response to the challenge novel analytical techniques and intricate sample clean up procedures have been developed but, if the analytical data generated are to be successfully applied to the understanding and management of environmental processes, validation of the methodology is essential.

In order to improve the quality of environmental analytical data a number of actions have been taken. These include the implementation of Good Laboratory Practice and accreditation systems, the introduction of Quality Assurance guidelines and norms (e.g., ISO 9000 and the EN 45000 series), the organisation of intercomparison exercises, proficiency testing and the production of certified reference materials (CRMs).

The Community Bureau of Reference (BCR) of the Commission of the European Communities (recently renamed the Measurement and Testing Programme) has been actively pursuing these quality objectives for the last twenty years. It has been particularly concerned with the organisation of intercomparison exercises and the production of CRMs for environmental, food and biomedical applications. It has also stimulated fundamental research to improve existing methods, investigate novel techniques and study analyte/matrix stability in support of the above functions.

The aims of the *BCR Workshop on Environmental Analysis* were to report on the status of current BCR projects in environmental analysis, to disseminate information on BCR activities in this field during the second framework programme (1987–1992) and to discuss future requirements and potential trends in the subject. The workshop was held at the Borschette Centre in Brussels on February 17–19, 1993. It was organised in such a way that (in most cases) the young researchers directly responsible for the various projects were able to present their work to an international audience and, subsequently, to be responsible for the preparation of the manuscripts. There were fifty-five participants representing most of the twelve EC countries and thirty-four oral presentations. The meeting was opened on behalf of the BCR by Dr. Griepink who outlined the aims of the Bureau and stressed the importance of its sponsored work programmes within the context of validation of environmental analytical data.

The themes of the Workshop are mirrored by the sub-headings used to classify the contributions in this special issues. Several presentations reported preliminary results and were therefore not suitable for inclusion in this issue but the Editors would like to thank all those who participated in the Workshop for their efforts in making it a success.

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Elsevier Science B.V.
SSDI 0003-2670(93)E0511-5

Analytica Chimica Acta, 286 (1994) 273–282
Elsevier Science B.V., Amsterdam

Preparation of filters loaded with welding dust. A homogeneity and stability study of hexavalent chromium

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(Received 16th February 1993; revised manuscript received 7th May 1993)

Abstract

One hundred filters were loaded with welding fume dust, collected in industrial working conditions, and the homogeneity and the stability of the materials with regard to Cr(VI) were verified. The filters were loaded, using a Sputnic air sampling unit containing 100 binderfree glass fibre filters. The filter load was determined by weighing before and after exposure. The mean amount of dust, charged on 98 filters amounted to 2.565 mg/filter (C.V. = 3.4%). The load of hexavalent chromium on the filters corresponds to loads usually found on occupationally charged filters (approx. Cr(VI) 100 μg/filter). The homogeneity was verified by analyzing 16 filters selected from the four rings of the Sputnic air sampler. The results proved the homogeneity of Cr(VI) on the batch of filters. Filters were stored at 25°C and −20°C and the stability of Cr(VI) and total soluble Cr was verified after 1, 4 and 13 week(s). The results showed no significant difference between the filters stored at 25°C and −20°C. In all cases no Cr(III) could be detected (below LOD) with the analytical procedure used, and no significant difference was found between the amounts of Cr(VI) and total Cr determined. Normalization of the amount of hexavalent chromium measurements according to the dust load substantially improves the analytical data.

Keywords: Chromium speciation; Quality control; Stability studies

It has been estimated that in industrialized nations about 1% of the workforce is engaged with the welding of metals [1], the effects of welding fumes on the health of exposed workers is of the utmost importance [2]. Recently, The International Agency for Research on Cancer (IARC) has classified fumes and gasses from welding as possibly carcinogenic to humans [3]. Particular concern has been focused on hexavalent chromium, which has shown to produce detrimental effects on the health of persons exposed to this species during welding [4–8]. Stainless steel welding fumes originating from manual metal arc welding (MMA) are found to produce higher contents of chromates than tungsten inert gas (TIG) and metal inert gas (MIG) welding fumes [9]. Welding fumes are a class of material which contains unpredictable forms of hexavalent chromium in a complex matrix [10,11] and the health effects due to chromate solubility are still not entirely understood, although phagocytosis may provide cell entry for insoluble compounds [12].

Besides being classified as carcinogenic to humans [3], hexavalent chromium also causes contact dermatitis [13], effects on reproduction such as delayed conception [14] and poor sperm quality [15], increased frequencies of both sister chro-

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SSDI 0003-2670(93)E0326-3

matid exchange and chromosomal aberrations are also reported [16–18].

The mechanisms of chromium metabolism, genotoxicity and carcinogenicity are extensively described by De Flora and Wetterhahn [19]. Nriagu and Nieboer [20] give a comprehensive assessment of the uses of chromium, its sources, and occurrence in the air, water and soil. The IARC Monographs [3] provide exhaustive reviews on chromium compounds and welding processes.

Much research has been carried out on the exposure of welders to chemical and physical agents and numerous articles have been published on the subject, with very inconsistent data reported in many cases [21]. There is inaccuracy in estimates of exposure to hexavalent chromium, particularly historical exposures [22] and sampling, storage and analytical procedure entail the possibility of changing the valences of chromium due to reduction and/or oxidation and hence are apt to over- or underestimate the Cr(VI) content [22–25]. This has led to a need to define sampling procedures, and optimize existing analytical techniques to quantify the Cr(VI) content in welding fumes [10,23,26–28].

In occupational health the occupational exposure limits (OEL) for water soluble and certain water insoluble compounds in indoor air is limited to 0.5 mg/m^3 for total Cr and to 0.05 mg/m^3 for Cr(VI). Continuous monitoring of the amount of Cr(VI) inhaled by workers has been imposed, as described in the EC Directive on exposure to carcinogenic substances [29]. In Denmark the OEL for Cr(VI) is 0.02 mg/m^3 and with portend changes to 0.01 mg/m^3 in 1993 and to 0.005 mg/m^3 in 1994 [30]. At present the reliability of Cr(III) and Cr(VI) determinations is very unsatisfactory. The lack of agreement between the results produced by different laboratories of the same samples clearly points to deficiencies in the methodologies for separation and measurement of the Cr(III) and Cr(VI) species.

No analytical method can be relied upon to produce reliable results, unless the accuracy of the measurement procedure is controlled and documented by the use of a certified reference material (CRM) with a similar matrix composition. Furthermore no valid comparisons can be made on the basis of analytical results obtained by different laboratories, unless the same reference materials have all been measured [31–34].

To ensure and objectively document the analytical performance for Cr(VI) analysis, external quality assessment and traceability should be applied using control materials and/or CRMs with a good matrix match and amounts of Cr(VI) species at similar levels to those that occur in field samples [35]. The development of these is therefore of paramount importance.

The National Institute of Occupational Health (AMI) in Denmark has developed and produces filter control materials for internal and external quality assurance. The filters are loaded with welding fume dust for improving the methodology in analyzing the metals Fe, Mn, Cu and Ti [36]. The control materials are used in the Danish External Quality Assessment Scheme (DEQAS) conducted by AMI [37,38].

The aim of this study was to develop methodology to produce a batch of filters with a homogeneous and stable distribution of Cr(VI). A new batch of filters, produced as described in this paper, is expected to be used as control materials in an international laboratory comparison to validate the state of the art of analytical methodology for chromium speciation and to asses the suitability of this product as a candidate CRM.

EXPERIMENTAL

Preparation of the loaded filters

The filters were loaded using a Sputnic air sampling unit containing 100 filters (Fig. 1). A smaller version of the air sampler is described in detail by Bjørgo et al. [39] and the Sputnic has been evaluated and shown to be suitable in preparing quality control materials [36]. The air sampler consists of a round vacuum chamber of stainless steel where 100 critical orifices are placed. Each orifice has a diameter of 0.4 mm and the 100 orifices are positioned in four rings with 36, 29, 22 and 13 orifices in each ring (Fig. 2). Two vacuum pumps secure a constant vacuum of 0.66 bar in the pressure chamber which again secures a homogeneous airflow of 2 l/min,

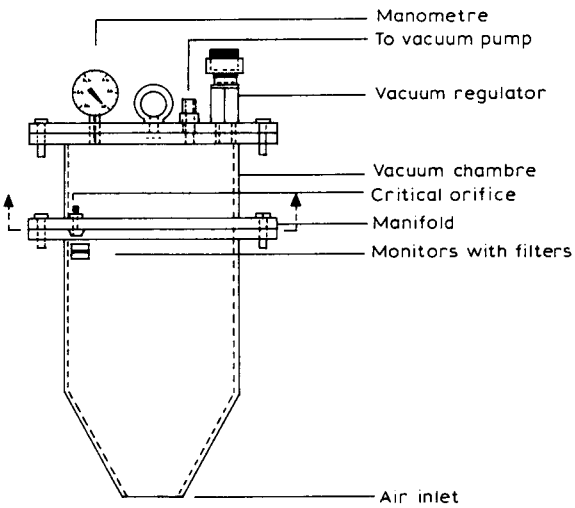


Fig. 1. "Sputnic" air sampling unit.

through each critical orifice and therefore also through the filter. The welding fumes were transported to the orifices through a symmetric conical tunnel mounted on the vacuum chamber. The collection occurs on borosilicate microfibre glass discs without resin binder. The 100 filters were placed in polycarbonate monitor cases on top of a support filter and assembled. To avoid losses of glass fibre on the monitor, when pressed together, a polytetrafluoroethylene (PTFE) ring (25 mm in diameter) was placed on top of the glass fibre filter.

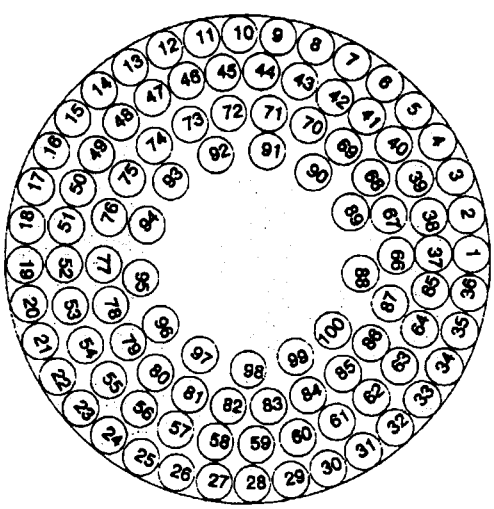


Fig. 2. The 100 orifices are positioned in four rings with 36, 29, 22 and 13 orifices in each ring.

The 100 numbered monitors, with filters, were acclimatized in an air-conditioned laboratory (23°C and 50% relative humidity) for 6 h. Prior to dust collection, the filters were weighed on an electronic semimicroweight Cahn 26 balance with a standard deviation of 16 μg/filter for filter loads less than 2.5 mg/filter. The 100 monitors were mounted on the critical orifices in the Sputnic air sampler and the flow through each monitor was measured with a calibrated 25-cm flow meter, uncertainty 1%. The data (in mm) were converted to flow rate (l/min) against a calibration curve. The air inlet of the Sputnic was positioned on top of a special fume box, similar to a "Swedish Model" [40], and the filters were loaded with welding fume dust originating from a manual metal arc (MMA) computerized welding method, using an electrode producing a welding fume dust containing approx 4.6% Cr(VI) under well-defined conditions. Pilot studies concluded the charging time to be 40 s, to obtain a load of approximately 115 μg Cr(VI)/filter. After charging, the Sputnic was flushed with argon (Ar) for 5 min, while the pumps still were on. Thereafter it was placed in the laboratory and a second flow measurement was performed. A second weighing was also performed following 6 h acclimatization in the air-conditioned laboratory. The monitors were assembled again and flushed with nitrogen (N_2) to store the filters in an inert atmosphere and sealed. Fifty monitors were stored at -20°C and 50 monitors at 20–25°C.

The particulate size distribution of the welding fumes determines the degree to which fumes may be inhaled and how they are deposited within the respiratory tract [3]. Scanning transmission electron microscopy (STEM) of the welding fumes (Fig. 3) shows an agglomerate of the fume particles. The particles, which tend to form long chains, have a diameter of approximately 0.2 μm and are alkalichromates and silicates of Na, K, Mn, Fe, Ni and Cu. Energy dispersive spectroscopy (EDS), has been used to give qualitative elemental analysis (Fig. 4), indicating inhomogeneous distribution in the agglomerate since Ni was not found in the chosen particle (0.6% Ni in the electrode).

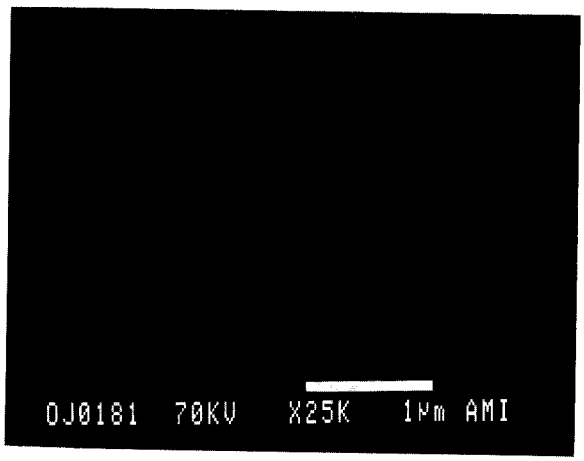


Fig. 3. Scanning transmission electron microscopy (STEM) of the welding fumes. The particles have a diameter of approx. 0.2 μm and form alkalichromates and silicate-aglomerates of Na, K, Mn, Fe, Ni and Cu.

Analytical procedure

The filter and PTFE ring were placed in a 25-ml beaker with the dust-loaded side facing upwards. The filter was covered with 10 ml of an alkaline buffer (2% NaOH–3% Na_2CO_3, pH 12.7) [27] and a small conical PTFE ring was placed on top to secure the filter beneath the surface of the liquid. This assembly was covered with laboratory film and afterwards subjected to agitation in a heated (70°C) ultrasonic bath for 30 min. Before separation and analysis by electrothermal atomic absorption spectrometry (ETAAS: Perkin Elmer PC 5100) [35], the supernatant was diluted 1000 times with a HCO_3^-–H_2CO_3 buffer (pH 6.4) [41] to reach the linear range in ETAAS. Aliquots of the supernatant were analyzed for total soluble chromium. After extraction with Amberlite LA-2, Cr(VI) was measured in the organic phase and Cr(III) in the aqueous phase respectively against matrix-matched calibration curves.

The Amberlite was diluted in methyl isobutyl ketone (MIBK) and stripped in 6 M HCl (LA-2–HCl–MIBK, 2:1:2) [42]. 1 ml of the diluted supernatant and 1 ml of anion exchanger were mixed on a whirling mixer for 1 min, centrifuged (2500 rpm) for 10 min and the two phases were separated and analyzed.

Reagents and utensils

All reagents were analytical grade. From Millipore (Bedford, MA) the borosilicate microfibre glass discs (Cat. No. AP40 024 05, type AP40, 24 mm in diameter), the polycarbonate monitor cases (Cat. No. M000 025 AO, type A, 25 mm in

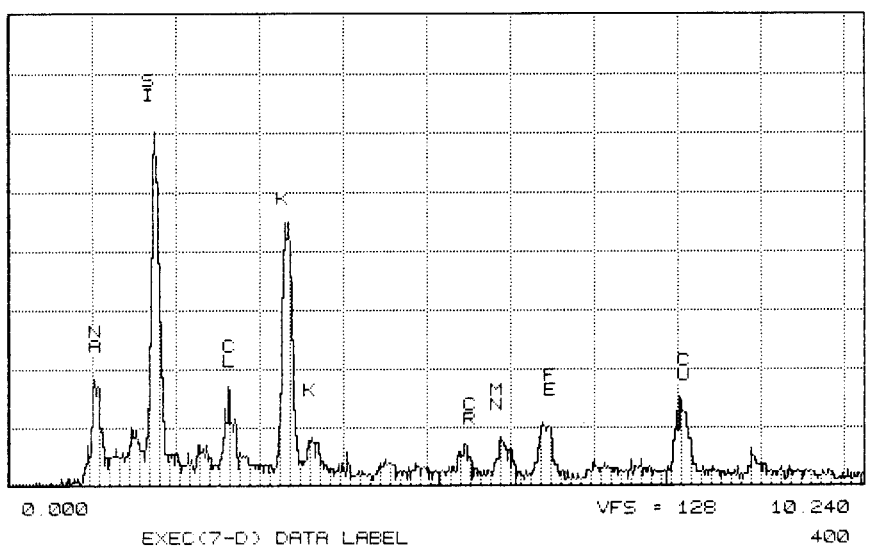


Fig. 4. Qualitative element analysis, energy dispersive spectroscopy (EDS), of one fume particle.

diameter) and the support filter (Prefilter, Cat. No. AP4002405, type AP40) was purchased. The welding electrode (Type 19.12.3.LR, diameter of 4 mm) was puchased from AB Sandvik Steel, Sandviken, Sweden.

The laboratory film (Parafilm "M") was from American National Can, Greenwich, CT.

RESULTS AND DISCUSSION

Van der Wal [21] indicates that chromium exposure to MMA/SS welders should be reduced by a factor of 10 in order to ensure that the probability of the occupational exposure limit, being exceeded, is less than 10%. Additionally welders may be exposed to much higher concentration of hexavalent chromium than would be indicated by collection of fume on a filter and subsequent analysis [43]. If excessive exposure cannot be avoided, then a programme of work place monitoring should be initiated. However, a number of considerations have to be taken into account to assure more dependable data and when evaluating such data.

Sampling

Interfering substances, reducing or oxidizing, e.g. SO_2, NO_x, O_2, O_3, Fe^{2+} (magnetite), must be taken into account, since they tend to cause erroneous results during sampling, sample storage, preparation and spectrometric measurement [10,45]. Different filter materials have been used during the last two decades, and due to reduction of Cr(VI) on the filters, inconsistent data originating from this problem have been reported. Cellulose nitrate, cellulose acetate, PVC, PTFE and glass fibre filters have been the ones widely used. All the materials reported have been found to reduce Cr(VI) under different circumstances [6,10,21,24], hence not found suitable to be used in this study. However, binderfree glassfibre filters showed reduction of Cr(VI) to a much lesser extend than the others [21].

A pilot stability study demonstrated, that binderfree borosilicate glassfibre filters caused no reduction of Cr(VI) due to organic substances and since the filters contain no binder, they retain structural integrity without weight loss when exposed to welding fumes.

To remove interfering substances, the Sputnic was flushed for 5 min with an inert gas (Ar) and after weighing flushed with nitrogen (N_2) to circumvent reduction of Cr(VI) during transport and long term storage [35].

Measurement procedure

The recommended *s*-diphenylcarbazide method to determining hexavalent chromium in welding fumes [27] has many drawbacks. Thomsen and Stern [10] point to the fact that this method is not suitable for use with welding fumes since low pH enhances the reduction of Cr(VI) so chromates will be found at levels which are significantly lower than their actual occurrence in the fumes. The reduction is significantly affected by the amount of Fe^{2+} from the magnetite (Fe_3O_4) which is highly soluble at low pH. The analytical method employed in this study involves a dilution in hydrogencarbonate buffer to pH 7–8. Dyg et al. [35,41] have performed stability studies with different parameters investigated such as stability of Cr(III) and Cr(VI) as a function of time, different solutions, possible losses due to adsorption, temperature dependency and choice of the material of the container. It was concluded that a HCO_3^-/H_2CO_3 (50 mmol/l) buffer solution was a suitable medium to achieve stable solutions of the Cr(III) and Cr(VI) species. This dilution was not only made to reach linear range in ETAAS, but it also decreases the pH value so that an eventual co-extraction of anionic Cr(III) ($Cr(OH)_{3+n}^{n-}$) and a polymerization of Amberlite in alkaline solution was prevented. To prevent air oxidation of Cr(III) to Cr(VI) during digestion in hot alkaline solutions [46] the beaker was covered with laboratory film and the solution was only heated to 70°C and subjected to agitation in a ultrasonic bath. This procedure proved to dissolves all chromates.

The methodological precision of the final determination was determined by 6 replicate analyses of a filter leachate, with a relative standard deviation of 1.2%.

The documentation of the analytical method accuracy has been proved by measuring lyo-

TABLE 1

Statistical treatment of all values in homogeneity study

	n	Mean	S.D.	95% CI	C.V. (%)	P [a]
Mass (mg)	16	2.586	0.045	2.562–2.610	1.7	
Flow (l/min)	16	1.99	0.03	1.97–2.00	1.5	
Cr(VI) (μg/filter)	16	115.9	2.2	114.8–117.1	1.9	0.08
Total Cr (μg/filter)	16	112.8	4.9	110.2–115.4	4.4	0.50

[a] ANOVA parameter. $P > 0.05$ the F test is accepted [44].

TABLE 2

Statistical treatment of all values in stability study

	n	Mean	S.D.	95% CI	C.V. (%)	P [a]
Mass (mg)	50	2.582	0.055	2.567–2.598	2.1	
Flow (l/min)	50	1.99	0.03	1.98–2.00	1.4	
Cr(VI) (μg/filter)	50	116.0	4.9	114.6–117.4	4.2	0.11
Total Cr (μg/filter)	45	115.3	6.0	113.5–117.1	5.2	0.00

[a] ANOVA parameter. $P > 0.05$ the F test is accepted [44].

philized Cr(III)/Cr(VI) reference materials (RMs) at each occasion [35]. The limit of detection and quantification (LOD and LOQ equal to 3σ and 10σ on the background signal, as defined by IUPAC) was respectively 0.1 μg/l and 0.5 μg/l.

Only filters with comparable flows and same load of dust were further studied. The mean mass load on 98 filters was calculated to be 2.565 mg dust/filter (coefficient of variance (C.V.) = 3.4%). The 50 filters, used in this study, were all in the mass range from 2.485 to 2.692 mg dust/filter (mean = 2.582 mg dust/filter, C.V. = 2.1%). Furthermore 82 filters out of 98 filters were within this range. The mean flow through the 50 filters used in this study, was 1.99 l/min. (C.V. = 1.4%). Normal distribution was found in all cases based on an N score test [44].

Homogeneity study

To verify the homogeneity of the batch, 16 filters, selected in equal number randomly from the four rings, were analyzed. Cr(VI) and total soluble Cr was determined by one measurement in each of the 16 filters selected from the four rings of the Sputnic air sampler. In all cases Cr(III) measurements was below the limit of detection, as expected, due to hydrolysis in alkaline solution (pH 12.7). Statistical treatment of all values is presented in Table 1. The 16 filters selected, had a mean mass load of 2.586 mg welding dust/filter (C.V. = 1.7%) and the mean

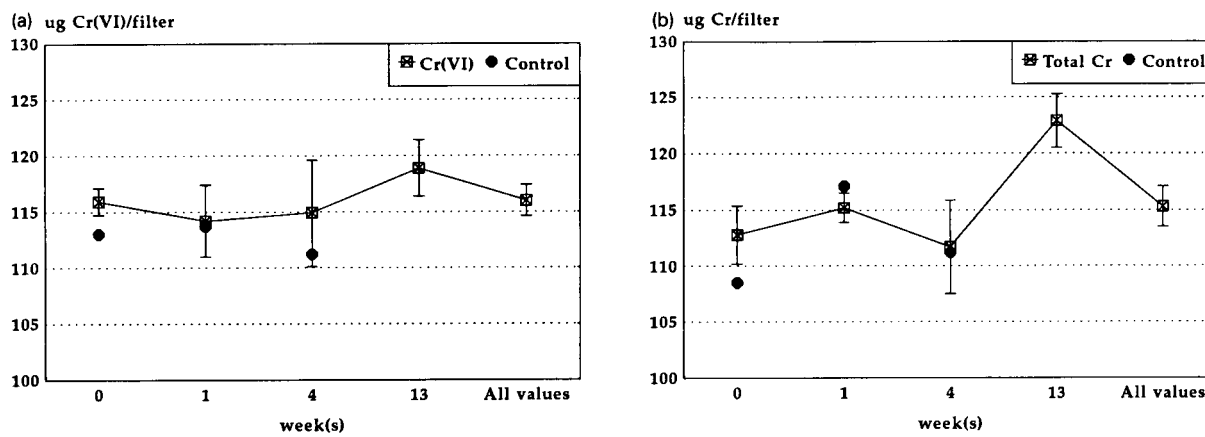


Fig. 5. Graphical representation of analysis results (± 95% CI) for (a) Cr(VI) and (b) total Cr after 0, 1, 4 and 13 week(s).

TABLE 3

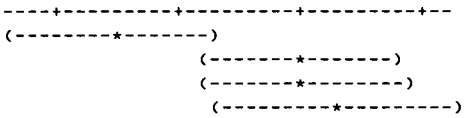
ANOVA on the ring number, corrected by mass and flow

(1) ANALYSIS OF VARIANCE ON Cr(VI), WITHOUT ANY CORRECTION

SOURCE	DF	SS	MS	F	p
RING NO	3	229.5	76.5	3.67	0.019
ERROR	46	960.0	20.9		
TOTAL	49	1189.4			

RING NO	N	MEAN	STDEV
1	13	112.5	6.5
2	15	117.1	4.3
3	13	117.1	3.5
4	9	117.9	2.5
POOLED STDEV =		4.6	

INDIVIDUAL 95 PCT CI'S FOR MEAN BASED ON POOLED STDEV

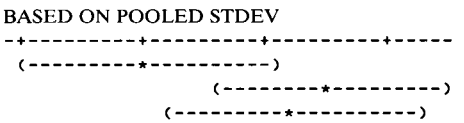


(2) ANALYSIS OF VARIANCE ON Cr(VI), CORRECTED BY MASS

SOURCE	DF	SS	MS	F	p
RING NO	3	0.1757	0.0586	1.65	0.190
ERROR	46	1.6296	0.0354		
TOTAL	49	1.8053			

RING NO	N	MEAN	STDEV
1	13	4.40	0.25
2	15	4.55	0.17
3	13	4.52	0.17
4	9	4.49	0.07
POOLED STDEV =		0.19	

INDIVIDUAL 95 PCT CI'S FOR MEAN BASED ON POOLED STDEV

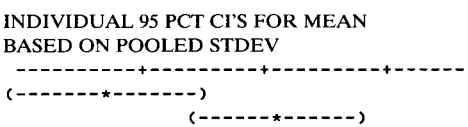


(3) ANALYSIS OF VARIANCE ON Cr(VI), CORRECTED BY FLOW

SOURCE	DF	SS	MS	F	p
RING NO	3	166.9	55.6	4.31	0.009
ERROR	46	594.2	12.9		
TOTAL	49	761.1			

RING NO	N	MEAN	STDEV
1	13	84.5	4.8
2	15	88.2	3.2
3	13	88.5	3.2
4	9	89.3	2.5
POOLED STDEV =		3.6	

INDIVIDUAL 95 PCT CI'S FOR MEAN BASED ON POOLED STDEV



flow was 1.99 l/min (C.V. = 1.5%). Statistical treatment of the results demonstrated a normal distribution and the analysis of variance (ANOVA) of the data from the four rings, establishes the homogeneity for both Cr(VI) and total Cr (P value > 0.05) (Table 1). The coefficients of variation were 1.9% for Cr(VI) and 4.4% for total Cr, respectively. A significant difference (95% confidence interval (CI) level) was found between the mean results ($n = 16$, $\pm$.S.D) 115.9 ± 2.2 μg/filter for Cr(VI) and 112.8 ± 4.9 μg/filter for total Cr, respectively.

Stability study

There were no significant differences (95% confidence interval) between analysis results of Cr(VI) and total Cr for filters stored at 25°C and −20°C. Therefore all results for Cr(VI) and total Cr, soluble in alkaline solution, respectively have been pooled together, represent storage at 25°C. Statistical treatment of all values is presented in Table 2. The mean dust mass of the filters ($n = 50$) used, was 2.582 mg (C.V. = 2.1%) and the mean flow was 1.99 l/min (C.V. = 1.4%).

To evaluate long term reproducibility of the measurement method, a batch of filter leachate from the homogeneity study was pooled. The filter leachate pool was filled in 15 polyethylene tubes, each containing 5 ml and stored at −20°C, and an aliquot was analyzed on each occasion. Graphical representation of analysis results for Cr(VI) and total Cr after 0, 1, 4 and 13 week(s) storage is shown in Fig. 5 including the leachate "control". There are no "control" results marked at 13 weeks storage due to failure of the freezer used for storage purposes. The "control" specimens were thawed for some days and this appeared, as expected, to cause erroneously low analysis results due to the reducing properties of the present electrolytes. This stresses the need to keep samples dry. Cr(III) was throughout detected to be below LOD. Analysis of variance (ANOVA) was performed on the ring number for each result obtained for Cr(VI) and compared to the corrected value, i.e., normalized by the dust load or the flow. The results are presented in Table 3 and Table 4 shows statistical treatment of these.

TABLE 4

Statistical treatment of results from Table 3

	n	Mean	S.D.	95% CI	C.V. (%)	P [a]
(1) Cr(VI) (μg/filter)	50	116.0	4.93	114.6–117.4	4.3	0.02
(2) Cr(VI)/Mass (% in fume)	50	4.50	0.19	4.44–4.55	4.3	0.19
(3) Cr(VI)/Flow (mg/m^3 air)	50	87.5	3.9	86.4–88.7	4.5	0.01

[a] ANOVA parameter. $P > 0.05$ the F test is accepted [44].

The stability has been established for Cr(VI) (95% CI) over the 13 weeks storage period. For total Cr, the result was significantly higher after 13 weeks compared to 0, 1 and 4 weeks storage (Fig. 5b). Unfortunately, this cannot be explained as the "control" specimen was thawed, owing to equipment failure.

No significant differences between all Cr(VI) and total Cr measurements (Table 2) were found. This result is based on 50 measurements and the significant difference found in the homogeneity study can be explained to the limited number of results ($n = 16$).

Table 3.1 shows a significant trend indicating that the outermost ring differs from the others, in respect to Cr(VI). This can be explained by the charge of the particles containing Cr(VI) and the inside design of the Sputnic, respectively. Normalization of the Cr(VI) results according to the dust load or flow (Tables 3 and 4) shows that the correction by mass dust load ($P = 0.19$) substantially improves and remedies the analysis results from without correction ($P = 0.02$) which confirms previous results obtained for other metals [36].

From analysis of variance an estimate of the sampling procedure between filters is calculated to approximately 4% based on a methodological precision amounting 1.2%.

Conclusion

Binderfree borosilicate glassfibre filter does not reduce hexavalent chromium when handled as described. The analysis result (Table 4.2): 4.5 $\pm$0.19% Cr(VI) in fumes and the predicted con-

tent of Cr(VI) in the electrode (4.6%) proves that no Cr(VI) has been reduced during sampling, transport and long term storage. Hence the procedure of storing the samples dry and in an inert gas prevents reduction of Cr(VI) due to reducing contaminants.

The analytical procedure described proved to be very trustworthy with a methodological relative standard deviation of 1.2%. Normalization of the Cr(VI) results according to the dust load substantially improves the analytical data. Besides hexavalent chromium, the filters can be used as control materials for other components, prerequisite of homogeneity, as well such as: Fe, Ni, Mn, Cu, F and Pb.

The aim of this study has been accomplished, namely to produce a batch of filters with a homogeneous distribution of stable Cr(VI). An estimate of the sampling procedure between filters is calculated to amount approximately 4%. A new batch of filters is expected to be used, as control materials, for an international laboratory comparison to validate the state of the art of analytical methodology for chromium speciation in welding fumes and to asses the suitability and stability of this product as a candidate certified reference material (CRM).

The project was supported by a grant from the Commission of the European Communities, Bureau of Reference Material (CEC-BCR). We thank Erik Beck Hansen, Danish Welding Institute, for donating the welding fumes and Ole Jørgensen for the "STEM" and "EDS". Rita Cornelis, Mike Campbell, Ebbe Thomsen and Stefan Herwald are gratefully acknowledged for their most valuable suggestions and advice.

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Analytica Chimica Acta, 286 (1994) 283–290
Elsevier Science B.V., Amsterdam

Chromium speciation with isotope dilution mass spectrometry

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(Received 17th February 1993; revised manuscript received 17th May 1993)

Abstract

Cr(III) and Cr(VI) were determined by positive thermal ionisation isotope dilution mass spectrometry (IDMS). From the spiked samples, Cr(VI) was separated from Cr(III) by extraction with the liquid anion exchanger Amberlite LA-2. Re-extraction of Cr(VI) was performed with an ammonia solution. After electrolytic deposition of chromium from both species the isotope ratio $^{52}Cr/^{53}Cr$ was determined. Detection limits of 1.2 ng g^{-1} for Cr(III) and of 2.4 ng g^{-1} for Cr(VI), respectively, were achieved. A HCO_3^-/CO_2 buffer solution of pH 6.4 containing Cr(III) and Cr(VI) enriched in ^{53}Cr was used as spike solution. The stability of the chromium species in this solution was tested in model experiments with labelled radioactive ^{51}Cr. The accuracy of the IDMS method could be shown in two interlaboratory comparisons. Several fresh and treated waste water samples with Cr(III) concentrations in the range of 2–55 ng g^{-1} and Cr(VI) concentrations of less than 2.4 ng g^{-1} were analysed. The results of the fresh water samples could be explained by the known geochemical behaviour of chromium in natural water systems.

Keywords: Mass spectrometry; Isotope dilution methods; Chromium; Interlaboratory studies; Waters

Chromium appears in the environment in three stable oxidation states. Apart from small amounts of metallic chromium Cr(III) and Cr(VI) are the most common chromium species [1]. Chromium in its trivalent form is an essential trace element for plants and animals. It is involved in the glucose metabolism and the nucleic acid synthesis [2]. Like the other essential trace elements, Cr(III) is toxic in higher concentrations. In contrast to the behaviour of Cr(III), Cr(VI) shows no essential character. Because of its high oxidation potential, Cr(VI) is toxic and carcinogenic even at very low concentrations [3]. As Farrell et al. [4] supposed, the carcinogenic effect of Cr(VI) results from an intermediate Cr(V)–DNA complex. The further reduction to Cr(III) in single electron steps leads to irreversible changes in the DNA structure. Because of these differences in the toxicity of Cr(III) and Cr(VI), determination of total chromium does not give full information about the health hazard of a certain chromium pollution. Correct evaluation of toxicities, bioavailabilities and chemical mobilities demand an exact and reliable species analysis. In principle there are different ways for achieving reliable analytical data. On the one hand, one can calibrate the results with a standard reference material and, on the other hand, one can use a precise and accurate analytical method. However, there is no suitable standard reference material available at the moment which allows chromium speciation. The Community Bureau of Reference (BCR) of the European Communities is going to develop such a standard reference material, which has to be certified by different analytical methods. Isotope dilution mass spectrometry (IDMS)

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SSDI 0003-2670(93)E0350-G

proves to be an analytical method which combines high accuracy and precision with good sensitivity [5]. The reliability of IDMS has been shown in many certification exercises. The greatest advantages of IDMS are the use of an internal isotope enriched standard for calibration and the lacking necessity for total or reproducible yields in the chemical separation and isolation procedures. The purpose of this work was to develop a method for chromium speciation with the known accuracy of IDMS.

EXPERIMENTAL

Chemicals

Deionized water is purified by a two-fold distillation in a quartz apparatus. HNO_3 p.a. and HCl p.a. (both Merck) are distilled below the boiling point in a quartz distiller. H_2O_2 p.a., NH_3 suprapure, $NaHCO_3$ p.a., and isobutyl methyl ketone (IBMK) p.a. (all Merck) are used without further purification. The liquid anion exchanger Amberlite LA-2 pract. (Serva) is distilled in a vacuum at about 180°C. $^{51}Cr(III)$ and $^{51}Cr(VI)$ labelled stock solutions are obtained from Amersham as $CrCl_3$ in 0.1 mol l^{-1} HCl and as Na_2CrO_4 in water, respectively. The oxidation state of Cr(III) and Cr(VI) and its completeness in the stock solutions were proved by the separation system represented in Fig. 2. The radioactivity is 37 MBq ml^{-1} in each of the solutions. For the radiotracer experiments these solutions are diluted with the buffer solutions described later to achieve the desired radioactive decay rates of about 10 kBq ml^{-1}.

Isotope dilution analysis

The elements to be determined by IDMS must have at least two stable or quasi-stable isotopes, which is fulfilled for chromium by four stable isotopes. An exactly known amount (about 1 g) of a spike solution enriched in ^{53}Cr is added to the sample. The used spike solution contains both Cr(III) and Cr(VI). The characteristic data of the spike solution is listed in Table 1. The species concentrations in the spike solution were determined by an inverse isotope dilution process using calibrated standard solutions of natural isotopic composition. After equilibration by mixing and shaking the spike solution with the sample the two different chromium species are separated. Each chromium species is determined to its $^{52}Cr/^{53}Cr$ isotope ratio. From this isotope ratio, the known data of the chromium species in the spike solution, and the known natural isotopic composition of chromium in the sample, the species concentrations in the sample can be calculated. The principles of IDMS are described in detail elsewhere [5,6].

TABLE 1

Characteristic data of the Cr(III)/Cr(VI) spike solution

Species	Concentration (10^{15} atoms g^{-1})	^{52}Cr (%)	53Cr (%)
Cr(III)	6.45 ± 0.03	2.01 ± 0.01	97.81 ± 0.02
Cr(VI)	9.83 ± 0.09		

Mass spectrometric measurements

All isotope ratio measurements are carried out with a single-focussing magnetic sector field mass spectrometer, type MAT 261 (Finnigan MAT, Bremen). A single-filament (Re) ion source is used for the formation of Cr^+ thermal ions. For achieving optimum ion currents the silica gel/H_3BO_3 technique [7] (10 μl of a silica gel suspension together with 1 μl of a 0.8 mol l^{-1} boric acid solution) is used with an additional admixture of $AlCl_3$ [8], which is deposited together with the isolated chromium on the rhenium filament and then heated until dryness. The principles of thermal ionisation mass spectrometry are given elsewhere [9,10]; more details on the formation of Cr^+ thermal ions are described in [7].

Sample treatment

Stability investigations. One of the greatest difficulties in chromium speciation results from the redox equilibrium between Cr(III) and Cr(VI). Its great sensitivity for changes in pH and for addition of oxidising or reducing agents must be considered in every step of the sample treatment until the species separation has been carried out.

Therefore, also the spike solutions required for IDMS have to fulfil different demands: stability of the species for at least several months; the matrix should be similar (e.g., in pH and HCO_3^- content) to natural waters; the matrix should be compatible with the chosen species separation procedure.

As Dyg et al. [11] have shown in ^{51}Cr experiments, 50 mmol l^{-1} buffer solutions of HCO_3^-/CO_2 and CO_3^{2-}/HCO_3^- can stabilise Cr(III) and Cr(VI). Our own investigations were also carried out with ^{51}Cr labelled solutions. The total Cr concentration in each solution was 25 ng ml^{-1}. We first investigated a ^{51}Cr labelled radiotracer solution in CO_3^{2-}/HCO_3^- buffer without CO_2 blanket, but this does not stabilise Cr(III) for more than a few days. These experiments were carried out in PE bottles at 25°C. Under the same conditions as described by Dyg et al. we found that the HCO_3^-/CO_2 buffer keeps Cr(III) stable for 160 days at 5°C and Cr(VI) for at least 120 days (Fig. 1). Obviously it is essential for the stability of Cr(III) to avoid loss of CO_2 by storing the buffer solution under a CO_2 blanket. As a consequence, the Cr(III)/Cr(VI) spike solution used in this work (Table 1) was prepared to be a 50 mmol l^{-1} HCO_3^-/CO_2 buffer solution. This spike is stored in Teflon-PFA bottles at 5°C under a CO_2 blanket. The Cr(III)/Cr(VI) content was checked immediately after production, after one month and after ten months of storage obtaining identical results.

Species separation. The Cr(VI) and Cr(III) species separation was investigated with different types of ion exchangers (cation and anion exchanger resin, as well as a complexing resin, Chelex 100) and by extraction using labelled $^{51}Cr(III)$ and $^{51}Cr(VI)$ compounds. The highest separation efficiency using Cr(III) and Cr(VI) compounds in a HCO_3^-/CO_2 buffer solution was found by an extraction of Cr(VI) with a liquid anion exchanger. 100 ml of the liquid exchanger Amberlite LA-2, which is stored in contact with 50 ml of 6 mol l^{-1} HCl [12,13], in 100 ml IBMK (isobutyl methyl ketone) is used. Under these conditions, Cr(VI) is completely extracted into the organic phase whereas Cr(III) remains in the aqueous solution (99–100% yield determined by

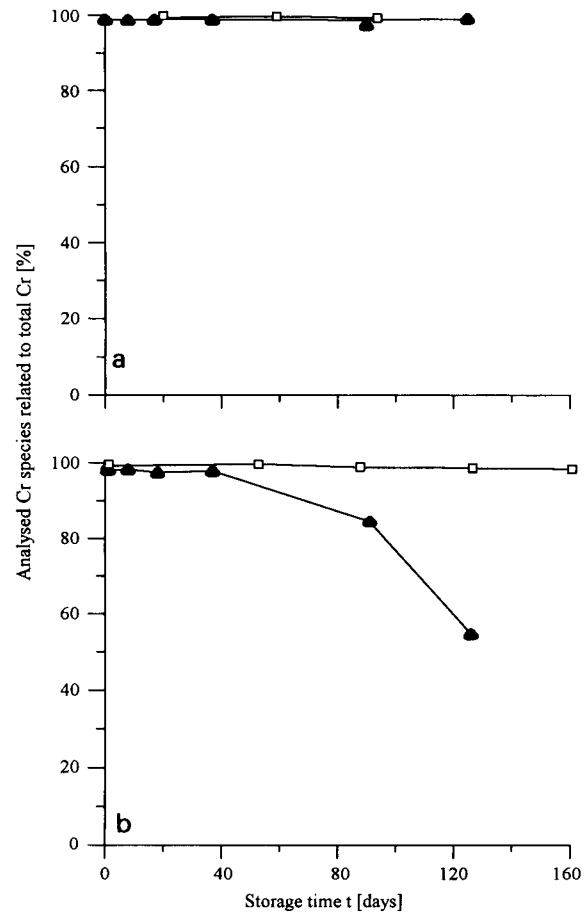

Fig. 1. Stability of Cr(III) and Cr(VI) in a 50 mmol l^{-1} HCO_3^-/CO_2 buffer solution at pH 6.4. (a) □, Cr(VI) stored at 5°C under CO_2 blanket; ▲, Cr(VI) stored at 25°C without CO_2 blanket. (b) □, Cr(III) stored at 5°C under CO_2 blanket; ▲, Cr(III) stored at 25°C without CO_2 blanket.

labelled ^{51}Cr compounds). A critical point using the extraction system is the separation of the two phases. A total phase separation is only possible by using sufficiently high salt concentrations in the aqueous solution. This is fulfilled, e.g., for all samples determined by the isotope dilution technique because the addition of the spike solution results in HCO_3^- salt concentrations of about 25 mmol l^{-1}. After separation, Cr(VI) must be re-extracted from the organic phase into the aqueous phase for isolation of chromium to be measured in the mass spectrometer. Different chemicals have been tested, e.g., by reducing chromate

with an acidic solution of sulfite, thiosulfate and hydroquinone, respectively. However, the re-extraction by a 6% (per weight) NH_3 solution has been found to be the most efficient system (98% isolation yield) with very fast kinetics. Other advantages of NH_3 are the high purity of commercially available ammonia solutions (suprapure), the relatively low costs, and its easy handling.

Isolation of chromium. For isolation of chromium from both solutions of the separation process, an electrolytic deposition at Pt electrodes is performed. This means that the electrodeposition is carried out in an ammoniacal solution, which also generates the most effective pH value for this type of isolation. Extensive studies are also carried out on the optimum electrolysis time and on other electroanalytical parameters. An additional improvement of the chromium isolation is achieved by optimisation of the vessels used for electrodeposition. Eppendorff microtest tubes with a volume of 1.5 ml are applied for sample volumes of 0.8–1 ml. The vessels are closed by self-made PTFE plugs, which include three holes, two adjusted for the Pt electrodes and one for the emission of the electrolysis gases. Electrodeposition for 6 h at 2.5 V in the microtest tubes is found to be sufficient for depositing more than 60% of the total chromium. This was proved in experiments with ^{51}Cr. The chromium deposit on the cathode is dissolved in

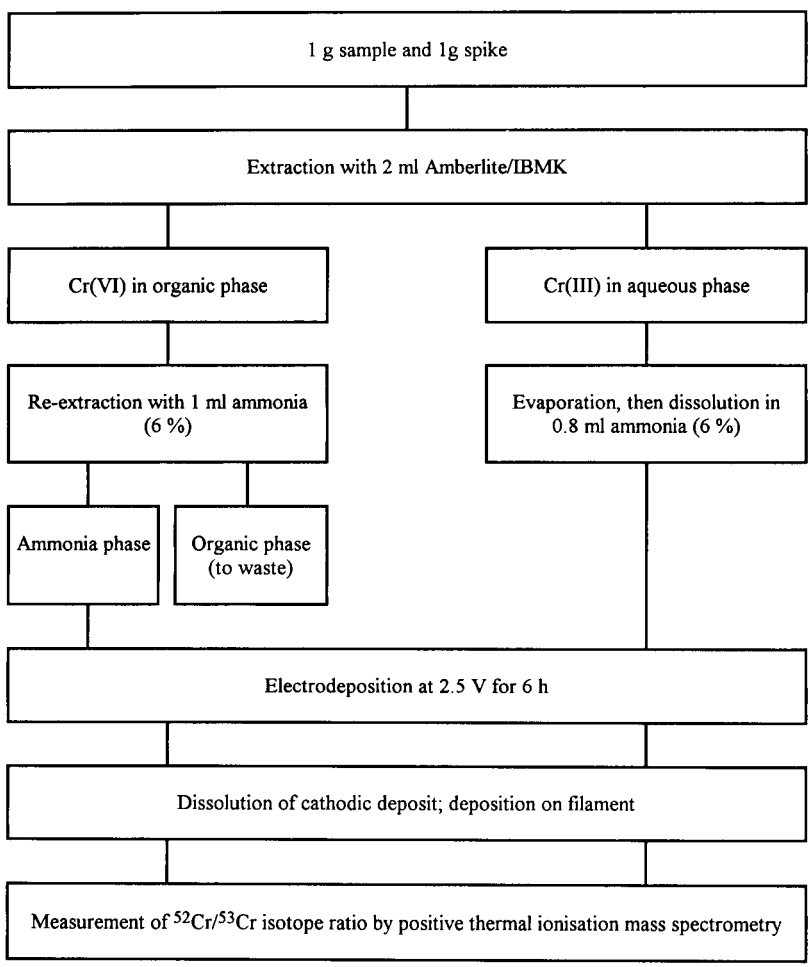

Fig. 2. Sample treatment for Cr(III) and Cr(VI) speciation with IDMS.

TABLE 2

Blank values and detection limits (for sample amounts of 1 g)

Species	Blank value (ng)	Number of analyses	Detection limit (ng g^{-1})
Cr(III)	1.4 ± 0.4	4	1.2
Cr(VI)	10.8 ± 0.8	7	2.4

50 μl of a mixture of concentrated HNO_3 and H_2O_2 (4:1).

Total procedure for the sample treatment. Figure 2 shows the scheme of the sample treatment. About 1 g of the sample together with about 1 g of the spike solution are weighed into a 15-ml polypropylene tube used for centrifugation. After shaking of the mixed sample/spike solution until equilibration about 2 ml of the liquid anion exchanger Amberlite LA-2 in IBMK are added. As described above, the Amberlite must be in its acidic form. After centrifugation for at least 2 min the phases are separated. Cr(VI) is re-extracted from the organic phase with 1 ml of ammonia (6%). Chromium is isolated from both solutions by electrodeposition at 2.5 V on Pt electrodes. The electrolytic deposits are dissolved in HNO_3–H_2O_2 (4:1) and then these solutions are evaporated to dryness. After dissolution in diluted HNO_3 the samples are deposited on the rhenium filament for the mass spectrometric measurement.

Blank determinations and detection limits. The detection limit in IDMS is either limited by the variation of the blank, or by the precision of the isotope ratio determination of the spike solution [9,10]. For chromium speciation the detection limit is restricted by the variation of the blank, whereas the precision of the $^{52}Cr/^{53}Cr$ determination of 0.3% (relative standard deviation) does not affect the determination power. In Table 2 the blank values and detection limits (3s definition) are listed for the determination of chromium species resulting from the sample treatment represented by Fig. 2. For the blank determination 1 g of the buffer solution is analysed instead of a real sample. Larger sample amounts per analysis than the applied 1 g samples will lower the detection limits. From the difference in the blank values for Cr(III) and Cr(VI) it can be followed that the extraction with Amberlite increases the chromium contamination significantly. If necessary, lower blank values can therefore be expected by further purifications of the liquid anion exchanger.

RESULTS AND DISCUSSION

Interlaboratory comparison

The results of the chromium species analyses performed in an interlaboratory comparison organised by the BCR are listed in Table 3. The results of this work [mean and standard deviation received from 5 (set A) and 3 (set B) independent determinations] are listed under laboratory No. 6. Two different sets (A and B) of samples were analysed. Set A contained both species in the ng

TABLE 3

Cr(III) and Cr(VI) determinations in an interlaboratory comparison of two solutions (concentrations by the weighed sample for A: Cr(III) = 24 ng g^{-1}, Cr(VI) = 16 ng g^{-1}; for B: Cr(VI) = 8 μg g^{-1}, no Cr(III) was weighed)

Laboratory No.	Method [a]	Concentration A (ng g^{-1})		Concentration B (μg g^{-1})	
		Cr(III)	Cr(VI)	Cr(III)	Cr(VI)
1	GFAAS	34.9 ± 2.3	21.9 ± 3.2	0.97 ± 0.29	7.96 ± 1.67
2	GFAAS	22.7 ± 0.1	19.1 ± 0.8	< DL [b]	8.53 ± 0.80
3	GFAAS	23.9 ± 0.3	15.5 ± 0.6	< DL	7.69 ± 0.20
4	FIA-AAS	14.8 ± 0.7		< DL	7.47 ± 0.10
5	ICP-AES	28.1 ± 1.2	12.3 ± 0.6	< DL	8.02 ± 0.07
6	IDMS	23.4 ± 1.5	16.3 ± 1.6	0.070 ± 0.003	7.99 ± 0.17

[a] GFAAS = Graphite furnace atomic absorption spectrometry; FIA = flow-injection analysis; ICP-AES = inductively coupled plasma atomic emission spectroscopy. [b] DL = Detection limit (values not stated by the laboratories).

TABLE 4

Intercomparison of two solutions containing Cr(III) and Cr(VI) by IDMS and GFAAS (GFAAS analyses were carried out at the University of Marburg)

Solution	Cr(III) concentration (ng g^{-1})			Cr(VI) concentration (ng g^{-1})		
	Weighed sample	IDMS [a]	GFAAS	Weighed sample	IDMS [a]	GFAAS
A	47	50.7 ± 5.4	49	73	72.7 ± 1.5	74.5
B	88	88.8 ± 3.9	79	52	49.9 ± 2.1	58

[a] The IDMS data are the mean of two independent analyses together with the deviation of the mean.

g^{-1} range. For the preparation of the solution of set B in the $\mu g\ g^{-1}$ range only Cr(VI) salt was used. Both sets of samples were delivered in freezedried form and reconstituted with the described HCO_3^-/CO_2 buffer solution directly before analysis. Most of the results are close to the weighed concentrations. However, for the lower concentration range (set A) only two laboratories (No. 3 using GFAAS and our laboratory using IDMS) fit the weighed values for Cr(III) and Cr(VI) within the given uncertainties. In the case of set B the analyses of Cr(VI) hit the weighed value mostly within the given limits of error. In the solution of set B Cr(III) could be detected by two laboratories although it has not been added. The question is, whether there has been a little change from Cr(VI) to Cr(III) during storage, or whether it is an analytical artefact. Because of the low concentration of Cr(III) beside the relatively high content of Cr(VI), an error of the IDMS analysis cannot be excluded although a second laboratory has also found Cr(III). The single-step extraction process in IDMS does not necessarily separate Cr(III) from Cr(VI) by exactly 100%. Radiotracer experiments showed that up to about 1% of the Cr(VI) can remain in the aqueous phase by extraction with Amberlite/IBMK. Therefore, a double-step extraction process should be employed when small amounts of one species are to be analysed beside a relatively high content of the other species.

Another interlaboratory comparison with the University of Marburg, Germany, was performed. Different solutions containing both chromium species in the ng g^{-1} level by weighing in the corresponding amounts of chromium salts were prepared. The results of the two solutions A and B were analysed by graphite furnace atomic absorption spectrometry (GFAAS) at the University of Marburg also using the Amberlite/IBMK extraction process for the separation of the species and by our laboratory using IDMS (Table 4). The IDMS data in Table 4 represent the means with deviation of the mean from two independent analyses. All IDMS results agree with the concentrations calculated from the weighed sample within the given uncertainties. They also agree with the GFAAS results of solution A.

Real water samples

Five different real water samples (river Danube, creeks Vils and Waldbach; treated waste

TABLE 5

Cr(III) and Cr(VI) determination in different fresh water samples by IDMS

Sample	Cr(III) concentration (ng g^{-1})	Cr(VI) concentration (ng g^{-1})	Total carbon (mg l^{-1})	Inorganic carbon (mg l^{-1})	Dissolved organic carbon (mg l^{-1})
River Danube, unfiltered	16.0	< 2.4			
Creek Vils, unfiltered	53.5	< 2.4			
Creek Vils, filtered [a]	2.6	< 2.4	51.2	46.6	4.6
Creek Waldbach, unfiltered	2.5	< 2.4			
Creek Waldbach, filtered [a]	2.0	< 2.4	10.5	2.2	8.3

[a] Sample filtered using a 0.45-μm PTFE filter. TC, IC, and DOC contents are determined in filtered samples.

water from a galvanising plant and a steel production factory) were analysed by IDMS. The results for the fresh water samples (always a single determination) are summarised in Table 5. As one can see, concentrations down to the lowest ng g^{-1} range could be analysed for Cr(III) whereas the concentration of Cr(VI) was always below the detection limit of 2.4 ng g^{-1}. In comparison with the other fresh water samples the unfiltered Vils sample shows the highest Cr(III) content, whereas the river Danube contains lower Cr(III) concentrations. The Waldbach is almost free from Cr(III). The results for the Cr(III) content of the fresh water samples can be correlated with the degree of pollution given in the "Gewässergütekarte Bayern" (water quality map of Bavaria) [14]. As mentioned there, the water of the Vils in the sampled area is highly polluted, the river Danube critically polluted and the Waldbach almost unpolluted. The graduation in water quality made in the "Gewässergütekarte" is affected by different factors, but in general it can be stated that the water quality is evaluated worse if the content of suspended particles and the pollution with organic and inorganic matter is rather high. The correlation between decreasing water quality and increasing Cr(III) content is therefore well established.

Cr(III) in natural waters tends to adsorb on suspended particles or to precipitate as $Cr(OH)_3$ [15]. However, in the presence of complexing agents, Cr(III) can remain for a long time in the dissolved form before sedimentation happens. The Cr(III) concentration in the dissolved phase of water systems containing complexing agents is therefore higher than it can be calculated by the solubility of $Cr(OH)_3$. Under the influence of complexing agents even deposited chromium from sediments can be remobilised [16,17]. In water analysis it is usual to separate soluble substances from suspended particles by filtration using a 0.45-μm filter [18]. The samples from the creeks Vils and Waldbach were therefore analysed with and without filtration. In the filtered Vils sample only 2.6 ng g^{-1} Cr(III) were detected, which is only 5% of the total concentration of 53.5 ng g^{-1} in the unfiltered sample. This means that about 95% of the Cr(III) are bound on suspended particles. Only a low portion of the total carbon (TC) in the creek Vils is due to potentially complexing agents determined as dissolved organic carbon (DOC). Herewith the great differences in the Cr(III) concentrations of the filtered and the unfiltered Vils samples can be understood. The DOC content of the creek Waldbach is about a factor of two higher than for the Vils. On the other hand, the TC content of the Waldbach is much lower than that of the Vils. The Waldbach transports higher amounts of dissolved organic matter, e.g., humic substances, but only a low portion of suspended particles. Most of the Cr(III) can therefore be dissolved as organic complexes. As expected under such a condition, the Cr(III) concentration of the filtered Waldbach sample is almost as high as that of the unfiltered sample. With respect to the organic carbon in the fresh water samples, no Cr(VI) could be expected. If there should have been a Cr(VI) input into these water systems, it should have been reduced to Cr(III) by the organic matter [15].

The Cr(III) and Cr(VI) contents analysed in two treated waste water samples are listed in Table 6. In both samples a relatively low Cr(III) content and no Cr(VI) above the detection limit could be analysed. It is remarkable that even in the waste water of the galvanising plant, where chromium plating is performed, only a concentration of 6.9 ng g^{-1} for Cr(III) could be found. This shows the high efficiency of the waste water treatment applied in this plant. In Germany the limit for an input of Cr(III) into sewage water is 2 μg g^{-1}, for Cr(VI) 0.5 μg g^{-1} [19]. By the developed IDMS method for chromium speciation it is no problem to control such concentration limits.

TABLE 6

Cr(III) and Cr(VI) determination in two different treated waste water samples by IDMS

Waste water from	Cr(III) concentration (ng g^{-1})	Cr(VI) concentration (ng g^{-1})
Galvanising plant	6.9	< 2.4
Steel production factory	10.8	< 2.4

Conclusion

IDMS proves to be a reliable method for chromium speciation. The discussed interlaboratory comparisons demonstrated the quality of this analytical method, which is therefore also an apt tool for the certification of standard reference materials. Because of the necessary species separation the amount of time consumed by a method like thermal ionisation IDMS is not much larger than for a method like GFAAS, which is normally much faster in total element analysis. The high accuracy linked with the IDMS results make IDMS of especial interest. Another important topic in the developed IDMS method is that both species are directly determinable. This is a great advantage compared with indirect determinations, where only one species is determined besides the total element content. The determination of the second species by calculating the difference between the total element content and the species content directly analysed can cause significant errors.

We thank the BCR of the European Communities for financial support, Prof. B. Neidhart and T. Müller, University of Marburg, for the excellent cooperation in the intercomparison study, and L. Rottmann for the determination of the TC, IC, and DOC concentrations in fresh water samples.

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Analytica Chimica Acta, 286 (1994) 291–296
Elsevier Science B.V., Amsterdam

Accurate and precise determination of chromium by isotope dilution mass spectrometry in some environmental materials

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(Received 17th February 1993; revised manuscript received 15th July 1993)

Abstract

A method for the determination of chromium with the help of thermal ionization isotope dilution mass spectrometry was developed. In this type of mass spectrometry a stable signal depends mainly on the purity of the loaded sample. Therefore, the main effort was the development of a method for the separation of the chromium from the matrix. A separation procedure based on ion exchange is described. Two alternative thermal ionization methods were investigated. With the first one (the so-called triple filament technique) the measurable amount of chromium ranges from 10 ng to 10 μg. The second technique (a single filament technique) needs 0.1–10 μg. For economical reasons the last technique was chosen. Combined with a blank value of 0.4 $\pm$ 0.2 μg and a sample intake of 1–2 g the detection limit of the described method is approximately 0.5μg/g. To test the method a number of certified reference materials were analyzed.

Keywords: Isotope dilution methods; Mass spectrometry; Chromium; Environmental materials

Together with elements like cadmium, lead, mercury and arsenic also chromium, defined as Cr(VI), appears very frequently in lists of toxic substances. This includes national regulations (water, sewage sludge, etc.) and EC directives. For instance EC directives concerning drinking and bathing water and concerning soils and sludges define the maximum tolerable chromium content [1].

In the VI form it is very toxic and has to be determined as such in environmental and biological substances [2]. But as the transformation from III to VI is not too difficult, the knowledge of the total chromium content is important.

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The knowledge of the chromium content is also important in the study and evaluation of long term trends and biochemical pathways.

The increasing level of quality assurance in analytical laboratories in the future will increase the demand for reference materials certified for chromium.

These needs combined with the difficulties around the determination of this element give reason to increase the number of materials with well defined values for the chromium content.

Chromium is a difficult element for the analytical chemist. The existence of two common oxidation states III and VI, its refractory character and the ability in the trivalent state to form very strong and stable complexes hamper the accurate determination of chromium. For elements like lead and cadmium the results obtained with vari-

SSDI 0003-2670(93)E0420-C

ous techniques usually agree. However sometimes for chromium the different techniques are not in agreement. Several techniques are applicable for the analysis of chromium, techniques like neutron activation, atomic absorption [3] and electrochemical methods [4,5]. Another possibility is the combination isotope dilution mass spectrometry (IDMS). The technique applied can be a combination of gas chromatography and mass spectrometry [6,7] or direct mass spectrometry with thermal ionization [8]. In this article a method of the last type is described.

EXPERIMENTAL

Apparatus

A Micromass 30B mass spectrometer has been used for the measurements. This instrument is a "double" focussing mass spectrometer with a radius of 31 cm and a 90° sector. The mass range is from 1 to 350 with an accelerating voltage of 10 kV. Ions are produced thermally. The machine has two detection systems: a Faraday cup and a Daly detector. After amplification the signals are measured by a digital voltmeter. The output of the voltmeter is read and processed by a computer.

Reagents and equipment

All dishes and beakers were made of PTFE and especially cleaned by leaching in hot hydrochloric acid and hot nitric acid. The high purity nitric, hydrofluoric, hydrochloric and perchloric acids were prepared by subboiling distillation. All other chemicals were reagent-grade. Manipulations were carried out in clean air benches.

Commercially available Econo (BioRad) anion exchange columns were used, 4 cm × 0.7 cm i.d., acetate form, Ag 1 × 8 200–400 mesh. The acetate form was prepared from the chloride form, by eluting the column in chloride form with 3 × 10 ml 2 M ammonia, 1 × 10 ml water and 2 × 10 ml 0.1 M acetic acid.

The chromium spike was prepared from chromium(III) oxide with an enrichment in ^{53}Cr of 97.9%, obtained from Oak Ridge National Laboratory. After fuming of the oxide with perchloric acid the material was dissolved in water.

The silica gel solution was prepared by leading silicon tetrafluoride gas into an aqueous solution saturated with ammonia gas. The silicon dioxide precipitate was filtered, washed and dried at 60°C. Before use 100 mg silicon dioxide was mixed with 1 ml ultra pure water. This mixture was stirred continuously.

The filament material was zone-refined rhenium, purity 99.995%.

Sample pretreatment and destruction

To 0.5–1 g sample, spike solution, 10 ml conc. nitric acid and 3 ml conc. hydrofluoric acid were added. The amount of spike added to the sample is dependent of the amount of chromium in the sample. In general the ratio Cr(sample)/Cr(spike) was chosen to be between 0.1 and 10. The destruction was performed in a steel lined PTFE bomb at 180°C during at least 72 h. After destruction the sample was evaporated till dryness and dissolved in 10 ml 6 M hydrochloric acid containing 0.1% hydrogenperoxide (w/v). As a last step evaporation till dryness and dissolution in 15 ml 6 M hydrochloric acid was applied.

Separation of chromium

The solution was put on a Ag 1 × 8 anion exchange column in chloride form. The solution passing directly through the column was evaporated very gently till dryness, the temperature should not exceed 60°C. The residue was dissolved in 25 ml 1 M acetic acid. 1 ml 5 % (w/v) potassium bromate in 1 M acetic acid was added. Oxidation took place at boiling temperature for at least 6–7 h. After the oxidation step a reduction of volume was performed by evaporation to approximately 3 ml, followed by addition of 25 ml water. The solution was put on an Econo column in acetate form, Ag 1 × 8. The column was rinsed by 8 × 10 ml 0.1 M acetic acid. The chromium was eluted with 11 ml 7 M nitric acid followed by evaporation till dryness and dissolution in conc. nitric acid and again evaporation till dryness (twice). The residue was dissolved in 1 ml conc. hydrochloric acid followed by evaporation till dryness. Subsequently, it was dissolved in 1 ml 6 M hydrochloric acid, and 0.1 ml hydrogenperoxide (30%, w/v) was added and the solution evapo-

rated till dryness. The residue was dissolved in 0.7 M nitric acid containing 0.1% hydrogenperoxide (w/v) and the solution was evaporated till dryness. The residue was taken up in 0.7 M nitric acid, the final Cr concentration should be approximately 1 μg/μl.

Filament loading

In thermal ionization mass spectrometry two methods are applied generally.

The first one is the so-called triple filament technique: the two side filaments of the sample holder, the so-called sample bead, are used for evaporating the sample while the center filament is used for ionization. For Cr, the temperature for the side filaments during measurements lies between 1200–1500°C while the center filament temperature is approximately 2100°C. The amount of chromium measurable with the triple filament technique ranges from 10 ng–10 μg.

In the second technique the sample bead has only one filament. This filament is loaded with the sample solution, a silica gel solution and a boric acid solution and evaporated till dryness. For Cr, the temperature of the filament during measurement is approximately 1250°C. Also with this technique good results were obtained. The amount of chromium needed was 0.1–10 μg.

Both methods have been tried but the materials have been analyzed using the single filament technique only (economical reasons, only one rhenium filament needed instead of three).

1 μl silica gel solution (100 mg silica per ml water) was put on the filament and dried by passing a current of 1.1 A through the filament. Next, 1 μl sample solution (approximately 1 μg Cr per μl) was put on the filament and dried at 1.1 A. As a last step 1 μl 0.8 M boric acid solution was added and dried at 1.1 A. Then the filament was heated at 1.5 A during 4 min, followed by heating at 1.75 A during 10 s. The sample beads were placed in a six-sample turret, which was placed in the mass spectrometer.

Mass spectrometric procedure

The sample was heated automatically in 30 min, the maximum current was 2.5 A. Manually the current was slowly increased to 2.6 A, approximately 1225°C. The measurement was started 40 min after starting the heating. In one measurement cycle the ion currents on the following mass positions were measured: 52–53–53–54–50–52–53.5–53.5–53.5. Measurements were performed with the help of a Faraday cup. The ion currents for the main isotopes were usually between 5×10^{-12} and 5×10^{-13} A. A measurement run consisted of ten sequentially measured cycles. From each sample two runs were measured, the second run was used for calculations.

Calculations

The measurements described were measurements of the blends (mixtures of target and spike), the unspiked samples were usually not measured. For the calculations the so-called natural composition was used. After correction of the ion intensities for the gradual change of the ion currents with the passage of time, using linear interpolation between two adjacent 52 isotope measurements, the ratios were calculated and checked for outliers using Dixon's test. The mean and the standard deviation of the remaining ratios were printed [9].

All acceptable results were used in an isotope dilution analysis program to calculate the mass ratio between the target and the spike. A correction factor which is applied in this program is the so-called mass discrimination factor. This factor corrects for non-equal sensitivities of the machine for different isotopes. For example in the measurement of chromium the intensity of the 52 isotope is usually somewhat too high, the machine favors it somewhat. To determine this factor, one needs isotope reference materials. For the element chromium such a material is available; SRM 979 chromium(III) nitrate of NIST (Washington, DC), which was used in this case. The correction factor is usually small and rather stable over a period of time. In this case the 53/52 ratio had to be divided by 0.99946.

At the end of the calculations the results of the blends were combined and when the repeatability was satisfactory the final result and its standard deviation was calculated.

RESULTS AND DISCUSSION

Calibration of the spike solution

The calibration of the spike solution was carried out with standard solutions obtained from pure chromium metal, purity 99.996%. The metal was etched with dilute nitric acid before use. The standard and spike were stored in cleaned polythene bottles. From every bottle the weight was recorded. In this way correction factors for evaporation during storage could be applied.

Every calibration was carried out by dissolving two pieces of metal and preparing from those two separate standard solutions. From each standard solution four mixtures with the spike solution were prepared on a weight basis. These blends were measured with the mass spectrometer as described. The results show the concentration of the spike solution obtained with both calibration solutions and are given in Table 1.

Blanks

The variation in the blank was, as in many other methods, often the restricting factor concerning detection limit and precision. Especially in IDMS with its frequently complicated chemical separation procedures it plays an important role. An advantage of IDMS is that an estimation of the variation of the blank value can be performed rather accurately. When a dilute spike solution is treated like a sample, a good determination of the blank level can be performed.

For chromium the level of the blank value was 0.4 ± 0.2 μg.

TABLE 1

Calibration of spike solution with two calibrants (A and B)

	Solution A (μg/g)	Solution B (μg/g)
	254.5	255.0
	254.2	254.6
	254.9	254.4
	254.5	254.2
Mean	254.5	254.6
S.D.	0.3	0.3
Total mean	254.5	
S.D.	0.3	

TABLE 2

Results of the determination of chromium in some reference materials

Materials	Reference value (μg/g)	Measured value (μg/g)
BCR 277 Estuarine sediment	192 ±7	197 ±3
BCR 320 River sediment	138 ±7	137.4 ± 1.5
BCR 414 Plankton	23.8 ± 1.2	27.1 ± 0.4
	Indicative value (μg/g)	Measured value (μg/g)
BCR 100 Beech leaves	6.7	8.4 ± 0.3

Reference materials

A number of reference materials have been analyzed in sixfold for their total chromium content, results are presented in Table 2.

Candidate reference materials

In the context of a program on the certification of soils and sludges, three materials have been analyzed, among other elements, on chromium. Not only the total chromium content was determined but also the aqua regia extractable part. The extraction was performed according to DIN 38414.

In Table 3 the mean value of the results of the other laboratories (mean of the means) is compared with the mean of the results obtained by IDMS. The results of the aqua regia extracts were in agreement with each other while the total Cr results of the IDMS were higher then the ones of the other laboratories. The disagreement between the IDMS results and the ones of the other laboratories in the case of the total chromium determination may be caused by incomplete destruction. In this study the destruction time was at least 72 h and the temperature 180°C which is a much more severe attack than used by any other laboratory in the certification study [10].

Detection limit

With the single filament technique the amount of chromium necessary for a reliable determina-

TABLE 3

The results of the determination of chromium in some candidate reference materials

Total chromium	Mean results (μg/g)	Measured values (μg/g)
BCR 142R Sandy soil	112.4 ± 4.7	124.7 ± 1.6
BCR 143R Sludge amended soil	543 ± 59	631 ± 5
BCR 145R Sewage sludge	312 ± 17	335 ± 4
Aqua regia extracts		
BCR 142R Sandy soil	84.2 ± 8.3	84.5 ± 0.9
BCR 143R Sludge amended soil	428 ± 13	420.0 ± 1.5
BCR 145R Sewage sludge	303 ± 21	316.6 ± 3.7

tion ranges from 0.1–10 μg. In this case reliable means a precision of the isotope ratio measurements better than 0.5% relative. Concerning the upper limit, not only a too small amount but also a too large amount gives instabilities in the signal. The blank of the applied method was 0.4 ± 0.2 μg. With a sample intake of 1–2 g the detection limit will be approximately 0.5 μg/g.

DISCUSSION

In isotope dilution the parameter to be determined is the ratio between added spike and the unknown amount of substance in the sample. When the amount of added spike is well known, it is quite easy to calculate from this ratio the unknown concentration. The measurement of a ratio implies that it is not necessary to work quantitatively like in many other methods, what is a great advantage.

Furthermore, in thermal ionization mass spectrometry integrated ion currents are measured. From those measurements ion current ratios are calculated. As the behavior of the different ions of one element in the mass spectrometer is much alike the ion current ratios are almost equal to the ratios of the corresponding isotopes in the sample and these ratios are fairly insensitive to changes in the experimental conditions during the measurement.

The two mentioned advantages of isotope dilution mass spectrometry are the main reasons for the fame of this technique as a very reliable technique.

The results demonstrate that the IDMS method described has a high degree of precision. The method itself shows a calibration precision of approximately 0.1%. However in the analysis of the reference materials the influence of other experimental factors like destruction is clearly shown: in those cases the precision ranges from 0.5–2%. Concerning accuracy a number of reference materials show good agreement between IDMS and the certified values but for some other reference materials the values of IDMS were higher. As already stated a reason may be that cases of incomplete destruction and dissolution of sample material were unwittingly included in some certification studies. Chromium has a refractory character and a severe chemical attack is necessary for complete success.

Concluding it may be stated that the use of the IDMS method is especially of great value for the certification of reference materials. During round robin tests it is one of the techniques suitable for determining target or reference values and in the case of contradictory results between methods, IDMS may give a helpful indication about the reason of the contradiction.

The project described in this paper has been undertaken under BCR-contract No. 5302/1/9/348/90/BCR-NL(10) from the Commission of the European Communities. The authors wish to thank the Commission of the European Communities and especially the Community Bureau of Reference BCR for this financial support.

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Analytica Chimica Acta, 286 (1994) 297–308
Elsevier Science B.V., Amsterdam

Development and interlaboratory testing of aqueous and lyophilized Cr(III) and Cr(VI) reference materials

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(Received 17th February 1993; revised manuscript received 22nd September 1993)

Abstract

This work succeeded in developing a reference material for speciation of Cr(III) and Cr(VI) in water. It consisted of a HCO_3^-/H_2CO_3 (50 mmol/l) buffer solution containing Cr(III) (40 μg/l) and Cr(VI) (10 μg/l) at pH 6.4. A second reference solution, typical for speciation of Cr(VI) from welding dust, contained Cr(VI) (5 mg/l) at pH 9.6. The parameters to obtain stable solutions were carefully studied. Two formulas were tested: first an aqueous solution kept under a CO_2 blanket in sealed quartz ampoules; this later evolved into a lyophilized version to be reconstituted in the buffer solution at the right pH. The stability studies demonstrated the reliability of the materials, which allowed to organize 2 interlaboratory studies between 7 European laboratories. The results of the intercomparisons confirmed the validity of the methodology for preparing such candidate reference materials for Cr(III)/Cr(VI) speciation.

Keywords: Atomic absorption spectrometry; Ion exchange; Radiochemical methods; Chromium; Metal spectation

The bioavailability and toxicity of chromium are of growing concern for the public health. The different oxidation states of chromium exert very dissimilar levels of toxicity, with the hexavalent species being highly toxic compared to the trivalent. Therefore, it is necessary to determine the species rather than the total element content.

The mechanisms of chromium metabolism, genotoxicity and carcinogenicity are extensively described by De Flora and Wetterhahn [1]. Nriagu and Nieboer [2] give a comprehensive assessment of the uses of chromium, its sources, and occurrence in the air, water and soil. Chromium toxicity to terrestrial and aquatic biota and to humans has been examined in relation to its chemistry and biochemistry.

The EC Directive [3] for drinkingwater states that total Cr should not exceed 50 μg/l. In occupational health the OEL (occupational exposure limits) for soluble and certain water insoluble compounds in indoor air are limited to 0.5 mg/m^3 for Cr and 0.05 mg/m^3 for Cr(VI). Hexavalent chromium is such a potent carcinogenic agent for the respiratory tract, that continuous monitoring of the inhaled amount has been imposed. This is stated in an EC Directive [4] on the exposure to carcinogenic substances. There exists convincing evidence that now the accuracy of the data is not satisfactory. To meet the requirements of the Directives, the reliability of the methodologies has to be substantially improved. There is an

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SSDI 0003-2670(93)E0526-D

urgent need for quality assessment of the Cr(III) and Cr(VI) routine measurements in water and welding dust. An effective and economical way to control the accuracy of the results consists of analyzing reference materials (RM) or better a certified reference material (CRM) [5]. A reference material is defined by the International Organization for Standardization (ISO) as a material one or more properties of which are sufficiently well established to be used for calibration of an apparatus, the assessment of a measurement method, or for assigning values to materials [6]. RMs are also useful for daily quality control and for checking the precision of the performance [7,8].

The ultimate goal is to obtain a certified reference material (CRM) which is an RM that has one or more property values certified by a technically valid procedure, accompanied by or traceable to a certificate or other documentation which is issued by a certifying body [6]. The property value is certified by well established laboratories who have improved their performance to an excellent level, applied quality control rules strictly and given proof for the traceability of the results [9].

This work aims at developing an adequate reference material for Cr(III) and Cr(VI) in aqueous medium. At first a water solution was made containing both species under stable conditions, but later this evolved into a lyophilized product. These materials were subject to a first and a second interlaboratory comparison on chromium speciation which allow to gauge the state of the art of the analytical methodology within Europe, improve the laboratory performances and test the aqueous and lyophilized reference materials for candidate CRMs.

EXPERIMENTAL

Analytical procedure

The present work relies on the separation of Cr(III) and Cr(VI) through anion exchange extraction of Cr(VI) with Amberlite diluted in MIBK (methyl isobutyl ketone) and stripped in HCl (6 mol/l) [10].

For the radiotracer experiments the measurement of $^{51}Cr(III)$ and $^{51}Cr(VI)$, both in a separate phase, is performed by measuring the 320 keV γ-ray of ^{51}Cr ($t_{1/2} = 27.7$ days) with a NaI well type scintillation detector, coupled to a single-channel analyzer. The counting system was provided with an auto-sampler changer. The advantage in ease of detection of trace amounts of radioactive isotopes is unsurpassed for γ-rays. Detection consists of a simple measurement of the radioactivity without requiring tedious sample preparation and calibration as is necessary in, e.g., electrothermal atomic absorption spectrometry (ETAAS). Additionally, the procedure avoids the hazard of detecting contamination from other sources of the trace element studied [11].

For the non-radiotracer experiments the measurement of Cr(III) and Cr(VI), both in a separate phase, was performed by ETAAS.

Reagents and laboratory ware

The reagents $NaHCO_3$, $Na_2CrO_4 \cdot 4H_2O$ and $CrCl_3 \cdot 6H_2O$ were all p.a. grade and respectively purchased from RPL (Leuven) and Merck (Darmstadt). The ^{51}Cr labelled solutions were purchased from Amersham.

The cleaning procedure for the glass ware and pipettes was as follows: (A) 3×3 mol/l HNO_3 + HCl (p.a.), (B) 2×3 mol/l HCl (subboiled) and (C) 4 × Milli-Q water. The cleaning procedure for the quartz ampoules and glass vials was as follows: soaking in detergent for 24 h, 3 times rinsing with deionised water, 8 h boiling in HNO_3 (3 mol/l) + H_2SO_4 (1.5 mol/l) (p.a.), 3 times rinsing in deionised water, 3 times rinsing in Milli-Q water, 8 h boiling in HNO_3 (3 mol/l) + H_2SO_4 (1.5 mol/l) (subboiled acids), 3 times rinsing with Milli-Q water, 8 h steamcleaning with Milli-Q water and finally drying in a clean bench.

Development of aqueous samples

Previous stability studies are described in detail by Dyg et al. [12]. Different parameters were investigated such as the stability of Cr(III) and Cr(VI) as a function of time, different solutions, possible losses due to adsorption, temperature dependency and choice of the material of the container. After preliminary experiments it was

concluded that a HCO_3^-/H_2CO_3 (50 mmol/l) buffer solution (typically HCO_3^- content in river water is approx. 1 mmol/l) at pH 6.4 under a CO_2 blanket could be considered as a suitable medium to achieve stable solutions of the Cr(III) and Cr(VI) species. By choosing hydrogencarbonate as a buffer and an agent to prevent the hydrolysis of Cr(III), a matrix very close to that of real waters was achieved. The results of the above mentioned experiments showed that for a 140 days period, stable solutions of Cr(III) and Cr(VI) at levels of 25 μg/l can be obtained in a hydrogencarbonate buffer (50 mmol/l) at pH 6.4 under a CO_2 blanket (to maintain constant pH value) stored in PTFE at 5°C (solution A) [12].

To serve the "occupational health analysts", whom are working in the range of mg/l of Cr(VI), a further stability study had to include the investigation of Cr(VI) in that concentration range (solution B). The "water analysts", complied with the type of solution already studied (solution A).

Preliminary studies in carbonate buffer solutions at different pH, showed that a solution at pH 9.6 could make up a stable medium for Cr(VI). One series of solution B containing Cr(VI) (5 mg/l) and stored at 5°C in quartz ampoules was prepared.

To confirm the previous findings, a new series of solution A was prepared and afterwards stored in quartz ampoules. Quartz was chosen instead of PTFE for practicality as quartz ampoules are easier to seal and for guaranteeing the CO_2 blanket not to evaporate through the wall. The new series of solution A contained Cr(III) (40 μg/l) and Cr(VI) (10 μg/l) and was stored at 5°C.

One series was spiked with ^{51}Cr radiotracers, another series without radiotracers.

Development of lyophilized samples

Due to its presumably indefinite shelf life, a lyophilized product of stable Cr(III) and Cr(VI) species could be most interesting as a reference material. This prompted us to investigate the feasibility of producing such a product.

The investigations were focused on the possible preparation of two different types of stable Cr(III)/Cr(VI) solutions at concentration ranges (A and B) which would comply to the needs of the two groups of analysts working in the field of Cr speciation already mentioned.

First special designs of the container needed to be developed to avoid losses of chromium as they occur out of an open vial during the freeze drying process. Quartz wool was used to prevent losses of specks of frozen material during the freeze drying step and proved to guarantee a 100% recovery of total chromium after reconstitution.

Considering the high cost of the quartz ampoules it seemed worthwhile to investigate the possibility of using glass vials. Two designs, to avoid losses of chromium during lyophilization, were tested.

At first special attention was paid to the recovery of total chromium and to the possible occurrence of adsorption of Cr(VI) on the glass wall. The first design simply consisted in putting a syringe needle through the PTFE-sealing to avoid losses of chromium during the freeze drying step. This procedure was found suitable, but considered unacceptable for this type of work due to Cr contamination risks. The second approach, which is currently used in all studies, consists of a laboratory film (Parafilm "M") cover with 3 perforations. After freeze drying the parafilm is first gently patted and then substituted with a PTFE seal especially designed for these glass vials. The presence of the radiotracer ^{51}Cr was measured in order to check if some chromium was present on the withdrawn parafilm, but no significant amounts could be observed.

Although a full recovery of total chromium was achieved throughout the freezedrying step, a major reduction of Cr(VI) was observed. It should be stated that the aim is to obtain freezedried material with a long term stability of the Cr(III)/Cr(VI) species, therefore it does not matter if a small amount of Cr(VI) is reduced during the freezedrying step.

Several parameters, which were supposed to influence the composition of the Cr(III)/Cr(VI) during the freezedrying step, were changed without any improvement. Finally, conditions for obtaining a reliable product were successfully achieved by performing all manipulations in the class 100 clean room and by using pure N_2 as

inlet gas after the freezedrying step. Therefore reduction of Cr(VI) to Cr(III) was prevented by storing the samples in this inert gas environment.

INTERLABORATORY COMPARISON

Information accompanying the aqueous and lyophilized samples

Quartz ampoules/glass vials were despatched by mail and upon receipt, the participants were requested to store the samples at + 5°C, until required for analysis, which should be carried out as soon as possible. Two sets of samples at two different concentrations were provided and the ampoules/vials were marked individually as A or B, with the approximate concentration ranges showed below, which were communicated to the participants on beforehand.

Sample A: concentration range of Cr(III) and Cr(VI) = 10–40 μg/l;

TABLE 1

Descriptions of techniques used by the participants

Lab. no.	Sample pretreatment	Technique	LOD
1	Cr(III) and Cr(VI) were separated by extraction of Cr(VI) into liquid anion exchange solution Amberlite LA-2, diluted 1:2 with methyl isobutyl ketone (MIBK). Sample B: Cr(VI): Coupling of chromate with 1,5-diphenylcarbazide (DPC).	ETAAS SPEC	0.2 μg/l 10 μg/l
2	Cr(III) and Cr(VI) were separated by extraction of Cr(VI) into liquid anion exchange solution–Amberlite LA-2, diluted 1:2 with methyl isobutyl ketone (MIBK). Volume ratio of sample and LA-2 1:1. Sample B was diluted a factor 500 for Cr(VI) and 1000 for total Cr.	ETAAS	Cr(III) and total Cr: 0.10 μg/l Cr(VI) 0.15 μg/l
3	Cr(III) and Cr(VI) were separated by using cation (Dowex 50W) and anion Dowex (1-X8) exchange resins, which were (approx. 1 ml) placed in separate PP columns (Bio-Rad).	ETAAS	0.2 μg/l
4	Sample A and B: Cr(III) and Cr(VI) were separated by extraction of Cr(VI) into liquid anion exchange solution–Amberlite LA-2, diluted 1:2 with methyl isobutyl ketone (MIBK). Volume ratio of sample and LA-2 1:1. Sample B was diluted by a factor of 500. Sample B: Cr(VI): Coupling of chromate with 1,5-DPC. Total Cr	ETAAS SPEC FAAS	0.2 μg/l
5	Column 50 μl aluminium oxide acidic activated, 56–170 μm Cr(III): on: KH_2PO_4/NaOH pH 7 off: 1.0 M HNO_3 Cr(VI): on: KCl/HCl pH 2 off: 0.5 M NH_3	FIA-AAS	2.0 μg/l
6	Sample A: Cr(III) diluted 1:1 with 0.02 M NH_4OH. Basic alumina column. Cr(VI) diluted 1:1 with 0.02 M HNO_3. Acidic alumina column. Sample B: Cr(III) and Cr(VI) diluted 1:10 with distilled water.	FIA-ICP-AES	0.93 μg/l
7	Extraction of Cr(VI) with LA-2/MIBK. Back extraction with NH_3 (6%). Spiking with Cr-53 enriched solution.	IDMS	

Sample B: concentration range of Cr(VI) = 5–10 mg/l.

The participants were asked to do 5 independent replicates and to report the results for both the Cr species as well as for total Cr on the enclosed "Report Sheet".

Preparation of aqueous samples

Volumes of 10 ml of A or B were pipetted into Suprasil quartz ampoules, which were immediately sealed under a CO_2 blanket.

The exact concentration of the Cr species, as prepared in clean room conditions (class 100), was as follows:

Solution A: Cr(III) = 37.9 μg/l, Cr(VI) = 9.3 μg/l, total Cr = 47.2 μg/l;

Solution B: Cr(VI) = 4.63 mg/l; total Cr = 4.63 mg/l.

Preparation of lyophilized samples

Six glass vials (or more if needed) each one containing freezedried aqueous Cr solution were despatched. To reconstitute the samples, $NaHCO_3$ was provided with the sample to prepare a buffer solution as reconstitution medium.

Preparation of buffer. In order to avoid excessive losses of CO_2, the buffer should be prepared by transferring 0.42 g of $NaHCO_3$ to a 100-ml volumetric flask and dissolving it with 40–50 ml of Milli-Q water. The flask should then be filled with CO_2 and 25 ml of HCl (0.1 mol/l) (analytical grade) added. The buffer is made up to volume with Milli-Q water. In order to maintain the pH of the buffer at 6.4, the flask should be flushed with CO_2 following use.

Reconstitution of samples. After opening the vial 10 ml of the HCO_3^-/H_2CO_3 buffer was added, the rubber seal replaced and, holding the vial between thumb and forefinger, the contents of the vial shaken gently for 30 s. The separation procedure shall take place immediately after the sample has been reconstituted.

The exact concentration of the Cr species in solution, when prepared in clean room conditions (class 100), were as follows:

Solution A: Cr(III) = 24.0 μg/l, Cr(VI) = 16.0 μg/l, total Cr = 40.0 μg/l;

Solution B: Cr(VI) = 8.00 mg/l, total Cr = 8.00 mg/l.

Analytical methods applied by participants

A summary of techniques applied in the determination of the Cr species in samples A and B is presented in Table 1. For the separation of the Cr(III)/Cr(VI) species, laboratories 1, 2, 4 and 7 used the same technique based on the extraction of Cr(VI) with liquid anion exchanger Amberlite LA-2 in MIBK. Laboratory 3 applied a cation exchange on DOWEX 50W for Cr(III) species and anion-exchange DOWEX 1-X8 for Cr(VI). Both laboratories 5 and 6 used aluminium oxide, respectively in the basic and acidic form for Cr(III) and Cr(VI) separations. Additionally laboratories 1 and 4 applied a complexation of the Cr(VI) of the B-type solution with 1,5-diphenylcarbazide.

The detection of the Cr occurred with 5 different types of analytical techniques: in case of solution A: ETAAS, ICP-AES (inductively coupled plasma atomic emission spectrometry), FIA-AAS (flow-injection analysis AES) and IDMS (isotopic dilution mass spectrometry), and additionally SPEC (spectrophotometry) and FAAS (flame AAS) for solution B.

The LOD (limit of detection defined as 3σ on the background signal) proved to be sufficiently low in all cases.

The diversity in separation procedures and detection techniques virtually eliminates the hazard that similar systematic methodological errors would bias the overall mean values for Cr(III)/Cr(VI).

RESULTS AND DISCUSSION

Stability studies of aqueous samples

A series of solution A containing Cr(III) (40 μg/l) and Cr(VI) (10 μg/l) was stored at 5°C in sealed and unsealed quartz ampoules under a CO_2 blanket. One series was spiked with ^{51}Cr radiotracers and the stability was studied over a 69-day period. The individual values are given as Mean ± C.V. (coefficient of variation) and illustrated in Fig. 1. The stability of both species are

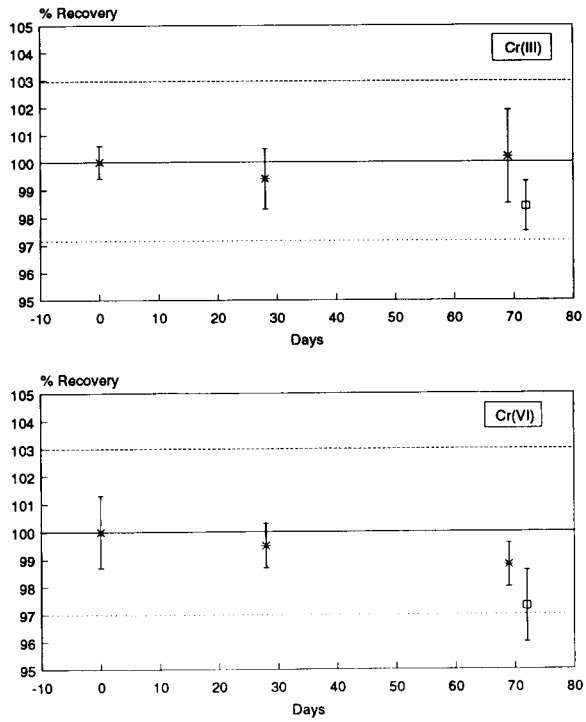

Fig. 1. Recovery of $^{51}Cr(III)$ and $^{51}Cr(VI)$ in hydrogencarbonate buffer (solution A containing Cr(III) (40 μg/l) and Cr(VI) (10 μg/l)). The recovery at $t = 0$ has been arbitrarily put to 100% (full line). The individual values are given as Mean ± C.V. and a 95% CI of the mean value is indicated as a dotted line. Key: □ = sealed quartz ($n = 2$); * = open quartz ($n = 5$–8).

confirmed with a 95% CI (confidence interval) of the mean value (dotted line). Stability of the chromium species as a function of temperature has been studied for solution B. Sealed quartz ampoules were stored at 20°C and 40°C for a period of 78 days (Fig. 2). Solution B turned out to remain stable.

Stability studies of lyophilized samples

13 quartz ampoules containing 5 ml of solution A spiked with $^{51}Cr(III)$ and $^{51}Cr(VI)$ were freezedried. Four specimens were analyzed (day 0) for total Cr, Cr(III) and Cr(VI) after reconstitution in 5 ml HCO_3^-/H_2CO_3 buffer at pH 6.4. The last 9 ampoules were sealed and stored at −20°C. Three ampoules were respectively analyzed after 14, 48 and 88 days. The results depicted in Table 2 display the stability of the freezedried material with regard to Cr(III) and Cr(VI) over a 88-day period and stored at −20°C. The fate of the Cr species during the freezedrying step was also investigated and no significant changes were observed.

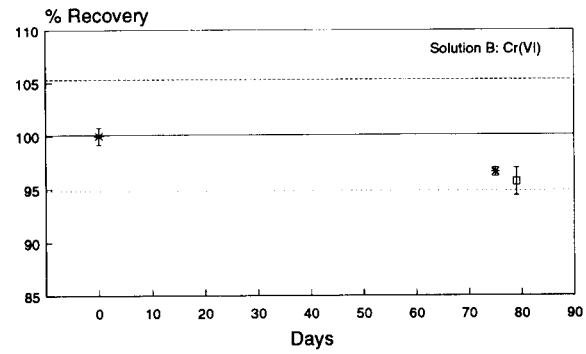

Fig. 2. Recovery of $^{51}Cr(VI)$ in solution B containing Cr(VI) (5 mg/l) and stored at (*) 20°C and (□) 40°C. The values are given as mean ± C.V. The dotted line indicates a 95% CI of the mean value. $^{51}Cr(III)$ measurement was below LOD.

To lower the freezedrying time the feasibility of lyophilizing 1 ml of a solution with 5-fold higher contents of Cr(III) and Cr(VI) and subsequent reconstitution and dilution with 5 ml hydrogencarbonate buffer was studied and proved to meet expectations.

TABLE 2

Recovery of total Cr, Cr(III) and Cr(VI)

[The freeze dried samples in quartz ampoules were stored at −20°C. The recoveries and ratios are given as percentages ± C.V.%. The recovery at day 0 has arbitrarily been put to 100%]

	Day 0	Day 14	Day 48	Day 88
	$n = 4$	$n = 3$	$n = 3$	$n = 3$
Total Cr	100.0 ± 0.7	103.0 ± 0.7	102.9 ± 0.7	99.9 ± 0.7
Cr(VI)	100.0 ± 0.9	101.7 ± 0.9	100.9 ± 1.0	99.4 ± 1.0
Cr(III)	100.0 ± 0.5	103.4 ± 0.7	103.2 ± 1.7	98.7 ± 1.5
Ratio [a] Cr(VI)	43.6 ± 1.1	43.9 ± 1.1	42.7 ± 1.2	43.3 ± 1.2
Ratio [a] Cr(III)	56.9 ± 0.9	57.1 ± 1.0	57.1 ± 1.8	56.3 ± 1.7
Σ [b]	100.5 ± 1.4	101.0 ± 1.5	99.8 ± 2.2	99.6 ± 2.1

[a] Ratio calculated from the gamma counts of the radiotracer ^{51}Cr at day x using the equation (Cr(III) or Cr(VI))100/total Cr. [b] Overall recovery of Cr at day x: Σ = ratio Cr(III) + ratio Cr(VI).

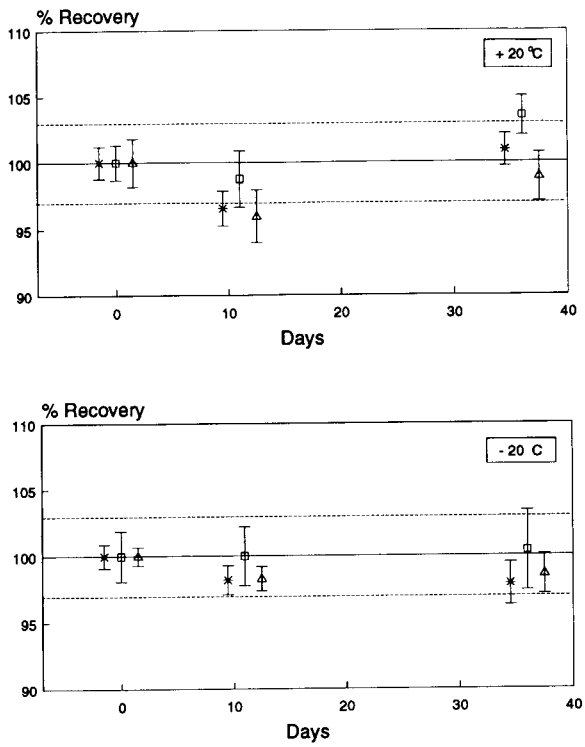

Fig. 3. Sample A. Recovery (±C.V.%) of (*) total ^{51}Cr, (□) ^{51}Cr(III) and (△) ^{51}Cr(VI) in lyophilized samples stored in glass vials at 20°C and −20°C.

Once the concept of lyophilizing smaller volumes of Cr(III) and Cr(VI) concentrate was tested, the glass vial concept was used to produce batches in which 2 ml of radiolabelled ^{51}Cr(III)/^{51}Cr(VI) solution A or B were lyophilized. The samples were reconstituted in 4 ml of buffer solution. The stability of the lyophilized material of solutions A and B, in glass vials stored at 20°C and/or −20°C, was checked over a period of 34 days. The results are shown in Fig. 3 (Sample A) and Fig. 4 (Sample B).

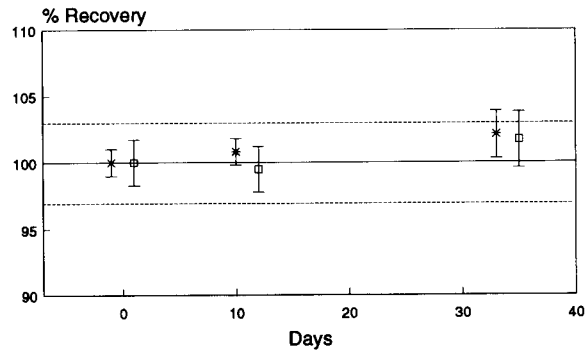

Fig. 4. Sample B. Recovery (±C.V.%) of total ^{51}Cr and ^{51}Cr(VI) in lyophilized samples stored in glass vials at 20°C. Key: * = Cr(VI); □ = total Cr.

Supplementary stability check of sample A over 160 days. Besides using the ^{51}Cr label counting technique, the stability of similar lyophilized samples of solution A has been validated using the ETAAS measurement technique. Two ml of solution A were lyophilized and stored at −20°C. The specimens were reconstituted in 10 ml of hydrogencarbonate buffer and have been analyzed after 0, 30, 64, 127 and 160 days. The results are shown in Table 3.

The results listed in Table 3 confirmed that the lyophilized water samples of A, contained unaltered Cr(III)/Cr(VI) species up to 160 days storage at −20°C.

The outcome of these experiments establishes the stability of the aqueous and lyophilized specimens of solution/sample A and B stored in

TABLE 3

ETAAS analysis of lyophilized Cr(III)/Cr(VI) specimens of solution A [Mean (μg/l) and 95% CI]

	Made-up conc.	Day 0	Day 30	Day 64	Day 127	Day 160
Total Cr	50.0	49.6 ± 3.0	46.3 ± 2.2	48.4 ± 6.2	49.2 ± 1.9	50.6 ± 1.9
Cr(VI)	10.0	9.2 ± 0.4	9.4 ± 0.9	10.0 ± 0.5	9.7 ± 0.3	10.6 ± 0.6
Cr(III)	40.0	40.8 ± 3.4	40.5 ± 5.4	40.4 ± 6.2	39.7 ± 1.8	39.5 ± 1.7
Σ [a]	50.0	50.0 ± 3.4	49.9 ± 5.5	50.4 ± 6.2	49.4 ± 1.8	50.1 ± 1.8
n		3	3	1 [b]	1 [b]	1 [b]

[a] Σ = Cr(III) + Cr(VI). [b] Calculated individual error estimate (95 % CI) on calibration curve.

quartz ampoules and glass vials respectively. Based on these results, the described concepts for aqueous solutions stored in quartz ampoules and lyophilized samples in glass vials were used to produce batches of solution/sample A and B for pilot interlaboratory comparison studies for chromium speciation including 7 European laboratories.

The interlaboratory comparisons

The results of the intercomparison of the aqueous RMs A and B are given in Figs. 5 and 6 and the mean of the accepted data in Tables 4 and 5. Those for the lyophilized materials are given respectively in Figs. 7 and 8 and the mean of the accepted data in Tables 6 and 7.

The sets of results found acceptable on technical grounds have been further submitted to the following statistical tests:

Kolmogorov-Smirnov test to assess the laboratory means to normal distributions;

Cochran test to detect "outlying" values in the laboratory variances;

Dixon test to detect "outlying" values in the population of laboratory means;

One-way analysis of variance (F-test) to compare and estimate the between- and the within-laboratory components of the overall variance of all individual results;

In all cases, the estimates of the within-laboratory standard deviation and the between-laboratory standard deviation as derived from one-way analysis of variance, demonstrated that the between-laboratory variation was the major source of variability between results.

The Kolmogorov-Smirnov test proved normal distribution in all cases.

Due to the small number of measurements Dixon's and Cochran's test for elimination of "outlying" measurements were only used for the aqueous solutions.

The aqueous samples

Cautious rejection of some data has been used though the strong relationship between the measurements of the chromium species (Cr(III) + Cr(VI) = Total Cr) implies that they cannot be handled as individual results.

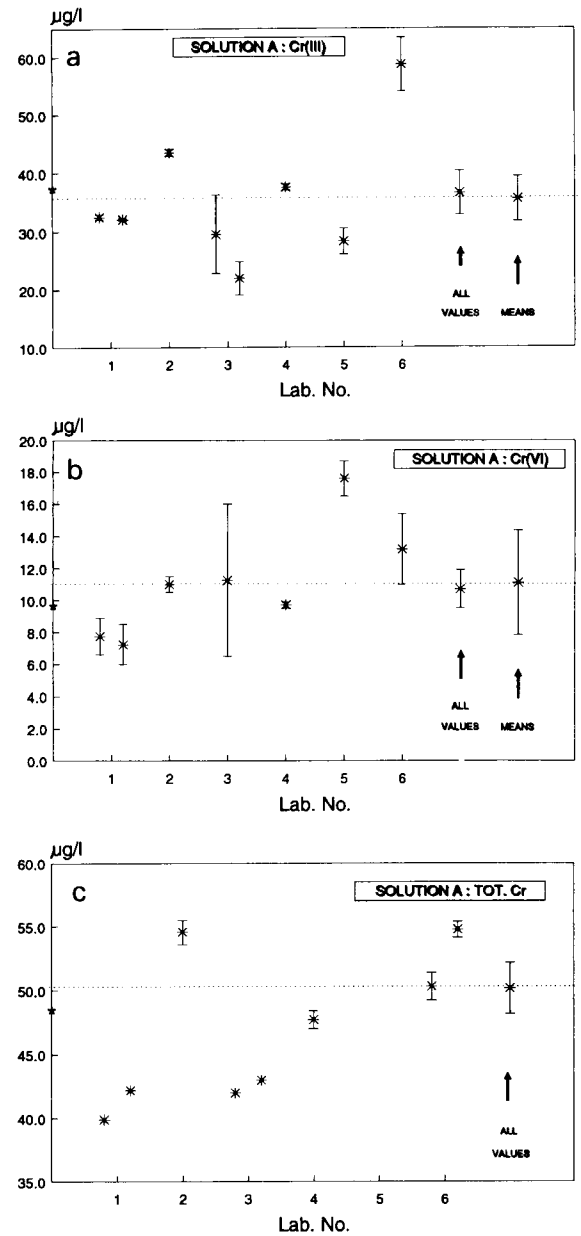

Fig. 5. Graphical representation of values (and 95% CI) submitted by the participants for aqueous solution A. Initial content is marked as * on the y-axis.

The outliers from the Dixon- and Cochran-tests were rejected, in most cases, with a 1% risk of error. For an outlying straggler the risk lies between 1% and 5%. Considerations that the stand-

TABLE 4

Results from analysis of solution A. [The results are given as mean (μg/l) and 95% confidence interval]

Solution A	Comments	n	Result [Mean (μg/l) and 95% CI]	Exact content (μg/l)
Cr(III)	Cochran test rejects lab. No. 2, 5 and 6	4	36.4 ± 8.5	37.9
Cr(VI)	Cochran test rejects lab. No. 3 and 6	4	8.9 ± 2.8	9.3
Total Cr	No statistical treatment has been applied due to few submitted replicate results.	4	50.2 ± 2.1	47.2

TABLE 5

Results from analysis of solution B. [The results are given as mean (mg/l) and 95% confidence interval]

Solution B	Comments	n	Result [Mean (mg/l) and 95% CI]	Exact content (mg/l)
Cr(III)	Measured below LOD by all participants			0
Cr(VI)	Dixon test rejects lab. No. 2	9	4.59 ± 0.19	4.63
Total Cr	Dixon test rejects lab. No. 2	5	4.73 ± 0.20	4.63

TABLE 6

Results for sample A. [The laboratory means and the mean of all values submitted are depicted. Mean (μg/l) and 95% confidence interval]

Sample A	n	Mean of means [Mean (μg/l) and 95% CI]	n	Mean of all values [Mean (μg/l) and 95% CI]	Initial content (μg/l)
Cr(III)	5	24.2 ± 2.8	32	23.9 ± 0.9	24.0
Cr(VI)	5	16.1 ± 3.1	32	16.3 ± 0.9	16.0
Total Cr	5	39.5 ± 1.2	33	39.9 ± 0.4	40.0

TABLE 7

Results for sample B. [The laboratory means and the mean of all values submitted are depicted. Mean (mg/l) and 95% confidence interval]

Sample B	n	Mean of means [Mean (mg/l) and 95% CI]	n	Mean of all values [Mean (mg/l) and 95% CI]	Initial content (mg/l)
Cr(VI)	6	7.98 ± 0.39	24	7.95 ± 0.22	8.00
Total Cr	5	8.02 ± 0.30	33	8.03 ± 0.08	8.00

TABLE 8

Dataset of Cr(III), Cr(VI) and total Cr determination in solution A after 0, 50 and 228 days period of storage at 5°C. [Mean (μg/l) and 95% confidence interval. (Recovery = (Cr(III) + Cr(VI)/total Cr)100)]

(μg/l)	n	Day 0	n	Day 50	n	Day 228
Total Cr	5	47.7 ± 0.7	7	46.5 ± 1.0	3	46.2 ± 0.6
Cr(VI)	5	9.7 ± 0.2	5	9.5 ± 0.4	3	9.2 ± 0.3
Cr(III)	5	37.5 ± 0.5	5	39.0 ± 0.7	3	41.2 ± 2.8
Recovery(%)		99.0 ± 0.9		104.3 ± 1.3		109.1 ± 1.1

ard error of the mean of the set exceeded the standard deviation of all the laboratory means have also been taken into account before rejections.

Solution A. The results considered for the Cr(III) and Cr(VI) determinations are in statistical agreement (95% CI) with the initial made up content. For total Cr there is a significant difference which most conceivably can be explained by the few submitted replicate results from the par-

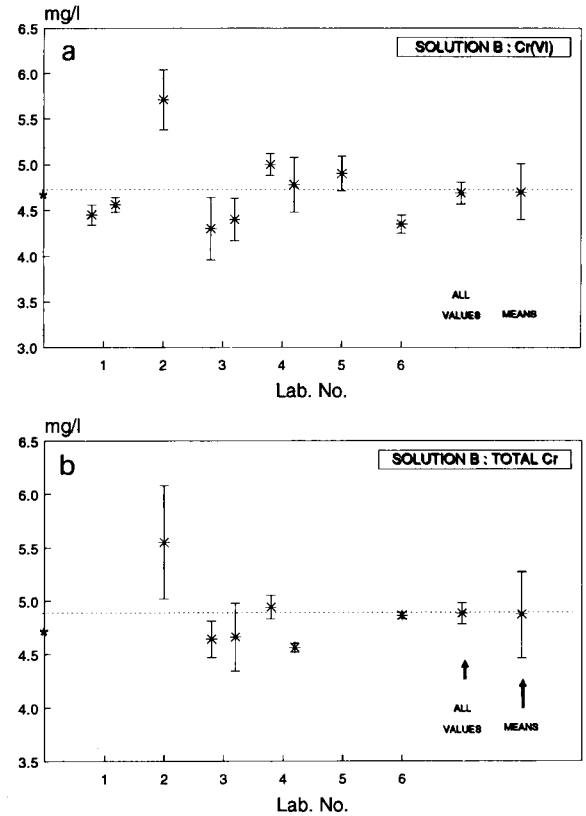

Fig. 6. Graphical representation of values (and 95% CI) submitted by the participants for aqueous solution B. Initial content is marked as * on the *y*-axis.

ticipants and accordingly the lack of being able to apply statistical treatment. Additionally the stability of the chromium species in the quartz ampoules was investigated after 50 and 228 days storage at 5°C (Table 8). The ampoules have been in the transportation system for 24 h without any coolant added.

Solution B. The results considered for Cr(VI) and total Cr are in good statistical agreement (95% CI) with the initial made up content. All participants measured Cr(III) to be below limit of detection.

The lyophilized samples

The Kolmogorov-Smirnov test proved normal distributions in all cases after exclusion of results reported by laboratory No. 1 for solution A.

In all cases, the estimates of the within-laboratory standard deviation and the between-laboratory standard deviation, demonstrate that the be-

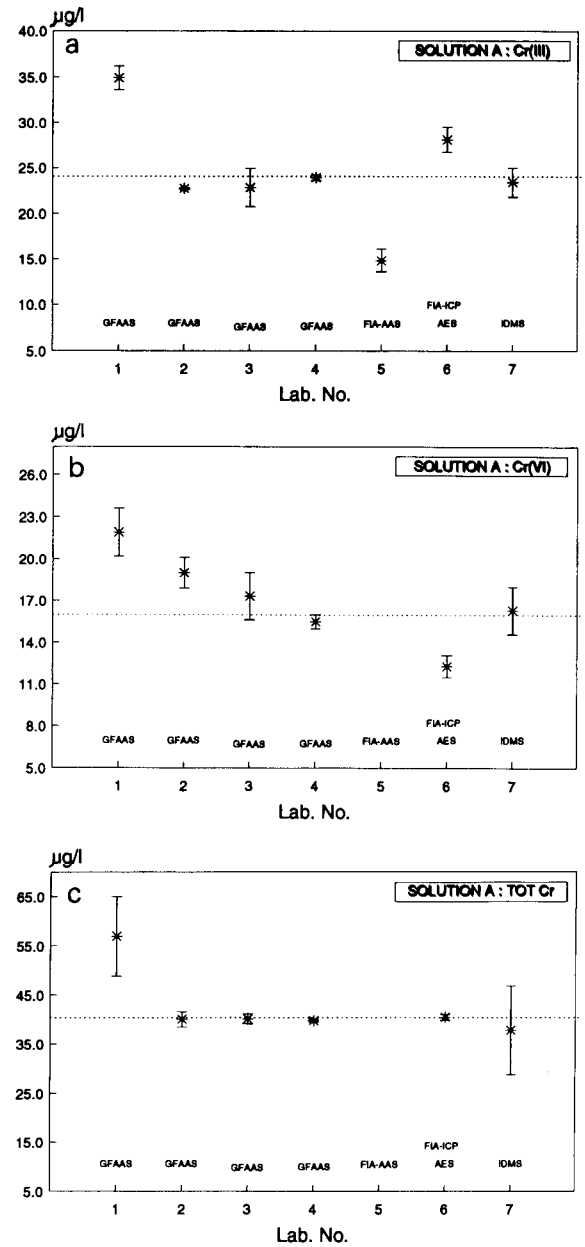

Fig. 7. Graphical representation of values (and 95% CI) submitted by the participants for lyophilized sample A. Dotted line represents the initial content.

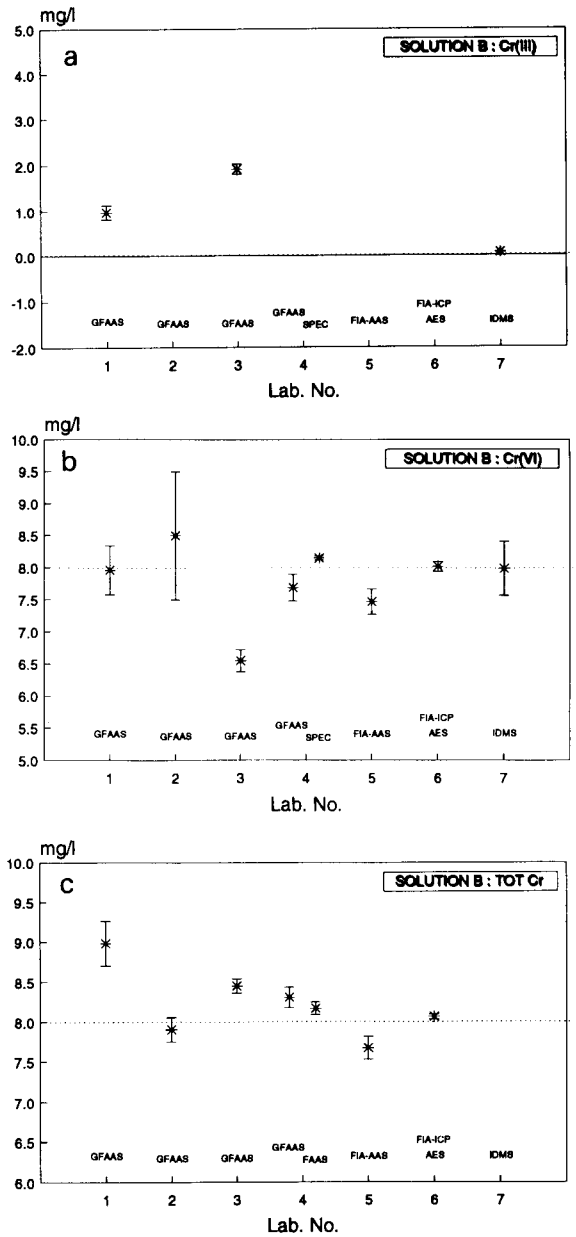

Fig. 8. Graphical representation of values (and 95% CI) submitted by the participants for lyophilized sample B. Dotted line represents the initial content.

tween laboratory variation was the major source of variability.

Sample A. As laboratory No. 5 reported problems to reconstitute all freezedried specks, it seemed justified to omit their Cr(III) result. No values for Cr(VI) nor for total Cr were submitted.

For laboratory No. 1 the results for total Cr exceed the initial content by 42%, these for Cr(VI) by 106% and for Cr(III) by 45%. Therefore their data were considered subject to systematic errors and withdrawn.

Due to the few measurements, elaborate statistical treatment was not feasible. The results reported by laboratories no. 2, 3, 4, 6 and 7 were used to calculate the mean of means and the mean of all values submitted. The results are shown in Table 6.

Sample B. Laboratories No. 1 and 3 reported to have detected a significant amount of Cr(III). Initially only Cr(VI) was added to the solutions, and this was the only species detected by the other participants. It was therefore very doubtful that any reduction of Cr(VI) should have occurred, which also the stability studies confirmed. The results from these two laboratories were consequently withdrawn.

Due to the few measurements, elaborate statistical treatment was not feasible. The results reported by laboratories no. 2, 4, 5, 6 and 7 were used to calculate the mean of means and the mean of all values submitted. The results are shown in Table 7.

For both samples the considered results are in good statistical agreement with the initial made-up content.

Conclusions

The data obtained for Cr(III)/Cr(VI) in the reference samples during the stability studies and the interlaboratory comparison demonstrate that the concept to produce batches of lyophilized samples of A and B appears reliable. The results meet the criteria of homogeneity, stability and accuracy, as requested for a reference material. It is therefore justified to organize a larger interlaboratory comparison to gauge the state of the art of the Cr(III) and Cr(VI) analytical methodology within Europe. These newly generated data will be submitted to a statistical evaluation, and subjected to scientific discussions with all the participants. When the outcome confirms the validity of the lyophilized reference material for Cr(III) and

Cr(VI) speciation, a definitive product can be made and certified by a selected group of laboratories. The availability of such a certified reference material will fulfil the urgent need for quality control of the amounts of Cr(III) and Cr(VI) routinely measured in water, and the amounts of Cr(VI) leached out of the filters used for personal monitoring to hexavalent chromium in the atmosphere.

S. Dyg received a BCR sectoral grant from the Commission of the European Communities (CEC). R. Cornelis is a research director of the National Fund for Scientific Research (NFWO), Belgium. Technical assistance of L. Mees and valuable suggestions from J.M. Christensen are gratefully acknowledged. The following laboratories are gratefully acknowledged for their contribution to these studies: Chemical Analysis Research Centre, Sheffield (UK); FB Chemie, Phillips-Universität Marburg (Germany); Institute of Occupational Health, Oslo (N); National Institute of Occupational Health, Copenhagen (Denmark); Laboratory of Analytical Chemistry, Universiteit Ghent (Belgium) Faculty of Sciences; Perkin Elmer, Überlingen (Germany) Department of Applied Research; Inorganic Institute, Universität Regensburg (Germany).

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Analytica Chimica Acta, 286 (1994) 309–318
Elsevier Science B.V., Amsterdam

Speciation analysis of organotin in water and sediments by gas chromatography with optical spectrometric detection after extraction separation

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(Received 17th February 1993)

Abstract

The state of the art of gas chromatography with atomic emission and atomic absorption spectrometric detection as a technique for speciation analysis of organotin in water samples and sediments is discussed. Extraction separation of ionic organotin compounds and their conversion to gas chromatographable species are reviewed. Recent advances in instrumentation with respect to sensitivity, selectivity and applicability to real sample analysis are highlighted. Particular emphasis is given to sources of error and accuracy of the data obtained.

Keywords: Gas chromatography; Environmental analysis; Hyphenated techniques; Organotins; Speciation

During the past several years organotin compounds have been extensively used as biocides, especially in antifouling paints [1]. The most widely used compounds tributyltin (TBT) and triphenyltin (TPhT) have been recognized as toxic also to non-target marine biota which was followed by environmental legislation restricting the use of these compounds [1–3]. Once released into the environment TBT and TPhT can undergo a variety of physico-chemical processes such as biodegradation, biomethylation, sorption on particulate matter or vaporisation [1,2].

The toxicity of organotin compounds is species dependent so a viable analytical method should allow the determination of the target compounds without an interference from other organotin species. It should also provide sufficient sensitivity (< 0.1 ng l^{-1} for water and < 1 ng g^{-1} for dry solid materials). The present techniques available combine a separation technique such as gas chromatography (GC), liquid chromatography (LC) or supercritical fluid chromatography (SFC) with an atomic absorption spectrometric (AAS), atomic emission spectrometric (AES), flame photometric detection (FPD) or mass spectrometric (MS) detection and are usually termed as coupled or hyphenated techniques [4].

The use of LC for the separation of organotins has recently been reviewed [5] and apparently the LC based techniques suffer from poor resolution and usually the lack of sensitivity. GC enjoys a wider popularity despite more complex sample preparation procedures often required because of insufficient volatility of the ionic organotin compounds.

In recent years a plethora of sample preparation methods and instrumental analytical setups have been developed for the speciation analysis of organotins in environmental matrices. These methodologies fall in two basic categories: these

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SSDI 0003-2670(93)E0400-2

based on in situ derivatization by sodium borohydride ($NaBH_4$) followed by purging and cryotrapping of the derivatized analytes [6] and those based on the extraction of the ionic organotin compounds, either native or after complexing them or in situ derivatizing. The purpose of this paper is to critically review the state of the art of extraction based sample preparation procedures and to highlight the recent instrumental developments in speciation analysis for organotin in various matrices.

Speciation analysis for organotin. General

Generally, every procedure for speciation analysis consists of five successive steps: (1) separation of the analytes from the sample matrix, (2) formation of volatile derivatives, (3) preconcentration, (4) cleanup and (5) determination. There is a strong trend to reduce the number of steps by combining some of them. The major consideration has been shifting away from the determination itself toward the quantitative separation of the analytes from complex samples. A schematic layout of sample preparation for organotin speciation analysis is shown in Fig. 1. Specific considerations with respect to water and sediment samples are discussed below.

Extraction of organotin derivatives

Water. Earlier methods based on acidification with hydrochloric (HCl), hydrobromic (HBr) and acetic acid (HAc) of the sample to release alkyltin compounds from the suspended particulate matter and to convert them into the respective halogenides which were then extracted into a variety of organic solvents usually of relatively high po-

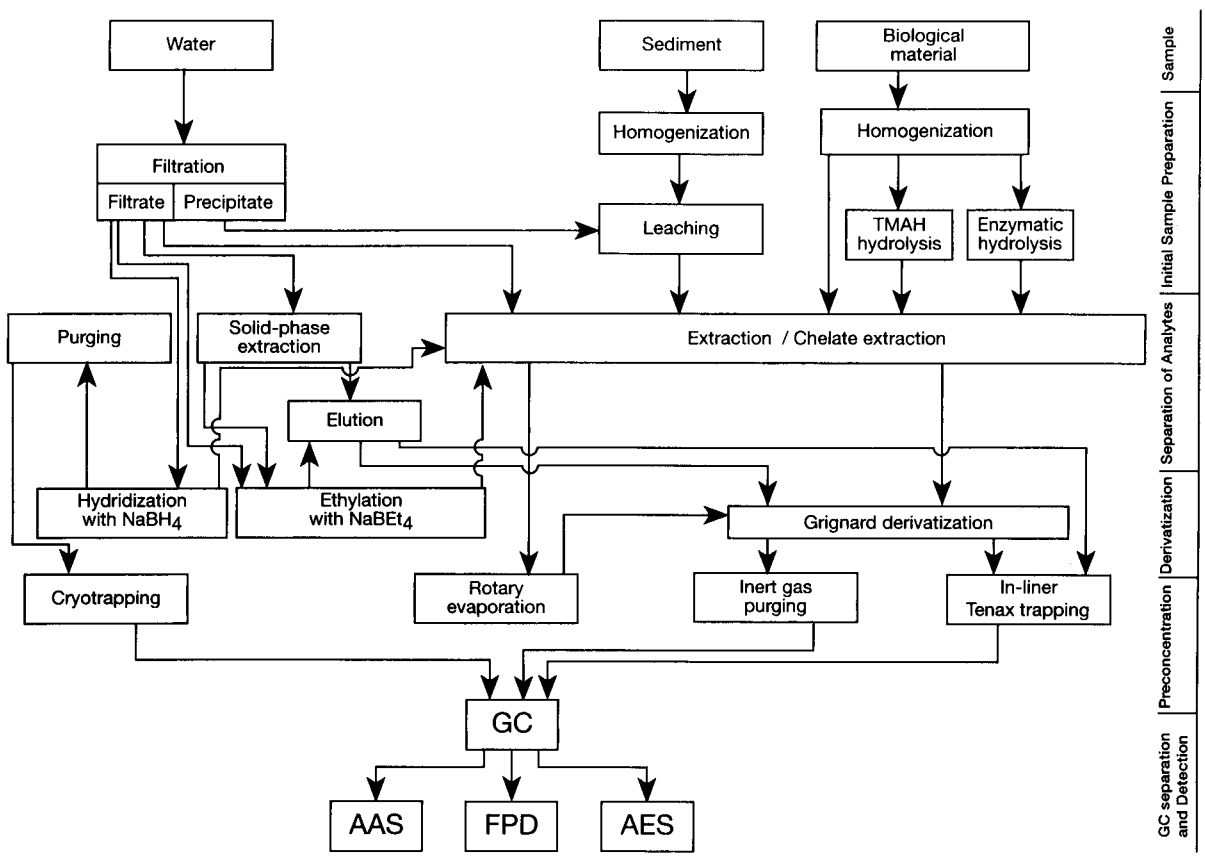

Fig. 1. Schematic layout of sample preparation procedures for organotin speciation analysis.

larity. This approach usually succeeds for trisubstituted organotins TBT, TPhT and tricyclohexyltin (TCyT) but fails for other species due to their higher polarity. In addition the more polar solvents are poorly compatible with the Grignard reagents used later for derivatization and favour co-extraction of organic interferents. Therefore the currently recommended procedures base on the extraction of low polar organotin complexes with tropolone or diethyldithiocarbamate (DDTC) using a non-polar solvent. Tropolone is preferred to DDTC as under acidic conditions (pH < 4) the latter undergoes decomposition, giving rise to extractable interferents [7].

An elegant approach which is rapidly gaining popularity is extraction of the analytes derivatized earlier in situ [8–12]. Sodium tetraethylborate ($NaBEt_4$) [10,11] is preferred to $NaBH_4$ [8,9] as a derivatization reagent. Hydride generation is more prone to interferences and in case of monosubstituted organotins it leads to very volatile derivatives which can hardly be further preconcentrated by evaporation of the extraction solvent. In addition, organostannanes are relatively reactive and tend to decompose when subject to cleanup or harsh instrumental conditions.

Liquid–liquid extraction methods often require large amounts of hazardous solvents and

TABLE 1

Selected methods for organotin speciation analysis in water [a]

Extraction reagents (solvent)	Derivatization agent	Experimental detection limit (ng Sn l^{-1})	Species (recovery, %)	Ref.
$NaBH_4$ (dichloromethane)	$NaBH_4$	FPD (1)	TBT, DBT, MBT (> 95)	9
Acetic acid–acetate, pH 5, $NaBEt_4$ (hexane)	$NaBEt_4$	MIP-AED (0.1)	TBT, DBT, MBT (85–97) TPhT, DPhT, MPhT (85–97)	11
Tris–acetic acid, pH 6, $NaBEt_4$ (isooctane)	$NaBEt_4$	FPD (0.4)	TBT, DBT, MBT (> 95)	10
Tropolone (dichloromethane)	PeMgBr	FPD (2)	TBT, DBT, MBT TPhT, DPhT, MPhT	25
HCl, tropolone (hexane)	MeMgCl	FPD (0.5–6.5)	TBT (61–93), DBT, MBT TPhT (56–89), DPhT, MPhT	18
HCl, ascorbic acid, Sep-Pak C_{18} cartridge, tropolone (diethyl ether)	EtMgCl	FPD (0.01) FPD (3.5–8) FPD (17–51)	TBT, DBT, MBT (60–95) TPhT, DPhT, MPhT (60–95) TCyT, DCyT, MCyT (60–95)	13–15
HBr, tropolone (pentane)	PeMgBr	FPD (20–50)	TBT, DBT, MBT (> 90) TPhT, DPhT, MPhT (> 90)	19
HCl, NaCl, tropolone (hexane), 1 h	EtMgCl	QFAAS (5)	TBT, DBT, MBT (84–95 ± 6) TMT, DMT, MMT	20
HCl, pH 1, NaCl, tropolone (toluene), 4 h	EtMgCl	QFAAS (40)	TBT (90–104), DBT (99–109), MBT (101–109)	21
HCl–THF (1 + 3), NaCl, tropolone (benzene)	PrMgCl	FPD (3)	TBT, DBT, TPhT, DPhT (92–100) MBT, MPhT (70)	22
Citric acid–phosphate, pH 5, NaDDTC (pentane)	PeMgBr	QFAAS (0.36–0.78) MIP-AED (0.008–0.013)	TBT, DBT, MBT (101–105) TMT, DMT, MMT (101–106)	23, 24
C_{18}, citrate–ammonia, pH 9, $NaBEt_4$ (methanol)	on-column $NaBEt_4$	MIP-AED (0.1–0.2)	TBT, DBT, MBT (80–100) TPhT, DPhT, MPhT (80–100)	17
Carbopack or LC_{18}, H_2O, tropolone (methanol)	PeMgBr	FPD (2) GFAAS (10)	TBT, DBT, MBT (98–100) Carbopack TBT, DBT, MBT (94–102) LC_{18}	16

[a] TBT, DBT, MBT: tri-, di- and monobutyltin; TPhT, DPhT, MPhT: tri-, di- and monophenyltin; TCyT, DCyT, MCyT: tri-, di- and monocyclohexyltin.

tend to be replaced by the solid phase extraction (SPE) procedures [13–17]. The advantages of the SPE include a higher preconcentration factor and ease of application in the field and in on line systems while a drawback is that only filtered samples can be analyzed.

Table 1 summarizes the methods enabling the quantitative recovery of organotin compounds in comprehensive speciation analysis of water.

Sediments. As organotin compounds are not involved in mineralogical processes and bind onto the surface of the sediment the complete dissolution of the latter prior to the analysis is not considered necessary. The basic approach to release organotin compounds from the sediment involves acid leaching (HCl, HBr, HAc) in an aqueous or methanolic medium by sonification, stirring, shaking or Soxhlet extraction with an organic solvent. To increase the extraction yield the addition of a complexing agent (tropolone, DDTC) is mandatory.

Table 2 gives a survey of analytical procedures for speciation analysis of organotins in sediments claimed to give the highest recovery. Apparently no reliable and efficient method for extracting all organotin species simultaneously from sediments has hitherto been developed. While the tri- and di-substituted compounds can be extracted quantitatively, only about 60% or less of the mono-substituted compounds are recovered. The reason for this is the incomplete extraction and/or derivatization because of a relatively high polarity

TABLE 2

Selected methods for organotin speciation analysis in sediments

Extraction reagents (solvent)	Derivatization agent	Experimental detection limit (ng Sn g^{-1})	Species (recovery, %)	Ref.
HCl–MeOH (cyclohexane) reflux 80°C	$NaBH_4$	FPD (50)	TBT (79 ± 26) DBT	8
0.1% NaOH–MeOH (hexane)	$NaBH_4$	FPD (10)	TBT, DBT, MBT	9
HCl, tropolone (dichloromethane)	$NaBEt_4$	QFAAS	TBT, DBT, MBT (94) TPhT, DPhT, MPhT TCyT, DCyT, MCyT	12
MeOH–HCl, tropolone, sonification (toluene–isobutyl acetate)	–	FPD (30)	TBT (94 ± 5), DBT (95 ± 2), MBT (86 ± 4)	26
HCl, tropolone (diethyl ether)	EtMgCl	FPD (0.01) FPD (0.02–0.7)	TBT, DBT, MBT (60–95) TPhT, DPhT, MPhT (60–95) TCyT	13–15
	MeMgCl	FPD (0.1–2)	TBT (61–93), DBT, MBT TPhT (56–89), DPhT, MPhT	18
HCl, HBr, tropolone (pentane)	PeMgBr	FPD (3)	TBT, DBT, MBT	27
Tropolone (dichloromethane)	PeMgBr HeMgBr	FPD (5) FPD (5)	TBT (94 ± 7) DBT (97 ± 16) MBT (40 ± 23)	28, 29
H_2O, NaCl, KI, sodium benzoate, tropolone (hexane)	EtMgCl	QFAAS (5)	TBT, DBT, MBT (84–95 ± 6) TMT, DMT, MMT	20
HCl, NaCl, tropolone (toluene), 4 h,	EtMgCl	QFAAS (2)	TBT (90–114), DBT (89–95), MBT (96–103)	21
HCl–THF (1 + 11), tropolone (benzene)	PrMgCl	FPD (0.5)	TBT, DBT, TPhT, DPhT (92–100), MBT, MPhT (70)	22
H_2O, acetic acid, NaDDTC (hexane)	PeMgBr	QFAAS (0.45) MIP-AED (0.005–0.075)	TBT (95–105) DBT (95–107) MBT (30–45)	23, 24

of MBT and its adsorption affinity to the components present in the sediment. The recovery is usually higher for butyltins than for methyltin compounds.

Formation of volatile alkyltin derivatives

The methods for the conversion of ionic alkyltins into gas chromatographable species include: (1) in situ hydridization using $NaBH_4$ or ethylation with $NaBEt_4$ [6,8–12], (2) derivatization using Grignard reagents: methyl- [18,30], ethyl- [13–15,20,21,31,32], propyl- [22,33], pentyl- [7,16,19,23–25,27–29] or hexylmagnesium chlorides/bromides [34,35], and (3) halogenation [26,36,37]. The methods of the first group mostly base on the formation of volatile organotin species in the aqueous phase and have become integrated with extraction separation. Some methods imply the formation of the volatile species in the extract [8,12] or on the solid phase support [17,38].

The Grignard alkylation reaction proceeds quantitatively leading to stable derivatives when it is carried out in a suitable solvent. Ethylation or pentylation are the usual choice as they allow a simultaneous speciation analysis of methyl-, propyl-, butyl- and phenyltin species. Pentylation leads to less volatile analytes than ethylation, which, on one hand, facilitates further preconcentration but, on the other hand, can account for condensation problems in the interface during GC-AAS analysis.

Gas chromatography of mono-, di- and tributyltin chlorides is less suitable for routine applications because of the rather extreme conditions required and the difficulty in the determination of MBT despite the addition of HCl to the carrier gas [26].

Preconcentration

Evaporation is the most popular method of preconcentration. Purging of the extract with a gentle stream of inert gas is preferred to Kuderna-Danish or rotary evaporation which can subject the sample to unnecessary heating or vacuum conditions [39]. Using a few microliters of a high boiling compound as a "keeper" cannot always be recommended as problems with the separation of the solvent peak from those of more volatile analytes may occur.

An elegant technique for on-line preconcentration of ethylated organotin species has recently been proposed [11,17]. The principle of preconcentration is based on the differences in volatility of the solvent and the analytes. Three consecutive processes taking place in the injection liner are involved: sample injection, solvent venting and transfer of the analytes onto the column. A solution of determinants (25 μl) in a volatile solvent (e.g., hexane) is injected onto a cool (0–10°C) Tenax packed liner. Then the temperature is slightly raised to increase the solvent vapour pressure and maintained for 1 min while a stream of the helium gas passes through the liner sweeping the solvent off the column. The less volatile analytes are kept in the liner until the volume of the solvent is reduced. Then the purge valve switches the carrier helium gas stream into the column while the temperature of the liner is raised to the effective injection temperature prompting the release of the analytes.

Evaporation of the derivatized extract leads to simultaneous preconcentration of interferents. When a sensitive detector is used, even the extraction solvent alone subjected to derivatization with Grignard reagent may show after preconcentration high background and the presence of some analyte peaks (Fig. 2). Differences in the purity of the Grignard reagents from different manufactur-

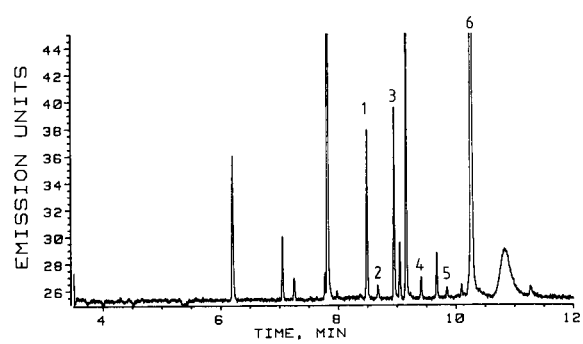

Fig. 2. Chromatogram of hexane derivatized with PeMgBr (500 μl of hexane, 100 μl of Grignard reagent, 20 μl injection, instrumental conditions given elsewhere). 1 = $SnBu_4$; 2 = $MeSnPe_3$; 3 = Bu_3SnPe; 4 = Bu_2SnPe_2; 5 = $BuSnPe_3$; 6 = $SnPe_4$.

ers apparently exists [40] but the occurrence of contamination problems is rather a question of instrumental sensitivity and the preconcentration factor employed than that of different reagents used. A further contribution to the blank value is likely to originate in glassware, complexing reagents and buffers used which, however, in contrast to the Grignard reagent can be purified prior to use.

Cleanup

Extracts of samples, rich in organic matter, contain coextractives (fats, high boiling hydrocarbons) which may deposit on the column influencing the separation, or increasing the detector background, thus negatively affecting the detection limits. The advent of more selective detectors has reduced the need for cleanup procedures but nevertheless in some cases they cannot be avoided. The cleanup procedures mostly base on ion exchange or reversed-phase chromatography (e.g., using Alumina, Silicagel or Sep-Pak C_{18} columns). An effective method of reducing the need for loss prone cleanup processes is to decompose the organic matrix by enzymatic hydrolysis or to separate it from the analytes by back extraction [39].

GC separation of organotin compounds

Non-polar phases have been recommended for the separation of derivatized organotin species [4]. Packed columns are being gradually replaced

TABLE 3

Comparison of detection limits reported for hyphenated techniques used in the speciation analysis of organotins

Detector	Instrumental setup	Detection limit		Ref.
		Definition	pg Sn	
FPD	Column: megabore column [25], capillary column [10,13,18]	3σ [a]	1.2 [b]	18
		2σ	1.1 [b]	13
		2σ	30	26
			0.016 [b]	41
			0.2	10
QFAAS	Column: packed column [30,43,44], megabore column [42,24], capillary column [45]	N_{p-p} [c] + 3σ	7.3–17.2	42
			4.8–13.8	30
	Transfer line: metal tube [12,43,44], fused-silica tube [24,30,42,45]	3σ	17.5–37.2	23, 24
		3σ	160 –400	43
	Interface: modified reducing union with support gas inlets [30,42], lab. made PTFE union [43]	a 3 mm high peak	80 –370	44
			100	45
			410 –640	12
CT-QFAAS [d]	Column: U-shaped trap, 3% SP-2100 on Chromosorb	3σ	30	6
		3σ	11 –45	6
	Transfer line: PTFE tubes			
	Interface: PTFE connectors			
GFAAS	Column: megabore column [7]	3σ	33 –71	7
	Transfer line: deactivated fused-silica tubing in heated metal tube			
	Interface: tantalum connector			
MIP-AED	Column: fused-silica capillary column [11,17,23,24,32,46,47]	3σ	0.05	23
		3σ	0.10–0.15	24
	Transfer line: column in heated metal tube [11,17,23,24,32,47]	3σ	0.15–0.2	11, 17
		2σ	6.1	46
	Interface: column to discharge tube in cavity		6	32
			0.35 [b]	47

[a] σ standard deviation. [b] Adjusted to be given as Sn. [c] N_{p-p}: mean peak-to-peak baseline noise. [d] CT-QFAAS: cryogenic trapping quartz furnace atomic absorption spectrometry.

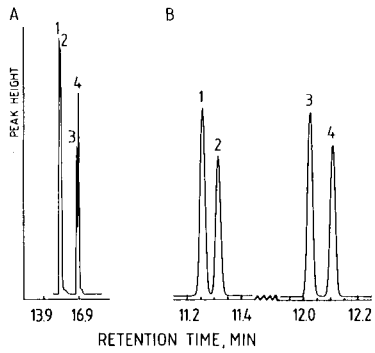

Fig. 3. Examples of separation of some organotin compounds on: (A) Megabore, (B) capillary column. 1 = Ph_2SnPe_2; 2 = Cy_2SnPe_2; 3 = Ph_3SnPe; 4 = Cy_3SnPe.

by open-tubular megabore or capillary columns. The latter are favoured due to better resolution, inertness and ease of use. An example comparing the separation of some pentylated phenyl- and cyclohexyltin compounds on different columns is illustrated in Fig. 3. The better characteristics of a capillary column are partly levelled by the smaller sample volume which can be injected without signal distortion.

Detection techniques

Non-specific GC detectors are almost useless in speciation analysis for organotins due to the high background from real samples extracts. The necessary selectivity is provided by atomic absorption and atomic emission spectrometric detectors. Flame photometric detection (FPD), quartz furnace (QF)-AAS and microwave induced plasma atomic emission detection (MIP-AED) are the most popular of them. The absolute detection limits obtained are dependent on the GC conditions and the interface characteristics and are compared in Table 3.

Flame photometric detection. FPD is the most popular detector for organotin speciation analysis due to its wide availability, low cost and competitive performance. Tin gives strong emission in a hydrogen rich flame in the 360–490 nm (blue) and in the 600–640 nm (red) regions. Measurements in the latter region are preferred. With appropriate interference filters (e.g., 610 nm) used good selectivity against hydrocarbons and good sensitivity (analytical detection limits to a few pg) can be reached. Excessive peak tailing and fouling of the detector with SnO_2 were evoked [41] but in a number of later works these side effects were apparently absent [10,13]. The use of a capillary column seems to be a prerequisite to avoid the above problems.

The sensitivity of flame photometric detection can be considerably (up to 100 times) increased by employing quartz-surface induced luminescence (QSIL) at 390 nm. The initial problems with the stability [41] of the emission intensity were apparently circumvented in a later work [48]. The detection limits with QSIL of 0.02–0.3 pg (as Sn) were reported. The specificity of GC-FPD can be enhanced by splitting the half of the GC effluent to a direct current plasma (DCP) emission spectrometer, forming a GC-FPD/DCP system [49].

Atomic absorption spectrometry. QF-AAS is a versatile detection technique used in speciation analysis. Its application to organotin analysis has been mostly restricted to the detection of stannanes [4,6] as for higher boiling species the interface and detection conditions are much more critical [7,24]. Early setups showed cold spots which caused peak broadening, tailing and uncontrolled losses. The introduction of a highly flexible quartz capillary as a transfer line that could be led directly into the detector cell circumvented these problems to a large degree and made the coupling much easier [7,30,42]. Nevertheless, the detection limits are by 1–2 orders of magnitude worse than for GC-AED and GC-FPD. An attempt was recently made to use the graphite furnace AAS as a detection technique [7]. The sensitivity obtained, however, was twice lower than that of QF-AAS and additionally signal quenching problems occurred during real sample analysis. Furthermore signal discrimination with the boiling point of the analyte has been hitherto an unsurmountable problem in GC–AAS interfaces.

MIP-AES. Atomic absorption spectrometry is a relatively unsensitive technique for the determination of tin due to the high atomization temperature needed and the possibility of the formation of refractory oxides. Plasmas providing higher

atomization temperatures are more advantageous. Different plasmas have been investigated for the atomization of organotins in the GC effluent but the widest popularity was gained by a microwave induced plasma (MIP) [50]. The recently marketed instrument compares favourably with its predecessors in terms of selectivity (owing to a better background correction) which has considerably improved the sensitivity by a factor of about ten. The instrumental detection limits reported so far vary considerably despite apparently identical systems used and were 0.05 pg [23], ca. 0.35 pg [47] and 6 pg [32] (as Sn). While in the latter work sub-optimal operating parameters were used, in the former two studies the same detector settings were employed and the same standards injected. The discrepancy in the values reported may result from some differences between the instruments with respect to individual components (injection system, cavity cooling system, purity of the gases) and also from the different method of calculation. For the same instrument a down-drift in sensitivity (from 0.05 to 0.20 pg as Sn) was observed during two years of operation [11,17,23,24].

Sources of error

Possible errors arising during sampling and storage were recently reviewed [51,52]. The most important problems in speciation analysis for organotin compounds are apparently the adsorption onto and the release of some organotin species from certain sample-container materials (e.g., polyvinyl chloride) and the poor recoveries of the analytes. The use of polyethylene [53] was discouraged while polycarbonate and glass were found to be suitable materials [54]. Sediment samples are to a lesser degree subject to changes in concentration during storage than water samples.

While spikes on pure water are usually recovered quantitatively, an increase in particulate load can make the recoveries lower and irreproducible which, in particular, are observed during sediment analysis. Individual organotin compounds may bind to components present in a sediment to a degree varying with the salinity and the amount of particulate matter present in the water column [55] which may make the method valid for one sediment ineffective for another [31]. Therefore the determination of an extraction efficiency by spiking and extracting the spiked sediment cannot be considered reliable as one is never sure whether the species added to a sediment are adsorbed in the same way as the species initially present. It is also inadvisable to use tripropyl- or tripentyltin as an internal standard to correct for poor recoveries as the recovery usually varies for different organotin compounds in different samples.

Zhang et al. [31] examined the recovery of butyltin compounds from the PACS-1 reference sediment in 10 different procedures. Quantitative yields could be obtained only for TBT and DBT in a few procedures but even then signal depression problems may lead to erratic results. The method tailored for one specific determination technique often fails when another detection system is used [31]. Siu et al. [36] observed a 50% reduction the of signal height for the TBT spikes on the PACS-1 sediment, although the TBT recovery was quantitative. This was also confirmed in our laboratory but only when GC-QFAAS was used for analysis. If the same sample extract was analyzed by GC-AED, no suppression was noticed.

Interlaboratory comparison studies

The methodology for organotin speciation analysis continues to be a subject of intense development. Many hyphenated techniques are currently in use and the number of environmental data published is constantly growing. The evaluation of the analytical quality of these data is the most important issue at the moment. The most practical way to control the accuracy and precision of analytical results is through the use of appropriate Certified Reference Materials (CRMs). Both the National Institute of Standards and Technology (NIST, USA) and the Measurement and Testing Bureau, formerly Community Bureau of Reference (BCR, EEC) have initiated round robin studies aiming to issue certified reference materials for organotin. For the moment, however, the only available certified reference material for TBT, DBT and MBT in sediment is

a marine harbour sediment (PACS-1) prepared by the National Research Council of Canada with concentrations of 1.21 ± 0.24 for TBT; 1.14 ± 0.20 for DBT and 0.28 ± 0.17 for MBT (mg kg^{-1} as Sn, in dry sample). The National Institute for Environmental Studies (NIES, Japan) issued a CRM 11, which is a fish tissue with a certified content 1.3 ± 0.1 $\mu g\ g^{-1}$ for TBT and a reference value of 6.3 $\mu g\ g^{-1}$ for TPhT (both values given as chlorides).

The BCR certification campaign on butyltins in a candidate CRM of estuarine sediment, has recently been concluded and this material (CRM 462) is expected to be soon certified. Another candidate material (harbour sediment, CRM 424) is still under investigation and will be certified as soon as the results obtained so far are confirmed by a sufficient number of independent methods [56]. No CRM is so far available for water analysis.

Conclusions

Recent advances in hyphenated techniques have brought the instrumental detection limits to the sub-pg level on the routine basis and now the limitations in speciation analysis for organotins are mainly related to sample preparation. The wider application of solid phase extraction and the development of in situ derivatization procedures have reduced the labour effort and increased the sample throughput enabling automation of organotin analysis in water. In the same time the procedures employed for biological samples and sediments remain multistep, interference prone and often unreliable. The intensified efforts in certification of reference materials for organotins by the agencies let expect the advent of sufficient number of CRMs in the near future to enable better evaluation of the accuracy of the data published.

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Analytica Chimica Acta, 286 (1994) 319–327
Elsevier Science B.V., Amsterdam

Development of a supercritical fluid extraction procedure for tributyltin determination in sediments

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(Received 17th February 1993; revised manuscript received 5th July 1993)

Abstract

The effect of extraction variables, such as extraction time, temperature and extraction agent composition (X), in supercritical fluid extraction (SFE) of tributyltin (TBT) from a spiked sediment, has been optimized by using a factorial–fractional experimental design. Under the optimum conditions (T = 60°C, P = 35 MPa, X = 5.1 M methanol in CO_2, t = 30 min) the TBT extraction efficiency was 82% with a coefficient of variation (C.V.) of 9.2% (n = 5). Furthermore, the analytical procedure was validated using a reference material (RM) certified for its TBT content and was successfully applied to the analysis of a Certified Reference Material (CRM). Furthermore, an intercomparison of the SFE with another extraction procedure based on atmospheric pressure liquid extraction (APE) for the TBT determination in sediments has been carried out. Although TBT recoveries were significantly higher in the SFE procedure, the precision of both methods was comparable, probably associated to the similar steps in the extract treatment procedure (derivatization and clean up).

Keywords: Sediments; Supercritical fluid extraction; Tributyltin

Analytical techniques for determining butyltin compounds in natural water are reasonably well established [1–6], however, analytical procedures for tin speciation methods in more complex matrices (i.e., biota and sediment) are not completely satisfactory, which was outlined in interlaboratory data variability found in intercalibration exercises [7].

Several factors can be responsible for the lack of interlaboratory agreement in the determination of organotins in complex environmental matrices.

(A) Quality control (QC) and quality assurance (QA) procedures are still not clearly established for such complex matrices, i.e., the lack of suitable reference materials often hampers the verification of the accuracy of method (using certified reference materials, CRMs) or its long-term reproducibility (e.g., using reference materials for establishing control charts).

(B) Extraction recovery studies of most of the analytical procedures are only based on the analysis of spiked samples and not on the determination of incurred species, which may lead to an over-estimation of extraction efficiency.

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SSDI 0003-2670(93)E0418-7

(C) Derivatization reactions are suspected to be not completely quantitative when real complex mixtures have to be analyzed and there is no means to control their yields.

(D) Finally, detector sensitivity is decreased by signal quenching during the analysis of complex mixtures [8].

All these steps have to be critically investigated in order to validate the multi-step techniques generally used for the determination of organotin compounds. Particularly, studies on extraction efficiency are still paramount for validating these analytical procedures. The method of extraction must be compatible with the technique of final determination used; in addition, the type of matrix (sediment or biota) dealt with might require special digestion procedures [9].

The occurrence of butyltins in sediment is either via superficial adsorption by binding to the organic matrix, or by strong association to the biological debris, which may trap these compounds strongly [10]. Nevertheless, there is no involvement in mineralogical processes. Therefore, complete dissolution or total breakdown of the sediment is not considered necessary [9].

A wide variety of extraction procedures have been used for the determination of organotins in sediment and biological samples, particularly when AAS is used as the final determination step; these involved acids (hydrochloric acid, acetic acid) for organotin compounds and bases (sodium hydroxide) [11–14]. Other procedures, i.e., techniques involving GC or LC, are based on extraction with an organic solvent, (i.e., dichloromethane, chloroform, toluene, hexane) [15,16]. The different sources of pitfalls that can be expected at the extraction step have been discussed extensively [17]. Moreover, the testing of ten different extraction procedures has shown that several methods are satisfactory for the extraction of DBT and TBT compounds, whereas the recoveries of MBT were erratic and non-reproducible for all the methods tested [9]. This difference in extraction recovery for different compounds highlights the need for a careful control of extraction efficiency for all the species determined.

A good assessment of QA implies that the extraction recoveries are verified; this can be done by spiking a sample of similar composition as the sample analysed with a known content of the analyte of concern, left to equilibrate and determine the analyte after extraction. The major drawback is that the spike is not always in the same physico-chemical form as the naturally occurring compounds. Alternatively, and only if the extraction procedure does not change the matrix composition and appearance, the recovery experiment may be carried out on the previously extracted real sample by spiking, equilibration and extraction. However, the recovery assessment can often be overestimated and this risk should be faced; CRM may be a tool to ascertain accuracy; they are, however, only useful in cases they contain incurred, and not spiked, species.

For the validation of extraction methods, materials with an incurred analyte (i.e., bound to the matrix in the same way as the unknown), which is preferably labelled (radiative labelling would allow to verify the recovery) would be necessary. Such materials not being available, the extraction method should be validated by other independent methods. Supercritical fluid extraction (SFE) is the method of choice due to the faster extraction rates, solvent waste minimization, and amenability to couple to other separation techniques (GC, SFC or LC). Supercritical fluids have unique physical properties, yielding to higher extraction efficiency in shorter extraction times than liquids [18,19]. Therefore, analytical-scale SFE has found many applications in the field of the extraction of organic contaminants in environmental samples [20–24]. Nevertheless, SFE has almost not been evaluated yet for the extraction of organometallic compounds from environmental matrices. Very recently a procedure based on SFE coupled to SFC has been reported for the determination of organotin compounds from food matrices [25] and another for the extraction of spiked organotins from topsoil [26].

The aim of this work was, therefore, to develop a SFE extraction procedure for TBT determination in sediments and to compare it with other procedures based on different desorption processes from the solid matrix (i.e., high-pressure liquid extraction, HPLE, and APE).

EXPERIMENTAL

Reagents and materials

SFC grade carbon dioxide was obtained from Carburos Metálicos (Barcelona). Pesticide grade hexane and methanol were purchased from SDS (France) and glass distilled diethyl ether from Carlo Erba-Farmitalia (Italy). Analytical grade 32% (v/v) hydrochloric acid, anhydrous sodium sulphate, sodium chloride, silica (70–230 mesh), and C_2H_5MgCl in THF were obtained from Merck and Aldrich (Germany). Methanol was doped with hydrochloric acid gas, generated from H_2SO_4 and NaCl. Organotin compounds: monobutyltin (MBT), dibutyltin (DBT), tripropyltin (TPrT), tributyltin (TBT), tetrabutyltin (TeBT), diphenyltin (DPhT) and triphenyltin (TPhT) were obtained in the highest grade (Fluka and Aldrich).

Sample preparation

A TBT-spiked sediment sample was prepared at the Joint Research Center of Ispra (Italy). This sample was collected in the Lago Maggiore (Italy), wet-sieved, air-dried and homogenized. The sediment was suspended in demineralized water and stirred overnight. 100 cm^3 of TBTAc in methanolic solution was then added to the mixture which was stirred twice for 30 min. The suspension was filtered with a water pump vacuum to a minimum water content and air-dried in the dark at 20°C during one week followed by drying at 40°C during several hours. Finally, the dry material was ground and sieved over a 90-μm sieve, mixed in a PTFE drum and bottled. A separate portion of the sediment collected (unspiked) was also prepared to serve as a blank in the intercomparison [27]. These two samples were used in the present study for the SFE method development.

Other samples were prepared in the frame of BCR projects for a possible certification of butyltins: a harbour sediment has been collected in the Sado Estuary (Portugal), air-dried and transported towards the Central Bureau of Nuclear Measurements (CBMN, Belgium) for further treatment: the preparation of this candidate CRM (CRM 424) has been described in detail elsewhere [28]. A second candidate (CRM 462) has been collected in the Arcachon Basin (France) and was also treated at the CBMN.

Finally, a SRM of harbour sediment (PACS-1) was obtained from the National Research Council of Canada (NRCC, Ottawa) which allowed to assess the extraction efficiency and accuracy of the methods discussed in this paper. This material was collected in the Esquimalt Harbour (British Columbia), freeze-dried, sieved at 125 μm, bottled and radiation-sterilized. Details on the preparation of this material are given elsewhere [29].

Atmospheric-pressure liquid extraction (APE)

3 g of sediment were extracted with 15 cm^3 of methanolic hydrochloric acid (10^{-2} M), obtained under a stream of HCl gas. Three extractions were performed by sonication during 20 min in a PTFE lined glass vial. Methanolic extracts were recovered by centrifugation at 3000 rpm and were concentrated to dryness by rotary evaporation at 30°C.

High-pressure liquid extraction (HPLE)

1 g of spiked sediment was extracted with a high-pressure Soxhlet system from J&W (Folsom, CA). Sediment samples (1 g) were transferred into a glass fiber extraction thimble, previously extracted, and inserted in the stainless-steel body. After screwing the cover, a vacuum source was applied to remove the air from the system, which was filled with 880 p.s.i. of CO_2 by opening a needle valve. Liquified gases were delivered by a dip tube tank, previously percolated through an activated charcoal filter. The gas filling level was checked according to the manufacturer directions. Following a pressure equilibration period of 2 min, a needle valve was closed and the cooling system (4°C) was switched on. When the pressure dropped, the system was refilled again until reaching the original pressure. Finally the extraction system was transferred to a water bath at 40°C and extraction was performed from 2 to 4 h. When the extraction was accomplished, the system was decompressed slowly to avoid extract losses, and kept at room temperature.

Supercritical fluid extraction (SFE) procedure

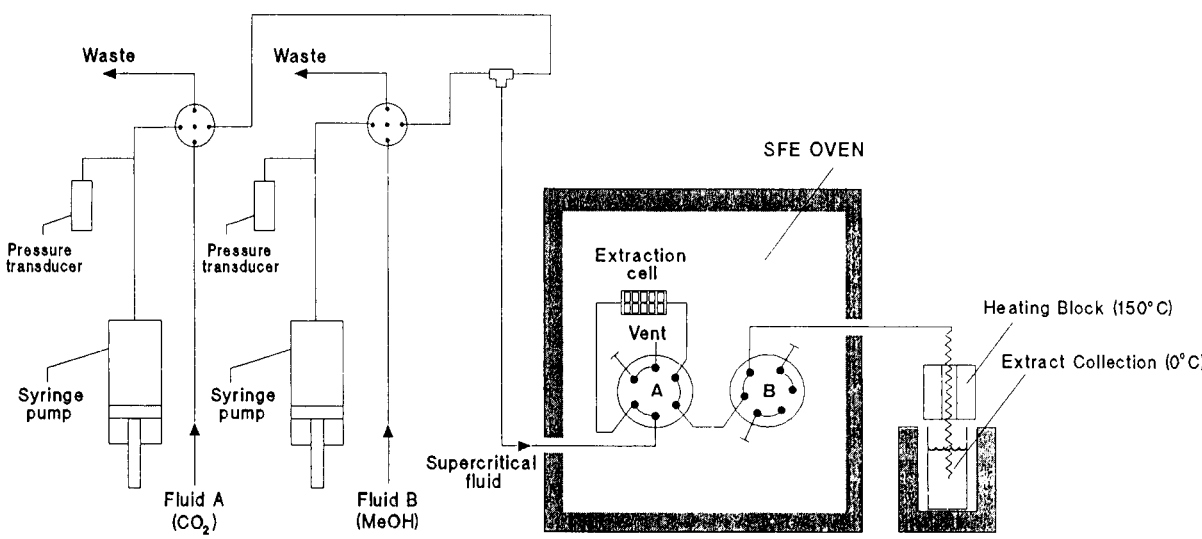
1–3 g of sediment were used for the method development. Extraction was performed in a SFE system coupled to a SFC–GC apparatus (Fisons Instruments, Milan). Supercritical carbon dioxide and modifier were delivered by two high-pressure syringe pumps, each one interfaced to a PC via pressure transducers (Fig. 1). Both pumps were operated under pressure or density programming modes. The syringe pump, which delivers the CO_2 was refrigerated at 2°C to keep it in the liquid state. Compressibility tests for every binary fluid mixture were performed in order to avoid fluid cross-contamination between the dual syringe pump system. A 5-cm^3 extraction vessel (Suprex, Pittsburgh) and two high-pressure valves (A and B, Fig. 1) were set up in a thermostatized chamber with circulating air. While valve A allows to replace the extraction vessel without the depressurization of the pumping system, by switching valve B it is possible to perform static or dynamic extraction modes. Extracts were collected into a 5-cm^3 screwed cap PTFE lined vial containing 2 cm^3 of isooctane and TPrT as internal standard. Flow-rate was monitored from the syringe pump LCD readings and was adjusted at 2 cm^3 min^{-1} (as supercritical fluid) by choosing a suitable length of linear restrictor (50 μm i.d. fused silica).

Extract clean-up and derivatization procedures

Extracts were derivatized with 1 cm^3 of 20% C_2H_5MgCl in tetrahydrofuran. The mixture was allowed to stand for 5–10 min and the excess of reagent was destroyed with 3 cm^3 of preextracted (hexane) bidistilled water at 4°C, and 0.5 cm^3 of 32% HCl. The organic phase was recovered by extracting with diethylether (2×5 cm^3), dried over anhydrous Na_2SO_4, and reduced to 0.5 cm^3 under a gentle stream of dry nitrogen. After the derivatization step extracts obtained from the APE and HPLE procedures were cleaned up by passing through 1 g of activated silica (Merck, 70–230 mesh).

Analytical determination

Organotin compounds were analyzed as ethyl derivatives in a Carlo Erba 6000 Vega gas chromatograph equipped with cold on-column and a single flame photometric detector (FPD). A 2.5 m $\times$ 0.32 mm i.d. deactivated fused silica tubing was coupled via a press-fitted back connector (Chrompack, Middelburg) to the analytical column, 30 m $\times$ 0.25 mm i.d. fused-silica coated with 0.11 μm of DB-5. An additional column was also used for compound confirmation and to determine the presence of interferences. The column temperature was 35°C for 2 min and then, programmed to 300°C at 8°C min^{-1}, keeping the



final temperature for 5 min. Hydrogen was used as carrier gas at linear velocity of 50 cm s^{-1}. The composition of FPD fuel gases were hydrogen (120 cm^3 min^{-1}) and air (100 cm^3 min^{-1}). Band-pass filter with peak transmission at 600 nm was used. FPD excitation voltage was 550 V and detector temperature was set up at 225°C.

Quantitation

MBT, DBT, TPrT, TBT and TPhT compounds were used for calibration using the internal standard procedure. Tripropyltin (TPrT) was used as internal standard and was added into the collection vial. The response factor of a standard mixture was monitored between real samples in order to assess the performance of the analytical system. Fresh standards were prepared monthly by derivatization. All the optimization experiments were performed in duplicate and reproducibility studies were done by 5 independent replicates.

Statistical optimization

A factorial–fractional experimental design [30] focused on four variables (extractant agent composition, pressure, temperature and extraction time) was applied for TBT extraction optimization, each one chosen at two levels (high and low), as shown in Table 1. Selected experiments were performed in duplicate. Response effects of variables and the two-way interactions were calculated according to the Yates algorithm [31].

RESULTS AND DISCUSSION

SFE procedure development and validation

A study of TBT recovery from a spiked sediment (see *sample preparation* section) by using liquefied carbon dioxide Soxhlet extraction (HPLE) was performed in the first stage of SFE method development. Extraction yield was poor using liquefied carbon dioxide (21.2%) (Fig. 2) and also with more polar fluids such as chlorodifluoromethane. Therefore, the addition of modifiers to CO_2 was considered to be imperative to obtain quantitative extraction of TBT by SFE. Taking into account the solubility of organotin chlorides in liquid methanol, methanolic hydrochloric acid (10^{-2} M) was chosen as a modifier of carbon dioxide. In fact, the sediment pretreatment with hydrochloric acid enhanced the recovery of TBT by HPLE (Fig. 2).

From data obtained in the fractional–factorial experimental design (Table 1), it was inferred that the strongly significant variables were modifier composition and in less extent temperature and no significant two-way interactions between variables was found. In order to improve the extraction efficiency several experiments were performed by increasing the methanol content within CO_2 since no two-way interaction between variables was found. Extraction efficiency increased asymptotically until 5.1 M (20%, v/v) concentration reaching an extraction efficiency in the range of 80% (Fig. 3), probably associated to

TABLE 1

Fractional–factorial experimental design for the SFE method development and TBT extraction efficiency from spiked sediment

Trial	Temperature (°C)	Pressure (MPa)	Time (min)	% (v/v) methanol in CO_2	Recovery [a] %
1	50	20	15	5	50.6
2	80	20	15	15	56.9
3	50	35	15	15	58.6
4	80	35	15	5	46.8
5	50	20	30	15	69.2
6	80	20	30	5	46.0
7	50	35	30	5	59.4
8	80	35	30	15	72.8

[a] Recovery calculated from the nominal spiking level.

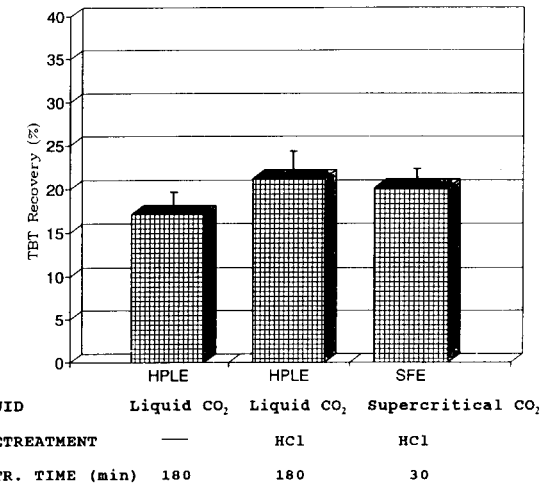


Fig. 2. Comparison of TBT recovery from the spiked sediment (see sample preparation) according to extraction procedure and sample pretreatment using liquid CO_2 in high-pressure liquid extraction (HPLE) and supercritical fluid extraction (SFE). The R.S.D. obtained with each extraction technique is indicated by vertical bars on the top of each histogram.

a parallel increase of the solubility of TBT with methanol concentration increase. On the other hand, the temperature was considered not suitable to be reduced in order to keep it above the critical point of the methanolic modified carbon dioxide. Concurrently, restrictor plugging was minimized by raising the extraction temperature from 50 to 60°C.

Optimum conditions were the following: $T =$ 60°C, $P = 35$ MPa, extraction agent composition $(X) = 5.1$ M (20%, v/v) methanol doped with

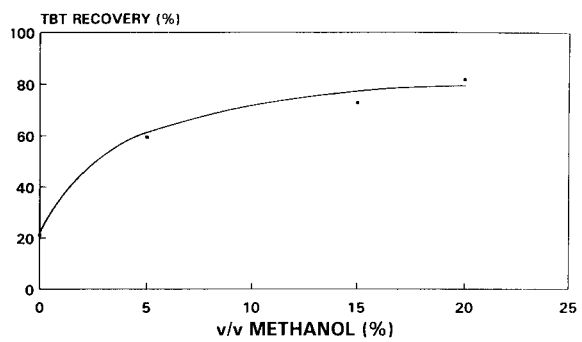


Fig. 3. TBT extraction efficiency from the TBT spiked sediment (see Experimental section) according to the amount of methanol added to the CO_2.

HCl in CO_2, v/v, $t = 30$ min. The extraction efficiency for TBT, referred to the spiking level, was 82%, which could be considered satisfactory according to spiking procedure used to prepare this sample (see *sample preparation* section). Figure 4A shows a typical cGC-FPD chromatogram of the SFE extract from the TBT spiked sample used in the method development, showing the absence of degradation products (DBT and MBT). It is interesting to notice that DBT was already present in the blank sample at similar concentrations that we found in the spiked sample. Consequently, it cannot be considered as a degradation product of the spiked TBT.

Very recently an analytical procedure enabled recoveries up to 94% pretreating a spiked top soil with sodium diethyldithiocarbamate following supercritical carbon dioxide extraction. However, this approach has not been tested in real samples [26].

In order to evaluate the accuracy and reproducibility of the SFE procedure, 5 independent replicates of PACS-1 were performed. Figure 4B shows a characteristic cGC-FPD chromatogram and Fig. 5 shows the results in terms of extraction efficiency. The reproducibility of overall method was considered satisfactory (R.S.D. 6.9%, $n = 5$) and the TBT concentration found (as Sn) was 1.08 ± 0.07 $\mu g\ g^{-1}$ (corrected for recovery), which falls within the certified value for this Reference Material (1.27 ± 0.22 $\mu g\ g^{-1}$ as Sn). The extraction efficiency of the SFE procedure for TBT in the PACS-1 according to the certified value was lower (69.4%) than the value found in the spiked sediment (82%). This fact could be attributable to a different binding behaviour of TBT between both matrices (easier extraction of artificially spiked TBT in comparison to incurred TBT).

Application of SFE to candidate CRM analysis

The developed SFE method was applied to the determination of TBT in the candidate CRMs 462 and 424 in the frame of the BCR project for tin speciation. A total of 5 independent replicates were performed in the candidate CRM 462 to check the method precision. Figure 5 shows the mean value for this CRM. The resultant value after correction for recovery was 90.34 ± 17.43 ng

g^{-1} (as TBT), which falls within the range of certified values for this CRM (75.5 ± 13.2 ng g^{-1} as Sn) [32]. The higher value of the C.V. (19.3%), could be accounted for to the lower levels of TBT (one order of magnitude lower than PACS-1); this level is, however, more representative of natural levels and hence gives a more realistic view of the SFE performance.

On the other hand, several attempts were carried out to determine the TBT content in the CRM 424 with the same SFE procedure. However, due to high organic carbon content (ca. 4 mg g^{-1}) several difficulties associated to this particular matrix arose. In particular, the matrix complexity and the low levels of TBT led to a lack of reproducibility of the procedure. Therefore, the SFE developed should be adapted to deal with complex matrices such as CRM 424.

The results of the SFE extraction procedure were compared with those obtained by the application of an APE (see Experimental section). Figure 5 summarizes the results showing a remarkable increase of extraction efficiency and precision by the application of the SFE proce-

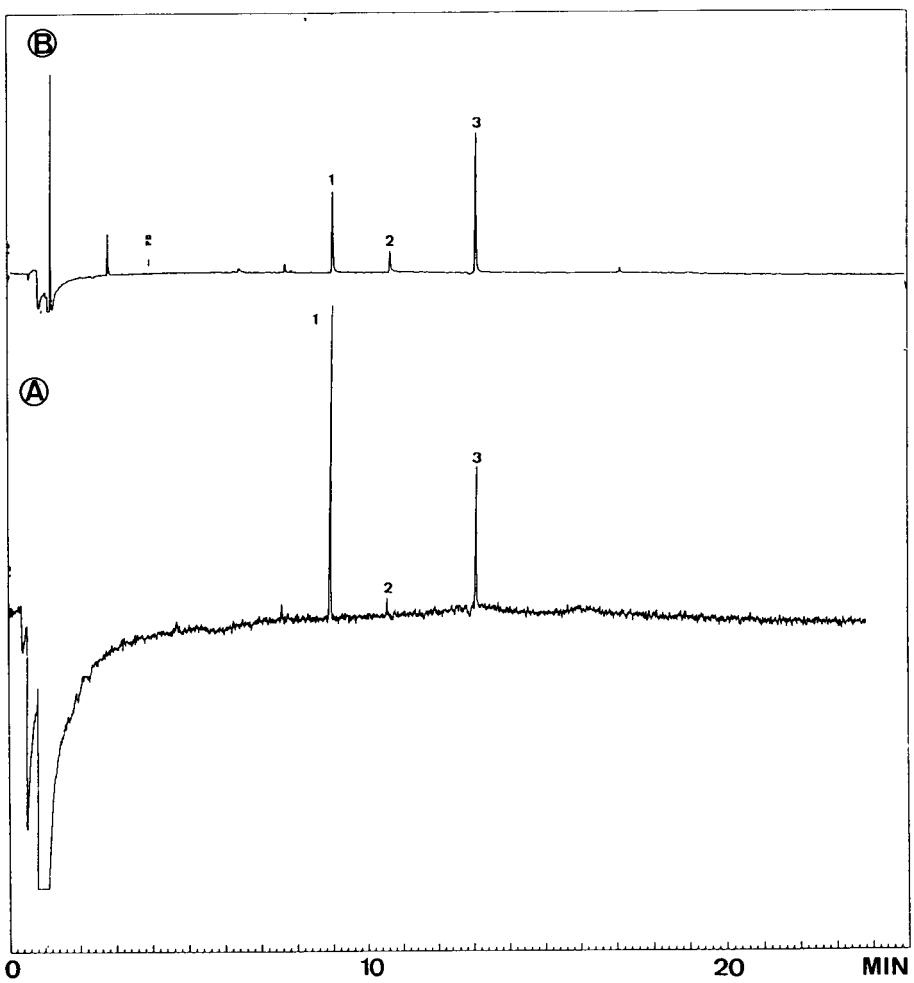


Fig. 4. cGC-FPD of the extract isolated from (A) TBT spiked sediment (JRC of Ispra), and (B) PACs-1, both obtained by using the developed SFE procedure. Compound identification: (1) tripropyltin (internal standard); (2) dibutyltin; (3) tributyltin as ethylated derivatives.

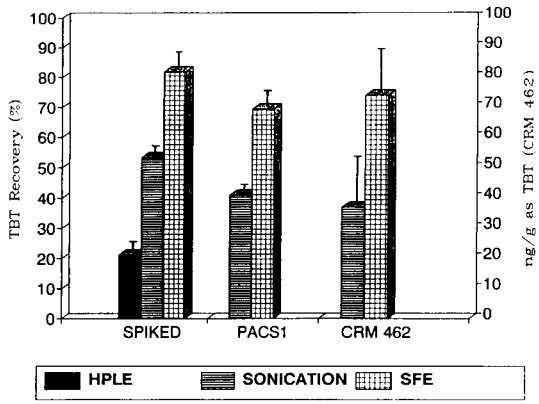


Fig. 5. Intercomparison of the TBT extraction efficiency from different matrices recovered by high-pressure liquid extraction (HPLE), sonication and supercritical fluid extraction (SFE) procedures. The TBT concentration in CRM 462 is also indicated. R.S.D. is indicated with vertical bars on the top of each histogram.

dure. Furthermore, the variance analysis (F-test) displayed significant differences between both extraction procedures for PACs-1 and CRM 462.

Conclusions

An SFE procedure for the determination of TBT in sediments has been developed, validated and applied to the analysis of candidate CRM. The extraction efficiency of the developed method was about 30% higher than an APE procedure applied to the same samples for comparison purposes. Nevertheless, the precision of both methods was comparable and most of the variability could be associated to the derivatization step applied to the extract previous to the cGC-FPD determination.

The main advantages of the developed procedure are the reduction of extraction time, the usage of solvent and the extraction selectivity, which avoids clean-up steps. However, in case of complex matrices, improvements in the hardware and sample handling are necessary to obtain more reproducible results. Further studies in extraction selectivity and on-line clean-up procedures are needed to deal with such matrices. Further research is in progress in order to extent the analytical procedure to the determination of mono- and dibutyltin in sediments.

Financial support was provided by the BCR EC (Brussels) under the contract No. 5367. J. Dachs and R. Alzaga are recipients of predoctoral fellowships from CSIC (Spain) and CONACYT (Mexico), respectively.

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Analytica Chimica Acta, 286 (1994) 329–334
Elsevier Science B.V., Amsterdam

Influence of storage conditions on the determination of organotin in mussels

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(Received 17th February 1993; revised manuscript received 5th July 1993)

Abstract

In order to check the possible occurrence of degradation of tributyltin and triphenyltin in mussel samples during their storage, a 1-year stability study was carried out. Analyses by gas chromatography with flame photometric detection and gas chromatography–mass spectrometry were performed on freeze-dried mussel samples stored under different conditions of temperature and light. The results showed that very good stability of butyl- and phenyltins can be achieved when samples are stored at −20°C in the dark. In contrast, samples stored at room temperature showed significant variations in organotin speciation, particularly in the presence of light.

Keywords: Gas chromatography; Gas chromatography–mass spectrometry; Biological samples; Mussels; Organotin compounds; Tributyltin; Triphenyltin

Tributyltin (TBT) and triphenyltin (TPhT) are widely employed as active components in antifouling paints on the hulls of boats and in the cooling pipes of industrial plants [1–3]. Owing to their high toxicity toward "non-target" organisms, such as oysters and mussels [4–9], these compounds can be responsible for environmental damage with economic consequences as observed in the past in areas of high shellfish production [10–12].

Both TBT and TPhT undergo degradation processes in the marine environment, such as microbial and UV degradation, consisting of progressive dealkylation to inorganic tin [13], although there is evidence for a faster rate for DBT → monobutyltin (MBT) degradation under some experimental and evironmental conditions [14–16]. As the toxicity of the organotins has a maximum for the trisubstituted compounds, the degradation can be considered as a mechanism of detoxification.

Many methods have been developed for the determination of organotin concentrations in waters, sediments and biota [17–27]. Suitable methods should be sensitive enough to determine organotins down to environmental concentrations (usually in the ng l^{-1} range for waters and ng g^{-1} for sediments and biota) and be able to discriminate among different chemical forms (owing to the different toxicities of the tin species). These techniques should also be accurate and precise. Hence it is necessary to check each step carefully before the final analysis in the laboratory for evaluating the quality of the results and there is a clear need for certified reference materials (CRMs) and laboratory RMs to implement truly efficient quality control practices. Storage of samples and materials is one of the most critical aspects of the whole analytical procedure, during which physico-chemical alterations could affect

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SSDI 0003-2670(93)E0419-8

the final determination. This is particularly true for organotins in biological and sediment samples, where degradation phenomena are well documented. Few data are available on the stability of organotin compounds in water and sediment samples on storage [15,28,29]. There have been no reports, to our knowledge, on organotin stability in mussel samples. The aim of this work was to study the stability of organotin compounds over a 1-year period in freeze-dried mussel samples stored under different conditions of light and temperature. Analyses were performed after 0, 1, 3, 6 and 12 months of storage, determining triorganotin compounds (TBT and TPhT) and the di- and monoorganotin compounds (DBT, DPhT, MBT and MPhT) (Ph = phenyl).

EXPERIMENTAL

Apparatus

Gas chromatography (GC) was carried out on a Varian Vista 6000 chromatograph equipped with a double flame photometric detector (FPD) operated without a filter. With respect to the standard configuration, the gas flows were modified as follows: air (1) 200 ml min^{-1}; hydrogen (1) 120 ml min^{-1}; and hydrogen (2) 80 ml min^{-1}. Other chromatographic conditions were as follows: carrier gas (helium) flow-rate, 9 ml min^{-1}; column, megabore DB-1 (methylpolysiloxane, 30 m × 0.53 mm i.d., 1.5 μm film thickness; J&W Scientific); temperature programme, 80°C for 1 min, then increased at 10°C min^{-1} to 280°C; injector, hot on-column, 240°C; and detector temperature, 240°C. Data were collected and integrated with a Hewlett-Packard HP 3396A.

Gas chromatographic–mass spectrometric (GC–MS) analyses were performed with a Hewlett-Packard HP 5890 gas chromatograph and an HP 5970B mass spectrometer system under the following conditions: electron impact ionization mode (70 eV); carrier gas, helium, 65 kPa head pressure; column, HP-5 (methyl–5% phenylpolysiloxane, 25 m × 0.20 mm i.d., 0.11 μm film thickness; Hewlett-Packard); temperature programme, 80°C for 2 min, then increased at 10°C min^{-1} to 280°C; injector, splitless, 240°C; transfer line temperature, 280°C; and selected ion monitoring (SIM) operation with the programme given in Table 1 (dwell time 100 ms for all ions).

TABLE 1

Programme for SIM

Compound	Start time (min)	*m/z*
TPrT	10	277, 275, 273
TBT	12.5	305, 303, 301
DBT	14	319, 317, 305
MBT	15	319, 317, 315
Sn(IV)	15.7	333, 331, 329
MPhT	17	339, 337, 335
DPhT	19	345, 343, 341
TPhT	20.5	351, 349, 347

Peak identification was based on the matching of retention times and isotopic mass ratios. The relative response factors were controlled by injecting standard mixtures on a regular basis (one injection every 3–4 samples) to compensate for any variations in the tuning conditions of the mass spectrometer.

Reagents and standards

Organic solvents were of pesticide grade from Carlo Erba (Milan, Italy).

Tropolone (2-hydroxycycloheptatrienone) was obtained from Lancaster Synthesis (Morecambe, UK), *n*-pentylmagnesium bromide from Aldrich (Steinheim, Germany), sulphuric acid from BDH (Poole, Dorset, UK), Florisil (100–200 mesh) from BDH, activated overnight at 180°C, anhydrous sodium sulphate (RPE-ACS) from Carlo Erba, treated at 550°C for 6 h before use, tributyltin chloride (TBT) from BDH, dibutyltin chloride (DBT), monobutyltin chloride (MBT), diphenyltin chloride (DPhT) and monophenyltin chloride (MPhT) from Aldrich and tripropyltin chloride and triphenyltin chloride from Merck (Hohenbrunn, Germany).

The purity of the tin compounds was based on tin only. They were used as received and were checked for the presence of degradation products by GC–FPD and GC–MS after Grignard derivatization.

Organotin stock standard solutions were prepared gravimetrically in methanol at about 500

mg l^{-1} (as Sn) and diluted 500-fold to give working standard solutions. When stored refrigerated in the dark, the stock standard solutions are stable for at least 3 months and the working standard solutions at least for 1 month, but the latter were renewed weekly.

Determination of organotin

The procedure used for organotin determination in mussels includes, after extraction in methanol–tropolone by sonication, liquid–liquid partitioning in water–methylene chloride, solvent exchange with isooctane, derivatization with pentylmagnesium bromide, a clean-up step and final determination by GC–FPD and/or GC–MS. Tripropyltin was used as an internal standard and relative response factors were calculated (by injecting standard mixtures) before the determinations.

The instrumental detection limit was 8 pg (as Sn) injected for both techniques, corresponding to 40 ng g^{-1} for GC–FPD and 80 ng g^{-1} for GC–MS for a 100-mg sample, final volume 1 ml and injected volume 2 μl (GC–FPD) or 1 μl (GC–MS).

More details of the procedure and the accuracy and precision of the method have been reported elsewhere [24,27].

Quality control procedures

As CRMs for organotins in biological materials are not available, with the exception of NIES (National Institute for Environmental Sciences, Japan) fish material for TBT, there is no traceable or completely defined means to assure the quality of analytical data in a long-term stability study. The following measures were taken. Pentylated organotin standard solutions were prepared at the beginning of the study and stored at −20°C in the dark [30], to serve as independent control solutions, as the stability of fully derivatized organotins far exceeds that of the starting compounds, particularly for phenyltins. Freshly prepared stock and working standard solutions were checked for degradation products by GC–MS after pentylation and analysed with respect to the stored pentylated standards. In order to control the method performance, organotin working standard solutions were regularly run through the whole analytical procedure, particularly in parallel with the stability study samples. Single analytical steps were carefully checked for performance problems or relevant changes in the materials used (e.g., clean-up for new batches of silica gel).

Sample collection

The samples were collected in La Spezia harbour (northern Italy), which is characterized by high maritime traffic and dockyard activity. One of the most important Italian mussel farms is located inside the harbour and mussels were obtained directly from the farm. Previous studies carried out on mussel samples from La Spezia harbour showed the simultaneous presence of all butyltin and phenyltin species at high concentrations, making these samples suitable for stability studies of both butyltin and phenyltin compounds.

Sample preparation

After collection, the mussel tissues were immediately frozen, then freeze-dried and homogenized. The material was neither sieved nor ground in a ball-mill.

Stability study

The initial concentrations of butyltins and phenyltins were determined by four replicate analyses, then the sample was divided into four aliquots and stored at three different temperatures in the dark: −20°C, +4°C and room temperature. In addition, samples were stored at room temperature in daylight (Pyrex glass). Analyses were performed on each subsample by GC–FPD (four replicates) after 1, 3, 6 and 12 months of storage. GC–MS was used to confirm the peak identification.

RESULTS AND DISCUSSION

The concentrations found at the beginning of the stability test ranged from 0.5 μg g^{-1} as Sn (dry mass) for monobutyltin to about 3 μg g^{-1} for tributyltin; monophenyltin was below the detection limit and the triphenyltin concentration was ca. 1 μg g^{-1}. Dibutyltin and diphenyltin showed

intermediate concentrations of about 1 and 0.2 $\mu g\ g^{-1}$, respectively.

The results for butyl- and phenyltin species concentrations (as percentages of the total butyl- and phenyltin content at time $t = 0$) as a function of the storage time are reported in Table 2 for the different storage conditions. The standard deviations, also reported in Table 2, were generally lower than 2% for butyltins (ca. 84% of the results) and 6% (ca. 80% of the results) for phenyltins.

Figures 1–4 summarize the trends of the butyltin species concentrations (as percentages of the total butyltin content at time $t = 0$) as a function of the storage time for the different storage conditions. The total butyltin content remains fairly constant for samples stored in the dark (Figs. 1–3) whereas the samples exposed to light at room temperature displayed a decrease in total butyltin concentration after 6 months (90% of the starting content recovered) and 12 months

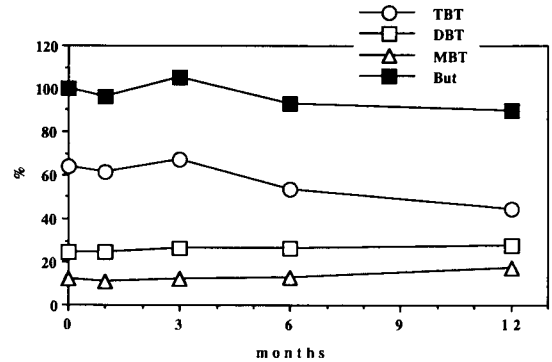

Fig. 1. Stability of butyltins in freeze-dried mussel samples stored at −20°C in the dark. ○ = TBT; □ = DBT; △ = MBT; ■ = Σ(But).

(80% recovered) (Fig. 4). Considering the individual compounds, no degradation occurred after 3 months of storage at −20°C; however, after 6 and 12 months a slight decrease in TBT concentration was detected whereas no significant variations

TABLE 2

Stability of butyl- and phenyltins in mussel samples as function of the time and conditions of storage, expressed as a percentage of the total butyl- and phenyltin content at time $t = 0$ (mean ± standard deviation, $n = 4$)

Temperature (°C)	Storage time (months)	TBT	DBT	MBT	Σ(But)	TPhT	DPhT	MPhT	Σ(Ph)
−20	0	64 ± 1.7	24 ± 0.6	12 ± 0.8	100 ± 1.1	87 ± 10.4	13 ± 3.8	0	100 ± 7.8
	1	61 ± 0.6	24 ± 0.3	11 ± 0.9	96 ± 1.1	116 ± 4.8	9 ± 1.2	0	125 ± 4.9
	3	67 ± 2.2	26 ± 2.0	12 ± 0.1	105 ± 3.0	82 ± 5.6	27 ± 4.7	0	109 ± 7.3
	6	54 ± 0.9	26 ± 0.8	13 ± 1.0	93 ± 1.6	82 ± 2.0	21 ± 0.4	0	103 ± 2.0
	12	45 ± 1.1	27 ± 0.2	17 ± 2.3	89 ± 2.6	77 ± 8.7	4 ± 0.6	0	81 ± 8.7
+4	0	64 ± 1.7	24 ± 0.6	12 ± 0.8	100 ± 1.1	87 ± 10.4	13 ± 3.8	0	100 ± 7.8
	1	61 ± 2.9	24 ± 1.3	16 ± 3.4	101 ± 4.7	104 ± 4.2	7 ± 4.3	0	111 ± 6.0
	3	55 ± 0.2	24 ± 1.6	17 ± 1.1	96 ± 2.0	52 ± 4.7	67 ± 5.9	0	119 ± 7.5
	6	45 ± 0.5	26 ± 1.5	21 ± 1.8	92 ± 2.4	51 ± 6.8	32 ± 7.5	0	83 ± 10.1
	12	43 ± 1.0	26 ± 0.3	28 ± 0.6	97 ± 1.2	43 ± 0.9	9 ± 0.5	44 ± 3.9	96 ± 4.0
RT(D) [a]	0	64 ± 1.7	24 ± 0.6	12 ± 0.8	100 ± 1.1	87 ± 10.4	13 ± 3.8	0	100 ± 7.8
	1	61 ± 0.4	25 ± 0.6	14 ± 0.4	100 ± 0.8	94 ± 2.9	9 ± 1.4	0	103 ± 3.2
	3	67 ± 4.2	40 ± 2.3	38 ± 0.9	145 ± 4.9	34 ± 2.7	21 ± 5.2	48 ± 3.7	103 ± 6.9
	6	44 ± 0.8	18 ± 1.4	30 ± 0.8	92 ± 1.8	29 ± 3.3	0	0	29 ± 3.3
	12	31 ± 0.4	21 ± 1.7	46 ± 2.1	98 ± 2.7	17 ± 1.9	0	74 ± 21.3	91 ± 21.4
RT(L) [b]	0	64 ± 1.7	24 ± 0.6	12 ± 0.8	100 ± 1.1	87 ± 10.4	13 ± 3.8	0	100 ± 7.8
	1	56 ± 1.4	26 ± 0.7	18 ± 1.9	100 ± 2.5	98 ± 6.6	14 ± 0.4	0	112 ± 6.6
	3	44 ± 2.9	18 ± 1.1	47 ± 2.0	109 ± 3.0	43 ± 6.2	8 ± 1.1	0	51 ± 6.3
	6	33 ± 1.3	14 ± 0.9	46 ± 2.5	93 ± 3.0	22 ± 0.1	0	0	22 ± 0.1
	12	20 ± 1.5	6 ± 0.4	55 ± 1.6	81 ± 2.2	0	0	72 ± 13.7	72 ± 13.7

[a] Room temperature in the dark. [b] Room temperature in daylight.

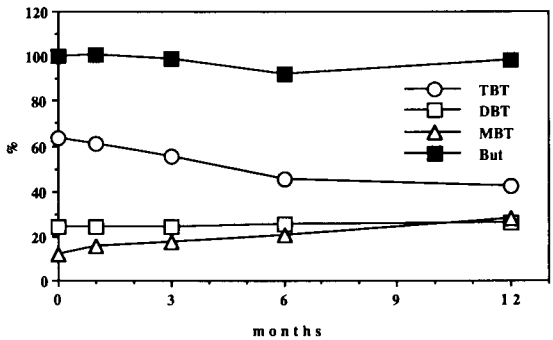

Fig. 2. Stability of butyltins in freeze-dried mussel samples stored at +4°C in the dark. Symbols as in Fig. 1.

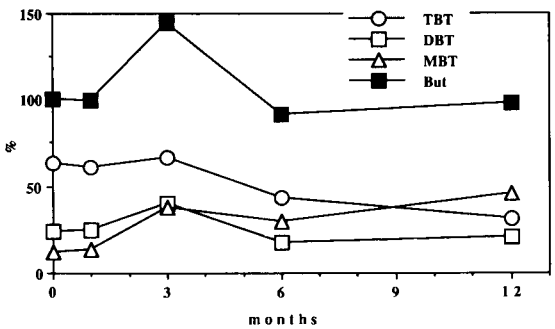

Fig. 3. Stability of butyltins in freeze-dried mussel samples stored at room temperature in the dark. Symbols as in Fig. 1.

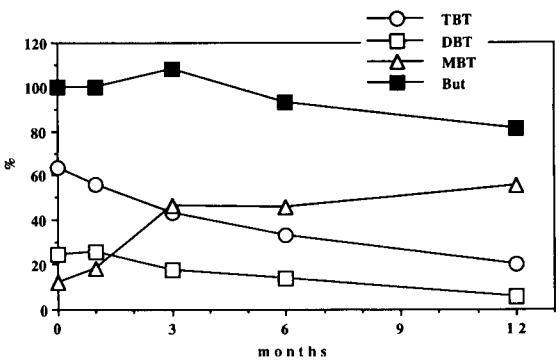

Fig. 4. Stability of butyltins in freeze-dried mussel samples stored at room temperature in daylight. Symbols as in Fig. 1.

were observed for DBT and MBT (Fig. 1). A similar trend was observed for the samples stored at 4°C. In this case, however, a slight decrease in TBT concentration was observed starting from the first month of storage, together with a slight

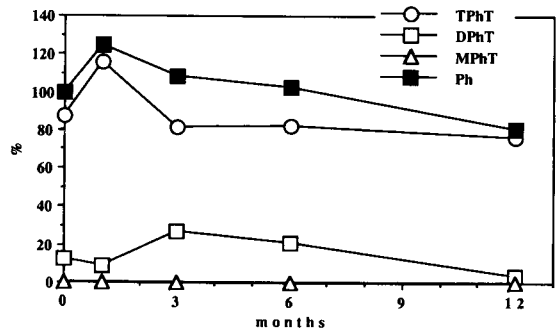

Fig. 5. Stability of phenyltins in freeze-dried mussel samples stored at −20°C in the dark. ○ = TPhT; □ = DPhT; △ = MPhT; ■ = Σ(Ph).

increase in the MBT concentration. Different results were obtained for samples stored at room temperature either in the dark or in daylight. In both instances significant variations in the speciation of butyltins were observed during storage: the TBT concentration decreased from 60% of the total butyltin content at the beginning of the study to about 20% at the end, whereas the MBT concentration increased from 10% to 50% in the samples stored in the dark and to almost 60% in those stored in the light (Figs. 3 and 4). Moreover, in samples stored in the dark, MBT became the predominant form after 12 months of storage whereas it became predominant after 3 months in samples stored in the light, confirming the effectiveness of UV irradiation in organotin degradation. The fate of DBT is more complex as two opposite effects should be considered: the decrease in concentration due to degradation to

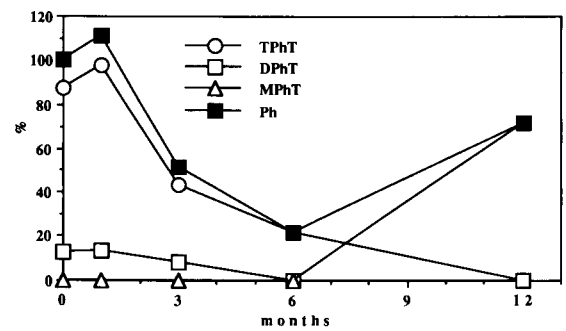

Fig. 6. Stability of phenyltins in freeze-dried mussel samples stored at room temperature in daylight. Symbols as in Fig. 5.

MBT and the increase in concentration due to TBT degradation, DBT being the primary degradation product.

Phenyltin compounds showed similar behaviour to butyltins. As an example, Figs. 5 and 6 show the stability of phenyltins in samples stored at −20°C in the dark and at room temperature in the light, respectively. No significant degradation occurred in samples stored at −20°C whereas substantial degradation of TPhT was observed for samples stored at room temperature in the light starting from the third month of storage. The unexpected value for MPhT obtained after 12 months of storage (Fig. 6) was probably due to contamination problems and/or analytical artefacts, as phenyltin measurements are usually characterized by higher uncertainties than those for butyltins.

Conclusions

The results showed that very good stability of butyl- and phenyltins in freeze-dried mussel tissues can be achieved when samples are stored at −20°C in the dark. Storage of the samples at room temperature must be avoided. Similar samples, if stored in the appropriate way and carefully homogenized, can be suitably employed to perform intra-laboratory quality control of analytical procedures in the absence of certified reference materials.

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Analytica Chimica Acta, 286 (1994) 335–341
Elsevier Science B.V., Amsterdam

Assessment of transport routes of triphenyltin used in potato culture in the Netherlands

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(Received 17th February 1993; revised manuscript received 26th July 1993)

Abstract

Organotin compounds (OTs) were analyzed after derivatization using a methyl Grignard reagent with gas chromatography with ion trap detection (GC–ITD) for detection. GC–ITD allows full scan mass spectrometry at trace levels and thus allows good verification of the presence of the OTs. With the described methods all currently used OT biocides can be determined in water, sediment and suspended matter: tributyltin (TBT), triphenyltin (TPT), tricyclohexyltin (TCT) and fenbutatin oxide (hexakis-(2-methyl-2-phenylpropyl)-distannoxane, FBTO). Additionally in water also some degradation products of the above mentioned biocides can be determined: dibutyltin (DBT), monobutyltin (MBT), diphenyltin (DPT), monophenyltin (MPT) and dicyclohexyltin (DCT). Detection limits are 1–40 ng/l (as tin) for water samples and 2–10 ng/g (as tin) for suspended matter and sediment samples. Transport routes of triphenyltin acetate (TPT) used in potato culture in the Flevoland polder in the Netherlands were studied. TPT levels were detected in freshwater and suspended matter and the sorption coefficient (K_d) was calculated. On the basis of concentrations in water and suspended matter it was calculated that only a negligible portion of TPT used is pumped out of the polder by the pumping-engines. Evaporation of TPT is probably a much more important transport route. Therefore a first attempt was made to investigate concentrations of OTs in rainwater. The results show that at distances of over 20 km from potato fields, TPT in rainwater is around the proposed general quality objective for freshwater of 10 ng/l (as tin). These observations support the modelling study by Baart and Diederen, indicating that evaporation of TPT is one of the transport routes of TPT.

Keywords: Gas chromatography; Organotin compounds; Triphenyltin; Potato

OTs are used worldwide in high quantities (40 000 tons/year in 1986 [1]) mainly as PVC stabilizers and in more limited amounts (10–15%) as biocides. OTs enter the environment directly via their use in agriculture and by leaching from antifouling paints. Until recently environmental analysis has focused primarily on butyltin compounds that are used in antifouling paints. The concentrations of OTs in freshwater systems resulting from their use in agriculture have received less attention. In the present study TPT sprayed on potato crop in the Flevoland polder was monitored at the pumping-stations where rain and sub-surface intrusion water are pumped out of the polder. It was calculated which part of the TPT leaves the polder via the pumping-engines.

Modelling studies [2] estimated that 40% of the TPT used is volatilized from potato crop.

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SSDI 0003-2670(93)E0440-I

Therefore the presence of TPT was investigated in rainwater samples, collected at distances of over 20 km from potato fields.

EXPERIMENTAL

Materials

Butyltin trichloride (MBT; 95% purity) was obtained from Janssen Chimica (Geel, Belgium). Tributyltin chloride (TBT; 90% purity), dibutyltin dichloride (DBT; no purity stated), phenyltin trichloride (MPT; 98% purity), diphenyltin dichloride (DPT; 96% purity) and triphenyltin chloride (TPT; 95% purity) were from Aldrich (Steinheim, Germany). Dicyclohexyltindibromide (DCT; no purity stated) was from Johnson Matthey Alfa (Karlsruhe, Germany). Cyhexatin (tricyclohexyltin hydroxide, TCT; 99% purity) was from Dr. Ehrenstorfer (Augsburg, Germany). Fenbutatin oxide (hexakis-(2-methyl-2 phenylpropyl) distannoxane, FBTO; 99.7% purity) was a gift from Atochem (Vlissingen, Netherlands). A solution of 0.1 g of sodium diethyldithiocarbamate (DDTC; 99% purity, Merck, Darmstadt, Germany) per ml of water was prepared. Before use this solution was extracted with an equal amount of pentane to remove non-polar contaminants. Alumina (Alumina B – super I, basic form, for column chromatography; ICN Biomedicals, Eschwege, Germany), was activated at 200°C for 24 h. The Grignard reagent methyl magnesium iodide (MeMgI; 2 M solution in diethyl ether) was synthesized by the dropwise addition of 0.4 mole of iodomethane (99.5% purity, Baker, Deventer, Netherlands) dissolved in 100 ml diethyl ether to 0.4 mole magnesium (> 99% purity, Merck) and 100 ml diethyl ether in a nitrogen atmosphere. Analytical-grade pentane, hexane, diethyl ether, acetic acid, sulphuric acid, sodium hydroxide and anhydrous sodium sulphate were purchased from Baker. A buffer solution of pH 5.00 ± 0.05 was prepared by mixing equal amounts of solutions of 5.40 M acetic acid and 3.40 M sodium hydroxide. Methylated organotin standards were synthesized and purified as described earlier [3]. These were employed to prepare calibration standards. For brevity, throughout this paper the organotin salts and their derivatives are

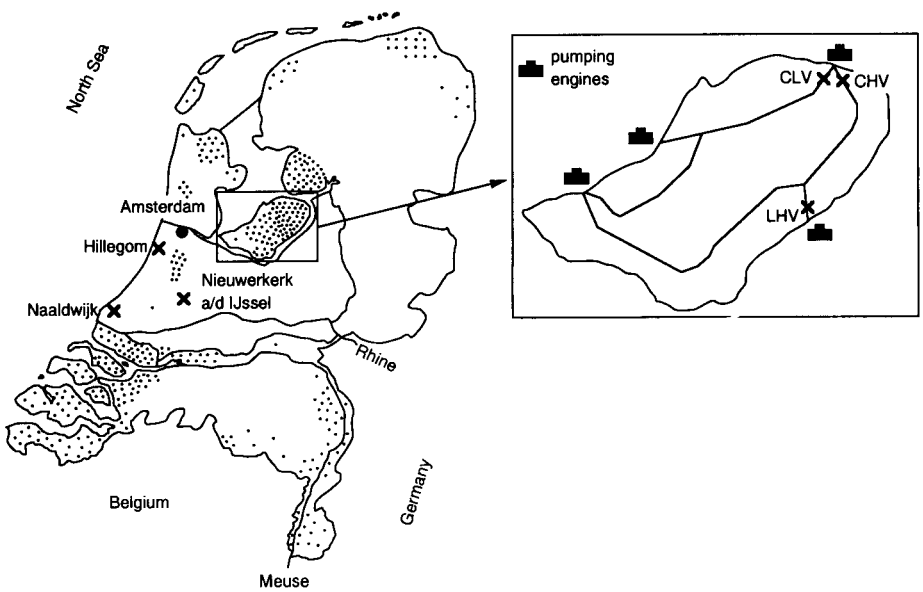


Fig. 1. Map of the Netherlands. Potato cropping areas are indicated with dots, rainwater sampling locations are indicated with ×. In the enlarged section the Flevoland polder is shown with the main canals and sampling locations at the pumping-stations indicated with ×.

indicated by the same abbreviations. OT levels given in this paper are expressed as tin.

Sample preparation

Water and suspended matter samples were taken in canal Hoge Vaart and canal Lage Vaart at the pumping-station Colijn (CHV, CLV) and in canal Hoge Vaart at the pumping-station Lovink (LHV; see Fig. 1). These pumping-stations provide for 60% of the total pumping capacity of the polder. Water samples were taken continuously using a PB-MOS (Edmund Bühler, Tübingen, Germany) flow proportional water sampler from June 1st 1992 until September 31st 1992. Once a week water was taken from the sampler and stored at −30°C in polyethylene bottles. Polyethylene allows frozen storage of the water samples. A disadvantage is that it releases MBT and DBT, but as the present study was aimed at TPT this was considered of minor importance. Rainwater was collected at three locations in the west of the country in an area with little or no potato cropping (see Fig. 1). Open rainwater collectors and wet-only rainwater collectors equipped with a automatic lid which only opened when it was raining were used for sampling. Water was collected once in two weeks from the sampler and was stored in polyethylene bottles at −30°C.

For water analysis 20 ml of buffer solution were added to 200 ml sample and if necessary the pH was adjusted to pH 5.00 ± 0.05 with a few drops of sodium hydroxide or acetic acid solution. The sample was transferred to a 250 ml liquid/liquid extractor, and 10 ml of pentane and 1 ml of DDTC solution were added. The sample was extracted twice with 10 ml of pentane (shaking for 3 min, phase separation for 7 min). The organic extract was dried over sodium sulphate and then reduced in volume to 1 ml by a gentle stream of nitrogen and subjected to derivatization.

Suspended matter was sampled with a Pennwalt continuous flow centrifuge at October 19th 1992. The centrifuge was operated at an inlet flow of 1000 l/h. After two hours the precipitate was collected and stored at −30°C. Approximately 3 g of suspended matter were dried with 15 g of anhydrous sodium sulphate (the water content was determined separately by drying at 105°C). The dry powder was extracted for 9 h by means of soxhlet extraction using 110 ml hexane–acetone (9:1). In this way only the trisubstituted (non-polar) compounds can be recovered because no complexing agents can be used with soxhlet extraction. The extract was dried over sodium sulphate and concentrated to 1 ml and subjected to derivatization.

The derivatization reaction was carried out in stoppered 15 ml reaction tubes. Methyl Grignard (1 ml) was added and the reaction was allowed to proceed for 30 min while shaking manually every 10 min. To stop the reaction 3 ml of hexane were

TABLE 1

Detection limits (as tin, $S/N = 3$) and recoveries of spikes added to water and suspended matter (detection was by GC–ITD)

Compound	Quantitation ion	Detection limits			Recoveries [a]	
		Standards (pg)	Water (ng/l)	Susp.m. (ng/g)	Water (%) ($n = 2$)	Susp.m. (%) ($n = 5$)
MBT	165	3	30	–	[b]	–
DBT	205	5	40	–	[b]	–
TBT	249	8	20	10	48 ± 2	44 ± 20
MPT	227	2	4	–	6 ± 6	–
DPT	289	1	2	–	25 ± 12	–
TPT	351	0.7	1	2	61 ± 3	80 ± 3
DCT	233	3	9	–	19 ± 27	–
TCT	301	3	9	3	55 ± 9	85 ± 11
FBT	401	3	2	2	123 ± 4	94 ± 5

[a] Recoveries were calculated for spikes added at the 0.6 μg/l (water) and 0.4 μg/g (suspended matter) level. [b] Recoveries of MBT and DBT could not be calculated reliably due to high background concentrations in the spiked sample.

added and the sample was cooled in ice. Subsequently 7 ml 1 M H_2SO_4 solution were added to destroy the Grignard reagent and dissolve the formed magnesium salt. Clean-up was done by sorption of the sample on 5 g activated basic alumina and elution with 15 ml of hexane–diethyl ether (8:2). The eluates were concentrated to an accurately known volume of approx. 1 ml of which 7.5 μl were injected in the gas chromatograph.

Apparatus

GC was carried out on a Varian 3400 gas chromatograph coupled to a Varian Saturn II ion trap detector (ITD). 7.5 μl on-column injections were done by means of a 8100 automatic liquid sampler (Varian, Walnut Creek, CA). A retention gap of 2 m × 0.53 mm i.d. deactivated fused silica was used (Chrompack, Middelburg, Netherlands). The analytical column was a 30 m × 0.2 mm i.d. SPB-1 column (film thickness, 0.2 μm) from Supelco (Bellefonte, PA, USA). The linear helium carrier gas flow was 40 cm/s (at 60°C). The temperature programme was: from 60°C (1.5 min) at 15°C/min to 285°C (10 min).

RESULTS AND DISCUSSION

ITD operation

Electron impact mass spectra of several alkylated OTs have been published previously [3–6]. The best signal-to-noise ratio was obtained by quantifying OTs at the fragment resulting after the loss of one organic chain from the molecular ion [7]. Quantitation masses are included in Table 1. Because for quantitation only masses above 150 were used, sensitivity was optimized for these masses. To minimize overhead time only two of the four scan-segments were used (set at 146–275, 276–412 amu). During GC analysis the mass range 155–355 a.m.u. was scanned and was switched to 395–412 amu after 22 min to allow for the detection of FBT-Me.

In ion trap mass spectrometry high concentrations of ions in the trap should be avoided because they influence each others trajectories (space charge effects) [6], which leads to bad mass spectral resolution. TPT and FBT spectra appeared to be the most susceptible to distortion by this effect. For this reason the amount of ions in segment 276–412 was optimized (via the reduction of the automatic gain control parameter with 50%), which resulted in good spectra up to amounts of 1 ng injected, while retaining high sensitivity for the less susceptible compounds, that have their quantitation fragments in the second segment.

OTs in freshwater and suspended matter

Detection limits and recovery values were assessed for the matrices investigated (see Table 1). Recovery values for mono- and di-substituted OTs are not satisfactory and need further study. For TPT detection limits, and recoveries were satis-

TABLE 2

OT concentrations (as tin) in freshwater and in suspended matter, collected at three sampling locations in the Flevoland polder, with calculated sorption coefficients (K_d) for TPT

Location	Freshwater TPT (ng/l)				Susp. matter [b]		K_d TPT (l/kg)
	June	July	Aug.	Sept.	TPT (ng/g)	TBT (ng/g)	
CLV	1.8 ± 0.4	1.1 ± 1.6	1.7 ± 0.3	4.3 ± 0.7	90	35	21 000–26 000
CHV	< 1	2.2 ± 0.2	4.3 ± 0.2	3.5 ± 1.6	140	100	40 000–60 000
LHV	3.3 ± 0.7	3.1 ± 0.2	3.9 ± 1.0	3.7 ± 0.8	105	30	28 000–30 000

[a] Water samples were analyzed in duplicate. Results are corrected for recovery 61 ± 3% ($n = 2$) for TPT. All other OT concentrations were below the detection limits, MBT and DBT detection limits were increased to 50–100 ng/l due to blank levels caused by the polyethylene storage bottles. [b] Results corrected for recoveries, 80 ± 3% ($n = 5$) for TPT and 44 ± 20% ($n = 5$) for TBT.

factory. TPT concentrations in water varied little during the sampling period (Table 2). Only a very slight increase in TPT concentrations was found from the beginning of the spraying season until the end. This minor increase indicates that direct run off of TPT is very little and that the TPT water concentration is mainly the result of slow desorption processes from soil and sediment. The proposed environmental quality objectives of 10 ng/l [8] for TPT are not exceeded. However, one has to bear in mind that these samples are average values for one month and (much) higher peak values may have occurred. Other OTs were below the detection limits given in Table 1. (Blank values arising from the polyethylene storage bottles prevented MBT and DBT determination below a level of 100 ng/l.) In two out of three water samples taken in may 1992 (before the beginning of the spraying season) TPT could also be detected at levels of 1.9 ± 0.2 ng/l (CLV) and 3.3 ± 0.9 ng/l (CHV), while at location CHV TPT was below the detection limit. This TPT probably arises from the spraying season of the year before and supports the assumption of slow release of TPT from soil and sediment sinks.

Suspended matter was sampled on October 19th after the spraying season when the highest TPT concentrations are expected. Suspended matter concentrations range from 90–140 ng/g for TPT and from 30–100 ng/g TBT (Table 2). These values are well above the proposed environmental quality objective of 1 ng/g for TPT and 1.5 ng/g for TBT.

Indicative sorption coefficients (K_d) for real field samples were calculated for TPT, by dividing the TPT concentration in the suspended matter by the concentration in water. For the water concentration the values obtained for the month September were used assuming that water concentrations in october were approximately the same. For the water analysis non-filtered water was used, while for reliable K_d calculation filtered water should be used. In this case the error is only small; using the known suspended matter concentration in the water and the TPT concentration in the suspended matter an estimation of the maximum error was made (Table 2). K_d values range from 21 000–60 000 l/kg. Literature data on sorption coefficients for TPT is still rather scarce. Loch et al. [9] reported K_d values for TPT of 500–2000 for sandy soils containing only 1–2% organic matter. The higher results obtained in the present study can be explained by the much higher organic content of suspended matter (approx. 60–80%). Fent and Hunn [10] estimated field K_d values for TBT using suspended matter and found values of 1500–27 000. These K_d values for TBT are in the same range as our results and thus seem to support the values found by us for TPT.

An estimation was made of the amount of TPT being expelled from the polder in the month September by means of the pumping-engines. The average use of TPT was estimated to be 24 kg km^{-2} $month^{-1}$ during the spraying season from June 1st till September 1st [11]. The consumption potato crop area was 144 km^2 [12] which gives an estimated use of 3500 kg TPT $month^{-1}$ (as tin).

The load of TPT being pumped out of the polder was calculated for the three sampling points using Eqns. 1 and 2.

$$\text{load TPT}_{(solution)} = \text{conc.TPT}_{(solution)} \times \text{volume} \quad (1)$$

$$\text{loadTPT}_{(sorbed)} = \text{conc.TPT}_{(sorbed)} \times \text{conc.susp.m.} \times \text{volume} \quad (2)$$

Where load $TPT_{(solution)}$ and conc. $TPT_{(solution)}$ is the transported amount and concentration, respectively, of solved TPT, load $TPT_{(sorbed)}$ and conc. $TPT_{(sorbed)}$ is the transported amount and the concentration, respectively, of TPT associated with suspended matter. Volume is the water volume pumped out during the month September and conc.susp.m. is the concentration of suspended matter in the water. Maximum and minimum estimates were made in the same way as for the K_d calculations. After correction for the non-monitored pumping-stations (assuming similar TPT load in the water), it was calculated that 170–190 g TPT (as tin) was pumped out of the polder during the month September. A conclusion at this point is that the transport of TPT via the pumping-engines is negligible compared to the amounts used.

TABLE 3

TPT concentrations (as tin) in rainwater from open rainwater collectors and wet-only rainwater collectors. Corrected for recovery (61 ± 3%, $n = 2$)

Location	Type of rainwater collector	Sampling date	TPT (ng/l)
Hillegom	open	29/7–12/8	5–8
Hillegom	open	12/8–26/8	9
Hillegom	wet only	12/8–26/8	10
Naaldwijk	open	12/8–26/8	9
Naaldwijk	wet only	12/8–26/8	16
Nieuwerkerk a/d IJssel	wet only	12/8–26/8	8

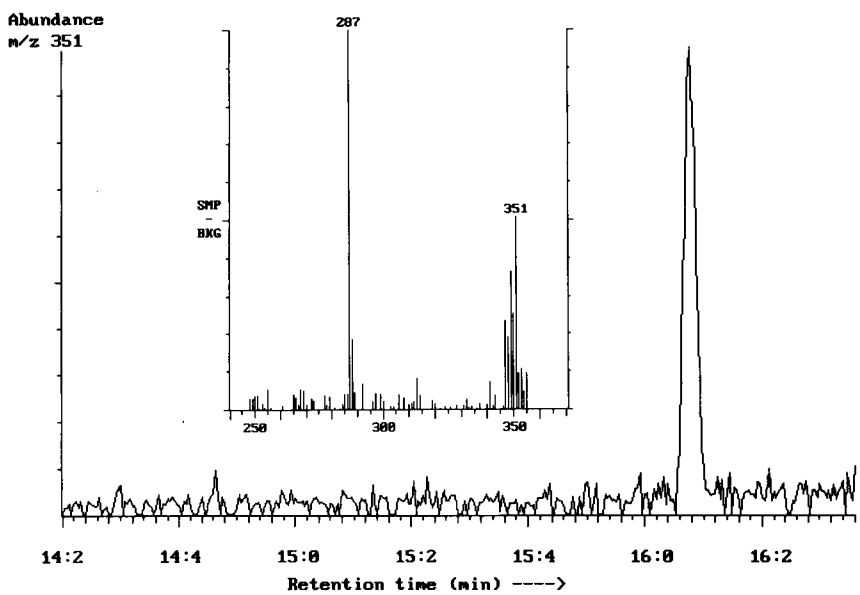
Rainwater samples

Results from rainwater analysis are given in Table 3. In Fig. 2 a chromatogram obtained from a rainwater sample is given. TPT is present at a concentration of 8 ng/l (as tin) and the spectrum obtained gives a good identification of the presence of the OT. In all samples TPT was found at concentrations above the detection limit. Because these samples were taken at distances of at least 20 km from potato cropping areas much higher levels can be expected in rainwater collected more close to the sources. No clear difference in TPT concentrations is found between open rainwater collectors and wet-only rainwater collectors, indicating that wet deposition is the most important for TPT deposition. More research is needed as these few samples do not allow final conclusions. Anyhow it is clear that rainwater concentrations support the predicted volatilization of TPT from potato crop.

Conclusions

Month-average freshwater samples showed TPT concentrations below the proposed environmental quality objective of 10 ng/l. In suspended matter TPT levels ranged from 90–140 ng/g and TBT levels ranged from 30–100 ng/g, which is well above the proposed environmental quality objectives of 1.5 ng/g and 1 ng/g respectively. K_d estimates for TPT gave values of 21 000–60 000 l kg^{-1}.

Calculations revealed that only a negligible portion of the TPT sprayed leaves the polder by means of the pumping-engines. Preliminary anal-



ysis of OTs in rainwater at remote locations showed detectable TPT concentrations supporting modelling studies that indicate that volatilization is an important transport route for TPT.

This work is supported by a grant from the University Stimulation Fund of the Free University (USF Grant 89–27). The Institute for Inland Water Management and Waste Water Treatment (RIZA; Lelystad, Netherlands) is acknowledged for their support and for providing water and suspended matter samples. Province Zuid-Holland is thanked for providing rainwater samples.

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Analytica Chimica Acta, 286 (1994) 343–355
Elsevier Science B.V., Amsterdam

Interferences generated by organic and inorganic compounds during organotin speciation using hydride generation coupled with cryogenic trapping, gas chromatographic separation and detection by atomic absorption spectrometry

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(Received 17th February 1993; revised manuscript received 7th September 1993)

Abstract

Hydride generation reactions are frequently used for the speciation analysis of organotin compounds in a wide variety of matrices. However, the organotin determination procedure may be altered by different interferences mechanisms. This work evaluates the interferences likely to occur in the determination of tributyltin in a harbour sediment candidate reference material for the EEC Community Bureau of Reference with the method of hydride generation combined on-line with cryogenic trapping, gas chromatographic separation and detection by atomic absorption spectrometry. First, a full geochemical characterization of the sediment and of its acetic acid leachate was achieved to evaluate interfering compounds that may inhibit the determination of TBT by hydride generation in this sediment. Second, interference studies on various organic and inorganic compounds likely to alter the overall organotin determination were carried out with simple model solutions. These model solutions were spiked successively with different possible interfering compounds mixed with the organotins under study, i.e., monomethyltin (MMT), monobutyltin (MBT), dibutyltin (DBT) and tributyltin (TBT) in a mixed solution at concentrations of 5 ng as Sn of each compound in 50 ml of water. Organic interferents studied were organic pollutants (organic solvents, polychlorinated biphenyls, pesticides, *n*-alkanes), humic substances and a complexing agent, EDTA. The inorganic interferents were represented by a mixture of fourteen inorganic metals. The addition of organic compounds to the hydride generation reaction had little effect on the signal suppression for all organotin species. Reproducibility was

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SSDI 0003-2670(93)E0512-6

significantly affected only by the presence of humic substances in solution. Signal suppression was observed only for the monoalkyltins (MMT and MBT) in the presence of EDTA. Solutions of mixed inorganic metals were found to be highly efficient in inducing severe signal suppression for all organotin compounds at a level of 100 μg of each element. Interference mechanisms during the speciation determination step are outlined and discussed.

Keywords: Atomic absorption spectrometry; Gas chromatography; Hydride generation; Interferences; Organotin compounds; Sediments

After its introduction by Chau et al. [1] in 1975 for the determination of methylated selenium compounds in the environment, the coupling of hydride generation (HG) combined with cryogenic trapping (CT), gas chromatographic (GC) separation and detection by atomic absorption spectrometry (AAS) (HG–CT–GC–AAS) has extensively been applied to speciation analysis. This technique has been used for the determination of chemical species of elements such as As, Se and Sb and also organic forms of Pb, and most often for organotin compounds [2]. The hydride generation method generally provides good sensitivity when compared with conventional nebulizer sample introduction systems [3]. This is mainly related to the separation of the analyte from the matrix and total transfer of the gaseous sample to the detector. However, in some instances this method has been reported to suffer from inhibition when applied to environmental samples, and most specifically with complex matrices [4]. Speciation analysis of organotins in sediments is becoming increasingly important as some of these compounds are highly toxic to marine life (e.g., trialkylated forms: tributyltin originating from antifouling paints). As a result, a wide variety of techniques have been developed in the last decade for the determination of organotins. Hydride generation-based techniques have proved to be generally successful [5].

Most techniques rely on the combination of several analytical procedures. When using multistep techniques such as HG–CT–GC–AAS for butyltin determination in environmental samples, different problems may occur at each stage of the analytical procedure: extraction, derivatization (hydride generation or ethylation), cryogenic trapping–separation or detection. Further, the extraction procedure prior to the determination is a critical point when dealing with complex sediment matrices. Acidic digestion procedures are usually recommended when the analytical protocol uses hydride generation, as the hydridization step is only highly efficient in aqueous media. These procedures have also been recommended in the presence of high organic contents in sediment matrices. Zhang et al. [6] evaluated butyltin extraction recoveries from a reference material (PACS-1) by several published procedures. Dibutyltin (DBT) and tributyltin (TBT) were efficiently extracted in an acidic extraction medium. However, none of the methods tested using either organic solvents or acid leaching gave satisfactory recoveries for monobutyltin.

Several problems may arise during the overall analytical procedure. Derivatization of organotins with sodium tetrahydroborate is likely to be inhibited in the presence of complex matrices, as evidenced by Valkirs et al. [4]. The presence of diesel fuel in sea water (0.4 mg g^{-1}) may inhibit the determination of volatile tin hydrides (1 μ l^{-1} of TBT) by HG–GC–AAS. Similar results were observed for the determination of TBT in sediments in the presence of increasing gasoline concentrations in the sediment extract [7]. However, Desauziers et al. [7] demonstrated that increasing amounts of $NaBH_4$ added to the same sediment acetic acid extract could limit these interferences. Sulphur compounds and pigments have also been suspected to produce some effects during the hydride generation step [8].

Interferences in hydride generation and atomic spectrometric detection have mostly been studied with respect to total tin determination [9–13]. Some workers have studied the effects of several cations and anions on the hydride reaction [14–16]. Dedina [10] suggested that interferences can either take place in the liquid phase (during the hydride generation reaction) or later when the analytes are in the gaseous state (alteration of the

atomization processes). However, these interference mechanisms are still not perfectly understood. The formation of tin hydrides is severely affected when these interfering elements are mixed with other hydride-forming elements, particularly when the latter are in excess in comparison with the analyte. Their occurrence in the liquid phase can prevent the hydride generation reaction and hence the conversion of the analytes into a volatile form. These interfering species may induce competitive reactions for the formation of the volatile species or the formation of insoluble complexes between interfering compounds and analytes.

Interferences do not necessarily take place only during the liquid reaction step. Once in the gaseous state, interferences may also occur by reaction with the volatile forms of interferents simultaneously produced by the addition of $NaBH_4$. These volatile interferents may alter the desorption and/or transport efficiencies of analytes from the hydride generator to the atomic spectrometer and also prevent the total atomization of the analytes. These effects may also take place indirectly via surface-mediated procedures after deterioration of the surface of the apparatus and specifically on the quartz cell surface of the atomizer. The heated quartz tube atomizer gives good sensitivity for tin hydrides, but the surface of the tube may deteriorate and decrease the response [17]. Full recovery of the signal can be achieved after the use of a silanizing agent to prevent the adsorption of tin on the quartz tube surface [18,19].

Providing TBT certified reference material in sediment is now a major challenge owing to the high toxicity of this compound and its ubiquity in this type of matrix. Different round-robin exercises between expert laboratories in the EEC have been performed in order to produce such material. The scatter of the results obtained in some cases and the sources of errors have been discussed during several interlaboratory trials organized by the Community Bureau of Reference (BCR) on TBT harbour sediment sample. On one particular sample, dispersion of the results highlighted the important analytical difficulties associated with the determination of TBT when using derivatization procedures such as hydride generation or ethylation in the presence of large amounts of organic compounds [20].

The aim of this work was to investigate possible sources of interferences associated with the determination of organotins in a harbour sediment matrix using hydride generation as the derivatization technique. First the geochemical composition of the candidate reference material with reference to its total metals and organic matter content was extensively characterized. Second, since for most of the methods used organotin compounds were extracted from the sediment matrix by an acetic acid leaching procedure, the occurrence of the most likely interfering compounds in the leachate was also evaluated. Finally, the effects of different organic and inorganic compounds on the hydride generation procedure for organotins were evaluated. These interference studies were performed on simple model solutions. Individual or mixed interfering compounds were directly added to the reaction flask with the analytes prior to the standard analytical procedure. Interferences observed can then take place either in the liquid phase or in the gaseous phase of the determination procedure if interferents are simultaneously volatilized by the addition of $NaBH_4$.

The effects of organic pollutants [organic solvents, polychlorinated biphenyls (PCBs), pesticides and *n*-alkanes] on organotin speciation were individually investigated. The impact of organic ligands, humic and fulvic acids and a complexing agent (EDTA) was also evaluated. Finally, the interferences generated by the presence of inorganic species were studied with a mixed solution of metals.

Interferences have been evaluated for a mixture of four organotins [monomethyltin (MMT), monobutyltin (MBT), dibutyltin (DBT) and tributyltin (TBT)] at concentrations of 5 ng as Sn for each compound in 50 ml of water (100 ng l^{-1}). These concentrations are low and representative of the levels obtained in some sediment leachates.

EXPERIMENTAL

Apparatus

Organotin speciation analyses were performed by the technique of hydride generation, cryogenic trapping, gas chromatographic separation and detection by atomic absorption spectrometry [21]. The hydrides were generated in acidic medium (pH 2.5) using sodium tetrahydroborate solution (50 g l^{-1}) in a glass reaction flask. The glass column was packed with Chromosorb W HP (60–80 mesh) coated with 10% Supelco SP2100. The column has been silanized with pure hexamethyldisilazane prior to use. Helium was used as carrier gas and its flow-rate was 300 ml min^{-1} for both purging and the subsequent desorption step. Heating of the column was performed with an electrical power supply through a Nichrome wire.

Detection was achieved with a PE 5000 atomic absorption spectrometer fitted with an electrically heated quartz furnace. The addition of hydrogen and oxygen (flow-rates 150 and 20 ml min^{-1}, respectively) was used to improve the atomization efficiency. The wavelength selected for detection was 224.6 nm, supplied by a tin electrodeless discharge lamp and gated through a 0.2-nm slit. The signal was recorded on an Intersmat integrator.

Reagents and calibrants

Individual stock standard solutions of organotin calibrants (MMT, MBT, DBT and TBT) (Ventron) were prepared in methanol (Merck, spectroscopy grade) in sealed flasks to avoid evaporation. Mixed working calibrant solutions of 100 ng ml^{-1} as Sn were prepared daily in pure acetic acid and obtained from the stock standard solution (250 μg ml^{-1} as Sn). All dilutions were performed on a mass basis. All solutions were stored at 4°C in the dark. A solution of $NaBH_4$ (Fluka) was prepared in water purified with a Milli-Q system (Millipore) every 3–4 h at a final concentration of 50 g l^{-1}. The acetic acid used for extraction, acidification prior to reaction and dilutions of calibration solutions was purchased from Merck (100%, analytical-reagent grade). A multi-element standard solution used as inorganic interferent mixture (Al, As, Cd, Co, Cr, Cu, Fe, K, Mg, Mn, Na, Ni, Pb and Zn at 100 μg ml^{-1} of each element) came from Spex Analytical Division. Humic substances were purchased from IHSS (Suwanee River). Fulvic acids and humic acids were diluted in Milli-Q-purified water to final concentrations of 26.8 and 26.3 mg l^{-1}, respectively, and used individually. A solution of the complexing agent EDTA was prepared in Milli-Q-purified water at a concentration of 2.5×10^{-3} mol l^{-1}. Other organic interfering compound solutions were prepared in methanol (Merck, spectroscopy grade). Their concentrations in the individual solutions were adjusted to give the following concentrations: pesticide (carbaryl, 99.5%), 502.5 μg ml^{-1}; PCB (2,2′,4,4′,5,5′-hexachlorobiphenyl), 369.2 μg ml^{-1}; pyrene working solution, 471.2 ng ml^{-1} (after successive dilutions from solutions of 553 and 10.3 μg ml^{-1}); *n*-hexadecane (*n*-C_{16}) was diluted in methanol–diethyl ether (89:11) because it is not soluble in pure methanol at this concentration, the final concentration being 13.8 mg ml^{-1}. The organotin calibrants and the interfering compounds were added to the solution with a motorized microlitre pipette (Rainin).

Sample collection and storage of the harbour sediment

The sediment studied was collected in the Sado estuary (Portugal) in 1989, air dried, stored in the dark, oven dried at 80°C, homogenized and bottled in the dark in glass bottles at BCR facilities [22].

Organotin speciation analysis in sediment

General procedure for the speciation analysis of organotin compounds. The following steps are used for the determination of organotins using hydride generation. Organotins are added to 50 ml of Milli-Q-purified water acidified to pH 2.5 with 2 ml of acetic acid. The volatile hydrides are generated by reaction with 10 ml of $NaBH_4$ at 50 g l^{-1}. This total amount of reductant has previously been shown to be efficient in overcoming some interferences in sediment matrices [7]. The hydrides are then flushed by a flow of Helium (300 ml min^{-1}) from the reaction flask. They are then trapped in the chromatographic column im-

mersed in liquid nitrogen. The species are separated after warming of the column on the basis of their boiling points and the chromatographic properties of the column. They are then swept by the carrier gas into the heated quartz cell aligned in the beam of the atomic absorption spectrometer, where they are atomized and detected. Quantification is based on peak areas.

Blanks are run periodically (every two analyses) and all analyses are performed in duplicate during the whole study.

The analytical performance of the technique is as follows: the linear range for calibration extends from 1 to 100 ng as Sn for a sample volume of 50 ml; the detection limits are 120 pg of TBT as Sn and based on 3σ of the background noise and with a sample volume of 50 ml; and the reproducibility for all organotin species is 10%.

Sediment extraction procedure for organotin speciation analysis. Organotin compounds in sediment are extracted as follows: 1–2 g of dry sediment are mixed with 20 ml of pure acetic acid in capped Pyrex centrifugation tubes and stirred with a magnetic stirrer for 12 h at room temperature in the dark. After digestion, the mixture is centrifuged at 4000 rpm for 10 min and the supernatant is collected in clean glass flask. Portions of 2 ml of the sediment extract are then directly injected into the reaction flask and analysed [8].

RESULTS AND DISCUSSION

Evaluation of TBT inhibition in candidate reference harbour sediment sample

Previous results have shown that sediment extracts from the candidate reference harbour sediment may strongly inhibit the TBT signal during determination [8]. In order to evaluate the magnitude of inhibition, the quantitative recovery of a constant amount of TBT (50 ng as Sn) in the presence of increasing volumes of the harbour sediment extract was measured. The sediment leachate used for spiking was obtained after digestion of 1 g of sample in 20 ml of CH_3COOH as previously described. Increasing volumes (0.25–2 ml) of this sediment extract were successively and individually added in the reaction flask with 50 ng of Sn as TBT. The TBT (50 ng as Sn) determinations in the presence of different volumes of sediment extract in the reaction flask were performed under the usual analytical conditions. Results for inhibition of the TBT signal in the presence of increasing volumes of sediment leachate are presented in Table 1. They show that the relative sensitivity of the TBT signal decreases with increasing amounts of extract. In the presence of 2 ml of the sediment leachate, only 55% of the signal of TBT is recovered. These results illustrate that this sediment extract contains interfering agents that can later seriously inhibit the hydride generation of TBT calibrant, even in the presence of high levels of TBT spikes.

TABLE 1

TBT recoveries (50 ng as Sn) in the presence of increasing volume of the acetic acid extract of the candidate reference material

Volume added (ml)	Recovery (%) [a]
0	100
0.25	94 ± 24
0.5	80 ± 22
0.75	93 ± 14
1.0	87 ± 11
1.5	73 ± 40
2.0	55 ± 23

[a] Mean recoveries ± R.S.D. (%) ($n = 2$).

Harbour sediment characterization

In order to establish the origin and the mechanisms of interferences likely to take place during hydride generation on the sediment extract, a full geochemical characterization of this sediment was performed. This study was first focussed on the total content of the sediment, but also concerned the acetic acid leachate from this sediment.

Geochemical composition of the harbour sediment

Organic carbon content. The organic carbon content was determined using a LECO analyser for sediments with the Strickland and Parsons method as modified by Etcheber [23]. The concentration obtained was 41 mg g^{-1}, which can be

TABLE 2

Concentrations of PAHs (ng g^{-1} dry sediment) in Sado sediment and in other sediments (from [24]).

Compound (ng g^{-1} dry weight)	Harbour sediment (this study) [a]	Saudafjord (Norway)		Buzzards Bay (Massachussets)		Caseo Bay (Maine)	
		Pristine area	Contaminated area	Pristine area	Contaminated area	Pristine area	Contaminated area
Pyrene	139 ± 6	6	6899	100	960	38	1680
Benzo[*a*]anthracene	110 ± 7	21	6415	41	330	62	1325
Benzo[*b*]naphtho(2,1-2)thiophene	266 ± 14	nd [b]	nd	nd	nd	nd	nd
Benzo[*e*]pyrene	149 ± 9	14	11387	– [c]	–	–	–
Benzo[*b*]fluoranthene	52 ± 5	22	8212	–	–	–	2845
Benzo[*k*]fluoranthene	43 ± 3	6.5	2935	–	–	–	–
Benzo[*a*]pyrene	73 ± 1	16	7725	75	370	14	805
Indeno[1,2,3-*cd*]pyrene	61 ± 3	–	–	–	–	24	505

[a] Mean results ± S.D. ($n = 5$). [b] nd = Not determined. [c] Dashes indicate not detected.

considered as relatively elevated for this type of sediment. Part of this high organic matter content can probably be extracted by the acetic acid leaching procedure and later generate interferences during the organotin determination.

Polycyclic aromatic hydrocarbon (PAH) determination. Special attention was paid to the PAH content of the sediment as PAHs are suspected to inhibit the hydride generation of organotins [4,7]. The specific determination of PAHs was done by liquid chromatography (LC) with fluorescence detection. The extraction was performed after a Soxhlet extraction procedure on 10 g of sediment [24]. The extract was purified on a Florisil microcolumn. Separation was effected by reversed-phase LC with fluorescence detection. Results of the PAH determination in this sediment and data reported from other sites are compared in Table 2. The variety of compounds detected and their levels measured in the candidate reference material suggest that this sediment is not seriously contaminated by PAHs. The results also suggest that the interferences observed may not be induced by the occurrence of this specific class of compounds

Major and trace element contents. Major and trace elements were determined on the dry sediment by x-ray fluorescence spectrometry with a Philips Model 1400/1510 apparatus. The results are given in Table 3. The total metals content of CRM 277 is also reported for comparison. CRM 277 was collected in the Belgian part of the Scheldt estuary. It is known to be seriously contaminated by effluents from chemical and metallurgical industries in the Scheldt estuary. Metal concentrations detected in the candidate reference material are high in general and within the range of those detected in the contaminated CRM 277.

TABLE 3

Metal concentrations ($\mu g\ g^{-1}$ dry sediment) in the candidate reference harbour sediment and in CRM 277 (Scheldt estuary)

Metal	CRM 277: total metal content	Candidate reference harbour sediment	
		Total metal content	Metal content of CH_3COOH leachate
Al	nd [b]	13.7 as Al_2O_3	nd
Cr	192	150	48
Mn	nd	0.042 as MnO	15
Fe	nd	5.9 as Fe_2O_3	nd
Co	nd	3.9	3
Ni	43.4	66.1	5
Cu	101.7	60.8	5
Zn	547	439.5	37
Cd	11.9	nd	nd
Pb	146	122.2	5
As	47.3	21.6	0.6
Se	2.04	nd	0.4
Sn	nd	0.1	nd
Sb	nd	nd	0.0235

[a] Total metal concentrations were measured by x-ray fluorescence spectrometry. Metal concentrations in the CH_3COOH leachate were determined by ICP-MS. [b] nd = Not determined.

Characteristics of the acetic acid extract

Acetic acid is preferred for extracting organotin compounds from the sediment matrix [6]. However, other compounds may be co-extracted with the organotins from the sediment matrix and interfere during the hydride generation steps. Therefore, the presence of some compounds in the acetic acid extract of this harbour sediment likely to generate interferences was investigated.

Extracted organic matter characteristics. The characterization of the organic matter present in the acetic acid extract was determined after a liquid–liquid extraction in cyclohexane of the sediment acetic acid leachate. This acetic acid leachate was obtained using the procedure previously described and on 1 g of sediment mixed with 20 ml of acetic acid. Analyses of this extractable organic matter were performed by LC with UV detection and by molecular fluorescence spectrometry.

Determination of aromatic compounds. The presence of aromatic compounds in the cyclohexane liquid–liquid extract from the sediment acetic acid leachate was investigated. This determination was performed by LC with UV detection at 254 nm. The column used was amino-bonded Spherisorb (5 μm) (25 cm × 4.6 mm i.d.). The mobile phase was pentane at a flow-rate of 1 ml min^{-1}. Since aromatic compounds have a much higher molar absorptivity than most of the other compounds likely to absorb at this wavelength, the peaks recorded with this method could then mostly be attributed to aromatic species. A chromatogram of the extract is presented in Fig. 1. The levels detected were not high in comparison with the results obtained previously on the total sediment with Soxhlet extraction and also in comparison with other environmental sediment samples. The predominance of monoaromatic compounds may be favoured by the successive extraction procedures performed on the sediment. These low levels of polyaromatics detected in the sediment leachate support the fact that PAHs are certainly not likely to generate important interferences in the organotin determination process.

Fluorescent organic matter. The PAHs and part of the organic matter content present in the solvent extract are fluorescent material. This fluorescent fraction of the extract was also characterized by fluorescence spectrometry. Fluorescence spectra of the cyclohexane extract were recorded on a Spex Fluorolog 212 with a double monochromator. The most appropriate excitation wavelengths were determined after recording the corresponding absorption spectra. Spectra were corrected for the instrumental response and the

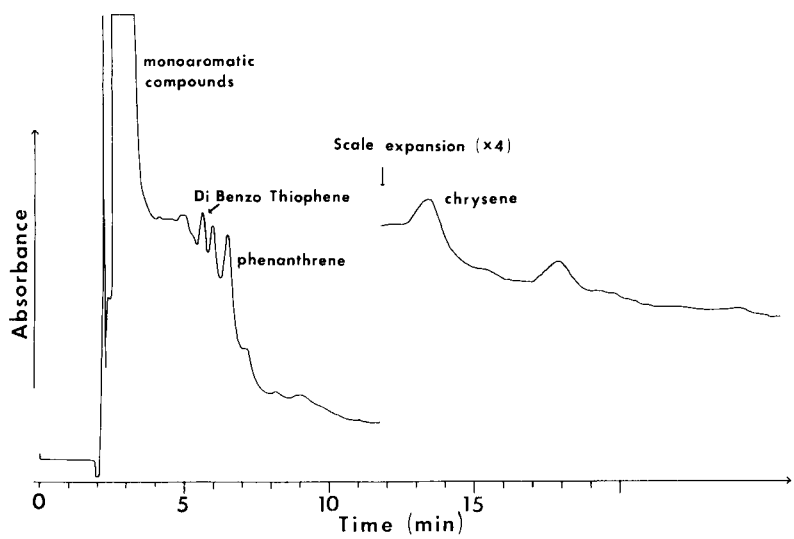

Fig. 1. HPLC–UV of the aromatic compound content in the cyclohexane extract after a liquid-liquid extraction of the acetic acid extract of the candidate reference sediment.

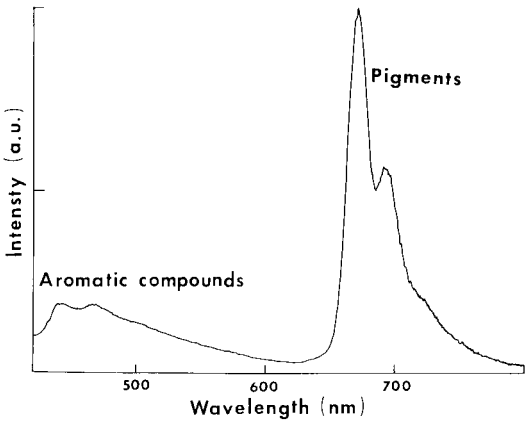

Fig. 2. Corrected emission spectrum [λ(ex) = 410 nm] of the cyclohexane extract after a liquid–liquid extraction of the acetic acid extract of the sediment.

blanks subtracted. A typical fluorescence spectrum of the extract is presented in Fig. 2. The most predominant bands observed can then be attributed to pigments. These results are not surprising as acetone is commonly used to extract pigments from sediments. It is also likely that acetic acid extracts humic material. However, as these substances are not soluble in cyclohexane, we did not pay particular attention to them.

Metal content. The total metal contents in the acetic acid extract were determined by inductively coupled plasma mass spectrometry (ICP-MS) (Perkin-Elmer, Elan 5000). The results are presented in Table 3 and clearly indicate that acetic acid does not efficiently extract metals from this sediment. Usually, the acetic acid leaching procedure is a standard step in sequential extraction procedures. The results obtained here are in agreement with those obtained using a sequential extraction scheme [25]. Typically, less than 10% of the total metal content is recovered with this extraction step.

Interferences associated with organotin speciation analysis

After the full characterization of the sediment, different sets of organotin determinations were carried out on model solutions with individual spikes of compounds likely to interfere during the determination steps.

Interference study design

For all interference studies, the interfering compounds were added directly to organotins in simple model solutions in the reaction flask prior to hydride generation. Blanks were also performed under the same conditions. Analyses of calibrants without any interfering compounds were performed periodically to check the overall sensitivity of the instrumentation.

Different classes of interfering compounds were successively tested by addition to the reaction flask in the presence of organotin standards. The compounds selected were individual organic pollutants (organic solvents, PCBs, pesticides, *n*-alkanes), humic material (humic or/and fulvic acids), a complexing agent (EDTA) and finally a mixture of inorganic metals. The concentrations of organic pollutants added to the reaction flask were chosen to match the levels found in sediment extracts originating from very polluted areas. During our extraction procedure, 2 g of dry sediment were typically used for a total extraction volume of 20 ml of CH_3COOH when performing organotin speciation analysis by hydride generation. If one can assume that for such a small amount of sediment, there is a linear extraction efficiency between the sediment volume and the amount of digesting aliquot, then under the previous conditions 2 ml from the sediment extract injected into the reaction flask would be representative of the acetic acid-extractable part originating from 0.2 g of sediment. Therefore, the levels of the organic interfering pollutants were generally based on worst case situations and would correspond to the amount obtained in 0.2 g of sediment.

For humic material, the amount added to the reaction flask was adjusted to natural levels previously recorded in various sediment matrices [26].

Experiments involving EDTA were performed slightly differently. The EDTA concentrations added to the reaction flask were such that the number of moles of EDTA present was a multiple of the total number of moles of organotins in solution.

The interferences induced by the inorganic metals were evaluated by adding increasing concentrations of the multi-elemental solution (from 0.01 to 100 μg of each element) to organotin solutions prior to hydride generation.

The relative sensitivities obtained in the presence of the different possible interferents are expressed as recoveries of the organotins. They were estimated by calculating the ratio between the peak areas of organotin calibrants in the presence of interfering compounds and the peak areas of the same organotin compounds in simple solution after blank subtraction.

Interferences of organic compounds in organotin speciation analysis

Effect of organic pollutants. Results obtained for the alkyltin recoveries in the presence of organic pollutants are presented in Table 4. The general trend shows that these organic compounds do not generate serious inhibition of the signal at the concentrations studied for all organotin species. DBT is the least affected by the presence of these compounds. Both MMT and TBT also present good overall recoveries. The compound presenting the worst recoveries is MBT. This lack of recovery could be attributed to the fact that this compound is the least stable compared with the other organotin species. It may easily degrade to inorganic tin and be converted into SnH_4 after the addition of the tetrahydroborate. This instability could be enhanced by the presence of the organic compounds in the reaction flask.

Further, some interferences detected with PCBs and pesticides may also be related to the presence of methanol, as it was the solvent used for addition of the pollutants spikes. When similar volumes of pure methanol are added in the reaction flask prior to hydride generation, the relative sensitivities measured are sometimes similar to those obtained with addition of PCBs or pesticides. After the addition of $NaBH_4$, the organotin hydrides formed may be decomposed because then the Sn–H bond is very reactive. Also, the instability of the Sn–H bond and therefore of the organotin hydrides is increased in the presence of methanol in solution. The occurrence of three Sn–H bonds with MBT after derivatization increases its instability and results in a decrease in sensitivity [27]. However, MMT, which also contains three Sn–H bonds, is not affected. This observation may be related to the fact that $MeSnH_3$ is more volatile than n-$BuSnH_3$ and is rapidly removed from the liquid phase, preventing its attack by methanol. The presence of methanol can also generate other reactions, leading to a decrease in sensitivity. Organotin hydrides have been reported to combine with methanol and produce non-volatile methoxyorganotins according to the following reaction [28]:

$$R_nSnH_{(4-n)} + (4-n)CH_3OH \rightarrow R_nSn(OCH_3)_{(4-n)} + (4-n)/2H_2 \quad n = 1, 2, 3 \qquad (1)$$

However, with other organic solvents, not miscible in water, interferences may be generated by partitioning of organotin hydrides in the organic phase owing to their higher affinity with most organic solvents.

Effect of humic substances. The results for the recovery of organotins in the presence of humic material are displayed in Table 5. Analytical problems generated here do not relate to a de-

TABLE 4

Recoveries (%) obtained on 5 ng of organotins as Sn for a 50-ml reaction flask in the presence of different concentrations of organic pollutants (average S.D. = 11%)

Organotin	3.7 μg PCB	96 μg PCB	38.4 μg pesticide	27.6 μg n-C_{16}	23.6 ng pyrene	76 μl CH_3OH	261 μl CH_3OH
MMT	97	84	88	110	90	81	80
MBT	106	50	52	100	85	77	41
DBT	103	103	96	98	100	88	109
TBT	98	79	87	84	90	62	87

TABLE 5

Recoveries (%) obtained on 5 ng of organotins as Sn in the presence of humic substances [a] (average S.D. = 22%)

Organotin	0.1 mg HA	2 mg FA	0.1 mg HA + 2 mg FA
MMT	83	83	95
MBT	67	87	84
DBT	98	113	111
TBT	72	107	nd [b]

[a] HA = humic acids; FA = fulvic acids. [b] nd = Not determined.

crease in the recovery of the analyte but rather to a decrease in the reproducibility between analyses. The average reproducibility of organotin analyses in the presence of organic pollutants was 11% and it increased to more than 20% in the presence of humic substances during the hydride generation procedures. These effects may be attributed to the fact that these compounds behave as surfactants, producing considerable foaming during hydride generation. During this foaming step, organotin hydrides may partially resolubilize in the organic phase, being removed for the stripping stage.

Effect of EDTA. EDTA was also directly added to the reaction flask with organotin compounds. Recovery data are presented in Fig. 3. The results show that the addition of EDTA does not affect the DBT and TBT recoveries. A slight effect on their recoveries can only be observed when the ratio between the number of moles of EDTA and of organotin compounds is very large, in the range 100–1000. In contrast, monoalkyltins (MMT and MBT) are severely influenced by the presence of this complexing agent, a signal suppression being observed with a ratio as above of only 10. With a ratio of 50, less than half of the MMT and MBT initially present in the reaction flask are recovered. Compared with DBT and TBT, monoalkylated organotins are more polar, and their smaller size probably facilitates complexation rates. The complexes then formed prevent hydride generation. This observation could explain part of the difficulties encountered with MBT determination in environmental samples.

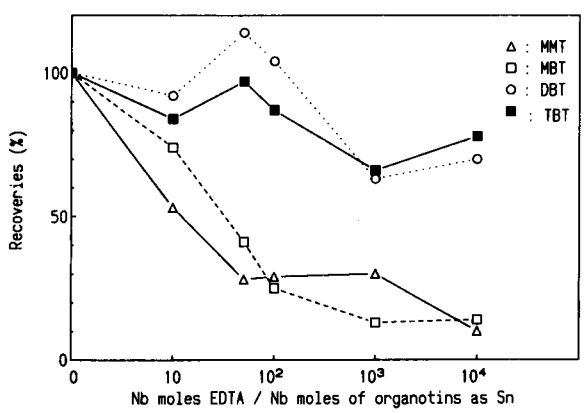

Fig. 3. Influence of EDTA on organotin speciation analysis (5 ng as Sn for each compound) after hydride generation. Standard deviation = 13% ($n = 2$) Δ = MMT; □ = MBT; ○ = DBT; ■ = TBT.

Interferences of inorganic elements in organotin speciation analysis

The effects generated by a mixture of fourteen metals on organotin speciation analysis were also evaluated as metals and more specifically hydride-forming elements are well known interferents in the hydride generation of tin [9]. The multi-elemental solution used contained a mixture of Al, As, Cd, Co, Cr, Cu, Fe, K, Mg, Mn, Na, Ni, Pb and Zn at concentrations of 100 μg ml^{-1} for each element. Some of these metals may form hydrides after the addition of $NaBH_4$. Increasing amounts (0.01, 0.1, 1, 10 and 100 μg of each metal of the multi-elemental solution) were successively added to an organotin compound solution in the reaction flask prior to hydride generation. Results obtained for the recoveries are shown in Fig. 4. MMT is not affected even with the highest concentrations of the mixed metal solution. In contrast, the total yield for butyltin compounds (MBT, DBT and TBT) starts to decrease significantly above a level of 1 μg of the mixed solution. The organotin signal can be totally suppressed for DBT and TBT with 100 μg of the mixed inorganic solution. A first hypothesis for this important decrease in the recovery for DBT and TBT could be associated with the differential kinetic rate of hydride generation. Several of the inorganic metals present in this solution can form hydrides under these conditions

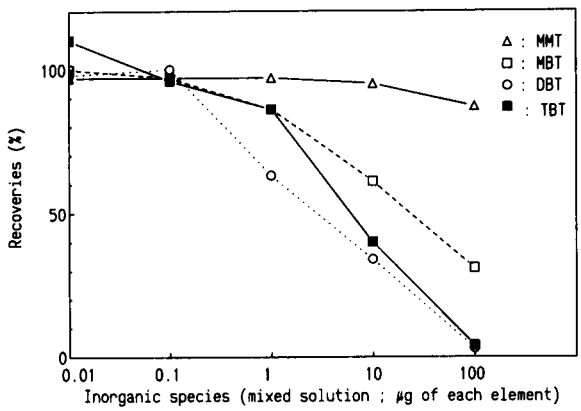

Fig. 4. Influence of multi-elemental solution (mixed solution of inorganic species Al, As, Cd, Co, Cr, Cu, Fe, K, Mg, Mn, Na, Ni, Pb and Zn) on organotin speciation analysis (5 ng as Sn for each compound) after hydride generation. Symbols as in Fig. 3.

[29]. The kinetics of hydride formation with these elements are probably faster than for the organotin compounds. When the interferents are present at high concentrations in the reaction flask, a considerable consumption of the $NaBH_4$ added may take place there via reaction with the inorganic species overriding the hydridization reaction of the organotins. A second hypothesis may be linked to reduction reactions taking place with the interferents in solution after the addition of $NaBH_4$. The reduced metals then may easily attack the Sn–H bonds of the organotin hydrides, resulting in their decomposition and to an overall decrease in the signal.

The discrepancies observed between the organotin species may also be related to their molecular size and solubility. When the size of the organotin molecules increases, the volatility of their hydride derivatives at room temperature decreases. The longer time of residence of the largest organotin species in water may favour their decomposition by reaction with inorganic species.

Finally, as most of the interferents are hydride forming elements, they may also be volatilized by the addition of $NaBH_4$ and could later interfere with organotins during the gaseous steps of the analytical determination.

It must also be noted that when high concentrations of inorganic interferents are mixed with organotins in the reaction flask prior to hydride generation, the DBT peak is split into two peaks, as shown in Fig. 5. Interfering agents may potentially catalyse a reaction occurring in either the liquid or gaseous phase during trapping and desorption of the column starting from dibutyltin and producing a new organotin compound with a slightly longer retention time (3.0 min instead of 2.8 min). This new compound does not come from a simple recombination of DBT with other organotins in the reaction flask, as this phenomenon is also observed when interferents are mixed only with DBT before hydride generation.

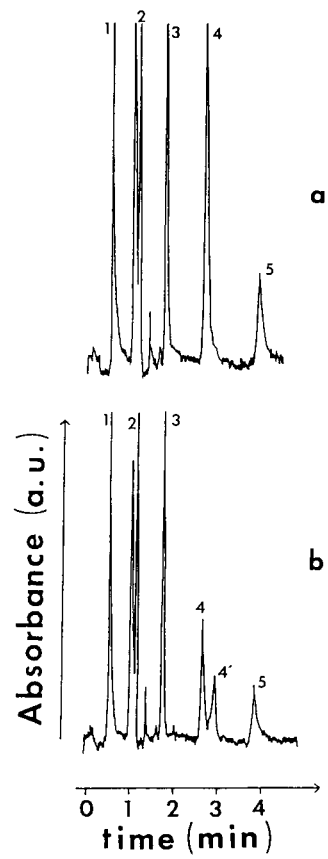

Fig. 5. Chromatogram of 5 ng as Sn of organotin compounds (MMT, MBT, DBT and TBT) by HG–CT–GC–AAS in a 50-ml reaction flask: (a) in simple solution and (b) in the presence of 10 µg of each element of the multi-elemental solution of inorganic metals (1 = SnH_4; 2 = MMT; 3 = MBT; 4 = DBT; 5 = TBT).

With a simple extrapolation between retention times of organotin hydrides and their respective boiling points, their molecular masses can be calculated [30]. This simple calculation for this new species suggests that it is a dibutylmethyltin compound. It seems unlikely that the methyl group would come from partial decay of a butyl group from DBT, as this alkyl group is very stable. On the other hand, the Sn–H bond is very reactive and may react in the column (during trapping or desorption) in the presence of other volatilized interferents. Inorganic interferents would then only act as solid catalysts. The ionic Bu_2Sn^+H could also then form a covalent bond with a methyl group coming from the chromatographic phase to produce Bu_2MeSnH. The column contains a methylsilicone-based coating. The compound could also originate from the silanization procedure performed prior to analysis.

Gas transport kinetics are not modified by the interferents in the gaseous phase, and no chemical redistribution occurs on the column as the retention times of other organotin hydrides are constant. Finally, the desorption and transport of the analytes are efficient as no significant memory effects were recorded throughout the procedures.

In conclusion, the most serious interferences are obtained in the presence of inorganic metal species. However, concentrations of elements generating significant signal suppression for organotin speciation analysis would translate into a total metal concentration of 500 $\mu g\ g^{-1}$ for each element present in the sediment. According to previous results, these levels are very unlikely to occur after an acetic acid leachate, except possibly for some major elements.

Conclusion

Hydride generation for the speciation analysis of organotin compounds is now often performed in a wide array of matrices (water, sediments, biological tissues). In this work, possible sources of interferences generated during the determination of organotin compounds from an organic matter-rich sediment (candidate reference material) were investigated. The geochemical characteristics and the acetic acid extract composition of this sediment were established with various analytical techniques (metal content and organic matter characteristics) in order to identify possible interferences during the determination of organotin compounds by hydride generation. Results obtained for both the organic and inorganic compositions suggest that most classical interferents cannot account for the TBT signal inhibition observed with the sediment studied. The role of organic and inorganic species likely to generate interferences in model solutions were also specifically evaluated.

Organic compounds in general (organic solvents, PCBs, pesticides, *n*-alkanes, humic substances) have only a small effect on the signal suppression. In the presence of organic pollutants, MBT is the compound most affected owing to its higher instability originating from the three Sn–H bonds. Also, the most serious effects of organic compounds are associated with the presence of humic substances which significantly alter the overall reproducibility of the analysis. The addition of an excess of a complexing agent (EDTA) has little influence on both the DBT and TBT signals. However, severe signal suppressions are observed for the monoalkylated species (MMT and MBT). This suppression can be attributed to the smaller molecular size of these species and their more polar behaviour, favouring rapid and stable complexation and preventing their derivatization.

The results obtained clearly demonstrate that under the experimental conditions used the most serious interferences encountered in the speciation analysis of organotin compounds by hydride generation are associated with the presence of inorganic metals in solution. With 1 μg of each element in the reaction flask, the signal depression can reach 90% for TBT and up to 60% for DBT. In the presence of 100 μg of each element in solution, both the DBT and TBT signals are totally suppressed. Interference mechanisms in the presence of inorganic metals during organotin determination would mainly originate from the severe reduction conditions associated with the $NaBH_4$ reaction and subsequent attack of the

Sn–H bonds of organotin hydrides by inorganic species.

Based on the results obtained after the sediment extract characterization and on the basis of the results obtained on model solutions, it was not possible to identify clearly some specific compounds likely to generate the organotin signal depression observed with the candidate reference material. Nevertheless, inhibition processes likely to occur in the determination of organotin compounds in this sediment could be generated either by the inorganic content of the extract or/and as a result of the presence of the high organic matter content of this sample.

The authors thank the Commission of the European Communities for providing financial support [contract No. 5526/1/9/313/91/08-BCR-F(10)]. They thank B. Jousseaume for helpful discussions.

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Analytica Chimica Acta, 286 (1994) 357–370
Elsevier Science B.V., Amsterdam

Review

Analytical techniques applied to the speciation of selenium in environmental matrices

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(Received 17th February 1993; revised manuscript received 19th July 1993)

Abstract

The toxicity and essential nature of Se in the environment depend on its concentration and the chemical forms in which it is present. Se and its derivatives may be widely dispersed throughout the environment as a result of anthropogenic inputs such as fossil fuel combustion and industrial, agricultural and natural processes. In order to evaluate the effects of the different Se species on living organisms, sensitive analytical methods are required for their determination in complex environmental matrices. An overview is given of the importance of Se speciation and the distribution, accumulation and transformation of the species in the environment. The state of the art of various techniques is discussed. The main sources of errors in each step involved in the analytical methods are reported and the importance of quality control and the need to use reference materials are also stressed.

Keywords: Environmental analysis; Review; Selenium; Speciation

Trace elements are present in the environment in a wide range of physico-chemical forms and the different species control their fate and impact in the environment. Frequently, trace metals are present in more than one valency state and/or in organometallic forms. Selenium is an element of Group VI of the Periodic Table, like sulphur. They have similar properties but the stronger electronegativity of selenium can explain the differences observed in the biochemical behaviour of the two elements. For selenium, the main reasons that justify the need to speciate the different forms in the environment are ambiguous: species can either be considered as essential (10–40 μg ml^{-1} in serum [1] and 0.1 μg ml^{-1} in urine [2]) or can be toxic when in excess [3]. Se is known to play an important role in mammalian systems (in the enzyme glutathione peroxidase, in selenoproteins, in carcinogenic processes, etc.) [1,4,5]. In the last few years, Se has been suspected to be an agent of cancer inhibition [2,6,7]. Se has a protective action towards cell membranes, preventing oxidative damage [8–11]. On the other hand, there is also some evidence of mutagenic effects of selenite and selenate [12,13]. The toxicity of Se depends on many factors:

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SSDI 0003-2670(93)E0422-4

chemical form, with different toxicity for organic and inorganic Se compounds [14–17]; pH, as organic ligands in natural waters can reduce the toxicity of metals by complexing them and this complexation is highly dependent on pH, with neutral pH favouring metal complexation [18]; transport mechanisms, where the lipophilic character of organometallic species allows rapid diffusion across biomembranes and generates toxicity [18,19]; interaction with other metals that may modify the toxicity of Se by complexation, e.g., As, Ag and Cu [13]; and nature of the matrix of ingested food (protein, carbohydrate composition, etc.) which can also affect the toxicity [20,21]. Selenium toxicity studies have been widely reported in the last few years [22–38].

In the environment, elemental selenium is generally associated with sulphur and it is found in metal–sulphur deposits [39–41]. Total Se levels in environmental samples range from 0.1–400 $\mu g\ l^{-1}$ in natural waters [42] to 1 ng l^{-1} in the atmosphere [43] and 0–80 $\mu g\ g^{-1}$ in soils [44–46].

Se may be released in the environment as a result of anthropogenic activity such as fossil fuel combustion and industrial and agricultural processes, and also by natural processes such as weathering of minerals. Se compounds are widely used in industry: glass manufacture [47], electronic applications, photocopy machines, inorganic pigments, rubbers, ceramics, plastics, lubricants, etc. [42,47,48]. These different uses are summarized in Table 1. The accumulation of total Se depends on the environmental matrix [39] and it is affected by factors such as climatic factors and soil pH [44,45]. In the atmosphere and sea water a global increase in total selenium has still not been documented but in soils this accumulation has been demonstrated.

The species identified in environmental matrices are selenite (SeO_3^{2-}), selenate (SeO_4^{2-}) as inorganic species and dimethyl selenide (DMSe), dimethyl diselenide (DMDSe), dimethyl selenone, selenomethionine (SeME), selenocysteine (SeCYS) and trimethylselenonium ($TMSe^+$) as organic species [40,41,48–50].

Inorganic Se compounds can be transformed into organometallic Se compounds through microbial action [51], being converted into dimethyl selenide, dimethyl diselenide and dimethyl selenone [52,53]. Different mechanisms and pathways leading to the formation of dimethyl selenide and dimethyl diselenide have been proposed [51] (Figs. 1 and 2). These methylation processes are regulated by factors such as temperature, oxygen concentration, sunlight, pH, salinity [54–65] and redox potential [66–71].

The legal regulations for total Se levels establish maximum permissible levels of 0.1–0.2 mg l^{-1} in air [39] and 8–10 mg l^{-1} in water [41]. No specific recommendations to date are available with regards to Se species. This lack of information can mostly be related to the difficulties asso-

TABLE 1

Industrial uses of different Se compounds

Compound	Uses	Ref.
Selenium	Solar batteries, photoelectric cells, xerography, stainless steel	42
Na_2SeO_4	Insecticide, veterinary agent, glass manufacture	42
Na_2SeO_3	Veterinary agent, glass manufacture, soil additive	42
Selenium diethyldithiocarbamate	Fungicide	
SeS_2	Veterinary agent, shampoos, semiconductors	42
SeS	Veterinary agent, fungal infections, eczema	42
SeF_6	Gaseous electric insulator	42
$SeOCl_2$	Solvent	42
$CuSeO_4$	Copper alloys	42
WSe_2	Lubricants	42
Al_2Se_3, Bi_2Se_3, CuSe, InSe	Semiconductors	48
$(NH_4)SeO_3$, As_2Se	Manufacture of red glass	48
CdSe	Photoconductors, semiconductors, photoelectric cells, solar batteries	48

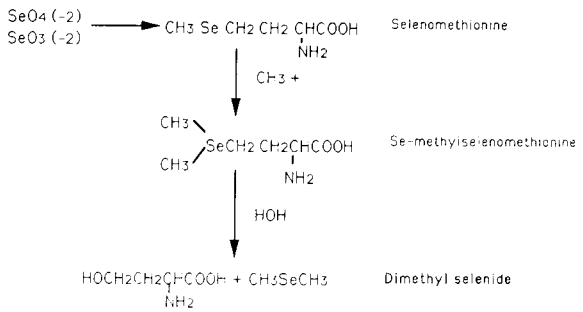

Fig. 1. Mechanisms of DMSe formation by natural methylation processes (from [51]).

ciated with the analytical techniques. Also, Se speciation is difficult owing to the low total concentrations, which may be below 10 $\mu g\ l^{-1}$. Significant errors may be introduced by contamination or loss of metal ions. Some chemical species are labile and not easy to stabilize. Therefore, special attention should be paid to the sampling and storage stages with regard to stability of the species and interconversions among them.

ANALYTICAL PROCEDURE

Critical factors affecting sampling and storage

Sources of errors introduced by sampling are very important owing to the low Se concentrations present in the environment, ranging from ng l^{-1} and ng g^{-1} to $\mu g\ l^{-1}$ and $\mu g\ g^{-1}$ in water and sediments, respectively [42–46]. There are several factors to consider at these ultratrace levels, such as the sample handling and storage steps. During storage, adsorption, desorption, volatilization processes, etc., may occur [19], altering the original concentration of the analyte.

Volatilization. Many organic Se compounds are relatively volatile [72,73] and losses by volatilization may occur at elevated temperature (over 120°C). Usually, samples are dried below this temperature and losses are not significant.

Adsorption and desorption. These phenomena are really important at Se concentrations in the $\mu g\ l^{-1}$ range and will subsequently determine the validity of the results obtained. Adsorption depends on pH, ionic strength, container material and the ratio of surface area per unit volume [74–77]. Se(VI) is more stable than Se(IV) in aqueous solution and less dependent on the acidic conditions of the sample. Preservation of Se(IV) and Se(VI) in natural waters at the 1 $\mu g\ l^{-1}$ level in Pyrex or polyethylene bottles at pH 1.5 (H_2SO_4) is satisfactory [75]. Acidification with nitric acid interferes seriously with the hydride generation method. Nitrite may supress the Se(IV) signal. This interference can be eliminated by adding sulphanilamide before the hydride generation [78,79]. Hydrochloric acid gives good recoveries for Se(IV) and Se(VI) at pH 2.5 [19,78]. Pyrex containers are better than polyethylene but the latter is preferable for handling and shipping. Freezing the samples at 4°C limits possible losses at room temperature [75]. Contamination by desorption from the container wall may occur if the vessel is not carefully cleaned. A recommended procedure to avoid this risk is to clean the vessel by soaking in a 0.08 mol l^{-1} HNO_3 bath and rinsing carefully with water purified with a Milli-Q system [80].

Algal growth. Under acidic conditions Se(IV) and Se(VI) concentrations remain stable for about 4 months, but at pH 5–7 algal growth may affect the Se stability [74,75,81,82]. Algae first appeared after about 6 weeks and became important after about 2 months. The addition of a few drops of $Cu(NO_3)_2$ preserves the stability of calibrant Se

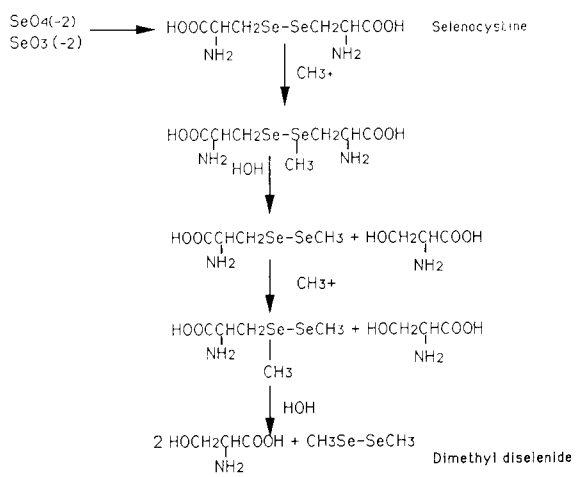

Fig. 2. Mechanisms of DMDSe formation by natural methylation processes (from [51]).

solutions stored in polyethylene vessels at room temperature, avoiding algal growth [83]. However, it may generate an interference problem when using the hydride generation method because of the presence of copper, which is a strong interferent for Se. The best storage condition for inorganic Se solutions is then direct acidification of the samples.

Gas sampling. The collection of air samples for Se determination is a significant problem because of the volatility of selenium compounds. Filters (Millipore, fibre-glass) and liquid impingers have been used to collect airborne selenium [84–86]. Further studies comparing filtration with solution impingement were undertaken and indicated that losses of Se occurred if filtration was used for its collection [87]. Recently, air samples have been collected by passing the air through an impinger containing water, which was sequently analysed for selenium. This method of trapping airborne Se is very efficient. However, when samples are collected with high-volume samplers, losses of Se may occur [87].

Sample treatment

Sample treatment must not introduce appreciable errors by contamination from reagents, laboratory air, containers, etc. [88–90]. Most methods for Se speciation analysis require the conversion of all species into Se(IV). Organic Se compounds [Se(−II)] must then be oxidized for the succesful application of these detection techniques [91] and Se(VI) needs to be reduced to Se(IV).

Wet digestion. Several sources of errors can be mentioned here: losses of volatile selenium compounds during the decomposition of the sample [81], which can be avoided by digestion under high pressure to reduce the temperature or by fitting a reflux condenser to the digestion flask [83]; some organoselenium compounds, such as selenomethionine (SeME), selenocysteine (SeCYS) and trimethylselenonium ($TMSe^+$), are very resistant to acid attack [81,92–94] and the incomplete digestion processes will result in an overall loss in the atomic determination step. SeME and $TMSe^+$ are among the most acid-resistant compounds found in human fluids. They can only be converted into inorganic selenium after a nitric–sulphuric–perchloric acid digestion at 310°C, leading to 100% recovery of both SeME and $TMSe^+$ [91] in biological samples (human body fluids).

Photo-oxidation. Photo-oxidation is an alternative method to convert organometallic forms of selenium [Se(−II)] into inorganic selenium(IV). This conversion step is performed by irradiation with UV radiation using a 1250-W medium-pressure mercury lamp for 5 h in the presence of hydrogen peroxide and borax solution [3,32,95–97]. This method has advantages over wet digestion procedures because it requires less time and minimizes the risk of losses. Also, the general operating conditions are simpler and hence the risk of contamination of the sample is lower. The mineralization efficiency for this method has been found to be about $100.0 \pm 1.6\%$ [98].

Combustion. Decomposition methods are currently used, combining the advantages of closed systems and dynamic mode operation to avoid losses through adsorption and volatilization [99]. Biomaterials, rocks and soils were analysed for selenium with a previous combustion decomposition method under dynamic conditions, which was shown to be a rapid method for volatilizing Se from these matrices [100]. During the combustion of these samples in a pure oxygen atmosphere and under dynamic conditions, Se is volatilized as SeO_2 and condensed in a cold finger. Thereafter, SeO_2 is dissolved with HNO_3 or HCl, which ensures complete dissolution. An admixture of silicic acid ensures slow and uniform combustion.

Clean-up. Clean-up procedures are frequently used to obtain low blanks and to improve the signal. A Chelex treatment for tap water has been successfully applied. Treatment for 20 min with a Chelex-100 column was sufficient to obtain clean blanks. Under these conditions, no adsorption of inorganic selenium species was detected; however, organic selenium species such as selenourea were adsorbed, requiring a previous oxidation prior the Chelex-100 treatment [98].

Extraction. Se(IV) can be preconcentrated by complexing with an ammonium tetramethylenedithiocarbamate solution, extracting into chloro-

form and back-extracting into nitric acid for fluorimetric detection [101–103].

Derivatization

Derivatization is an important step in the analytical scheme for the speciation of selenium compounds.

Hydride generation. In order to apply derivatization techniques for the determination of Se, it is essential to understand all the redox processes between Se species (−II, IV, VI). Hydride generation (HG) is the most frequently applied technique for the speciation determination of Se in biological materials (human fluids) and environmental matrices (sea and natural waters, sediments, etc.) [19,104–112].

Se(VI) is reduced to Se(IV) with HCl with an optimum concentration between 4 and 7 mol l^{-1} [19,113]. It is recommended to perform this reduction process at high temperature (90–100°C) as this redox equilibrium is strongly temperature dependent [114]. The optimum time is between 20 and 45 min. On the other hand, Se(−II) can be oxidized to Se(IV) with potassium peroxodisulphate solution (0.04 mol l^{-1}) in HCl media [115].

$NaBH_4$ is the most common reducing agent used to obtain Se hydride species. This reagent is relatively unstable and must be prepared daily or stabilized in alkaline solution, e.g., NaOH, and stored at low temperature. Its decomposition may be due to the high concentration of transition metal ions, probably generating interferences during the formation of SeH_2 [116,117].

Nitrogen-cooled trapping is a third efficient step when using hydride generation to preconcentrate Se(IV) [19,110]. Basically, it consists of a trap immersed in liquid nitrogen to retain the compounds (SeH_2) by condensation on a silanized glass column. Cryogenic trapping is a useful technique to preconcentrate the species and therefore to improve the overall sensitivity. This system has to be kept dry when the purging time is long because of possible accumulation of water and ice in the trap, which could block the system. $CaCl_2$ has been used as a drying agent but was shown sometimes to contribute to the removal of the analytes. The best means of water removal is to use of a 2-propanol–dry-ice bath. The use of a dry-ice water trap is especially convenient for low-boiling compounds [19,110,118].

Interferences from other metallic ions in Se determination by hydride generation can be divided into those originating in solution and those taking place in the gas phase. Se tracer studies have shown that As and Bi cause liquid-phase interferences (by consumption of the $NaBH_4$, by causing decomposition or adsorption of the hydrogen selenide, etc.); Sn, As, Sb, Bi and Te give gas-phase interferences; Cu, Co and Ni can also partially mask the selenium signal [119]. Addition of common masking agents such as EDTA or 1,10-phenanthroline is ineffective in the determination of selenium. The use of potassium cyanide or cyanoborohydride before the analysis leads to a significant improvement in the signal [119]. In the analysis of natural waters, the presence of nitrite completely suppressed the Se signal. This problem can be overcome by addition of sulphanilamide, which is diazotized by the nitrite [78,79].

Ethylation. $NaBEt_4$ has been used to derivatize Se species. This reaction cannot differentiate inorganic selenium species because a single derivative (Et_2Se) from the two oxidation states is generated. It has not yet been explored for organic compounds. The interest in this reagent is the absence of natural ethylation processes with environmental samples; the risk of interferences is lower than with $NaBH_4$. However, this approach needs further investigations [120].

Complexation. Fluorimetric detection has been applied to the determination of Se in river, estuarine and coastal waters. This method is based on the formation of a fluorimetric complex of Se. Se(IV) is complexed with 2,3-diaminonaphthalene to form 4,5-benzopiazselenol, which is extracted into cyclohexane. The fluorescent complex is determined by molecular fluorescence spectroscopy (MFS) [102,103]. Se(IV) can also react with 4-nitro-*o*-phenylenediamine to form 5-piazselenol [121] or with 3-bromo-5-trifluoromethyl-1,2-diaminobenzene to form 4-bromo-6-trifluoromethylpiazselenol [122]. These piazselenols are volatile and can be determined by gas chromatography with an electron-capture detector.

Separation

Chromatographic techniques coupled with different detector systems have been extensively used for the separation and determination of all Se species. This approach minimizes interferences from the matrix. However, errors may arise from a lack of efficiency in the separation, e.g., due to incomplete retention on the column, decomposition of the species, incomplete recovery of the eluate or peak overlap [41].

Gas chromatography. Several techniques have been applied to the determination of volatile Se species and inorganic Se using gas chromatography (GC) [95,105,121,123–126]. Volatile Se compounds, which include DMSe, DMDSe and DESe, can be determined without chemical modification directly in air and water matrices. Columns employed to separate these species include 10% polymetaphenyl ether (PMPE) on 80–100-mesh Chromosorb W or WAW support [123,124], 3% OV-1 on Chromosorb W [169] and non-polar methylsilicone SP2100 [127,128]. These chromatographic columns are coupled to atomic absorption spectrometric detection.

$TMSe^+$ can be detected by separation in a column filled with a strongly acidic cation-exchange resin, followed by conversion of $TMSe^+$ into DMSe with an alkaline solution and subsequent detection with GC–atomic emission spectrometry (AES) [126]. Other workers improved the separation provided by a cold-trap hydride generation AAS system to detect DMSe and DMDSe by insertion of a gas chromatograph between the cold trap and the atomizer [19].

GC interfaced with microwave-induced helium plasma detection (MWPD) has been reported for the determination of organometallic compounds in complex matrices such as oil–fuel mixtures. Columns generally used for this purpose are packed columns [129] but capillary columns are essential for complex environmental matrices. Fused-silica capillary columns are preferred because of their flexibility and inertness, properties which make them particularly suitable for handling chemically active organometallic compounds [121,130]. Results have been obtained recently using this method for organomercury and organoarsenic compounds and promising results are expected for selenium.

Other methods to determine Se(IV) and total inorganic Se in natural waters are based on the formation of volatile piazselenols, which are separated by GC and detected with an electron-capture detector [121,122]. Two types of column are commonly used: 6.7% OV-17 plus 8.2% QF-I on 80–100-mesh Chromosorb W and 3% 10C on 60–80-mesh Chromosorb W.

Liquid chromatography. Liquid chromatographic (LC) techniques offer the potential of determine all non-volatile selenium species without derivatization processes [131–137].

Mehra and Frankenberger [131] have developed a chromatographic procedure for the simultaneous determination of Se(IV) and Se(VI) in soil extracts by single-column ion chromatography (SCIC) with conductimetric detection (CD). The eluent used was *p*-hydroxybenzoic acid at pH 8.0. The resolution of this method depends on the ionic strength of the eluent [131,138]. In contrast to previous SCIC studies [139,140] which showed interferences from anions such as Cl^-, NO_3^- and SO_4^{2-} in the determination of Se(VI), this system was not reported to be affected by these anions [131].

Se(IV) and Se(VI) can be separated by anion-exchange LC. Selenite is eluted with ammonium acetate–ammonium hydrogenphosphate (pH 4.6); by changing the pH to 6.9 it is possible to elute Se(VI). Se(IV) and Se(VI) can also be separated prior to mass spectrometric (MS) detection using a column packed with an anion-exchange resin such as Dowex 50W-X8; the other organoselenium compounds were separated by varying the pH of elution from XAD-2 resin [141,142].

LC–HG-AAS coupled systems have been developed for the determination of Se(IV) and organic Se. Soluble organic matter present in some groundwater samples and soil extracts interfere with the determination of Se by HG-AAS. Chromatographic methods using XAD-8 resin and a Sep-Pak C_{18} cartridge retain the hydrophobic organic matter (adsorbed), removing the interference in the subsequent recovery of Se. The amount of Se isolated by XAD-8 columns is asso-

ciated with hydrophobic organic forms of Se. The dissolved organic matter associated with the hydrophilic fraction passing through the Sep-Pak C_{18} cartridge contains Se associated with free amino acids [105,143,144].

Interfacing LC with plasma emission detectors is among the most recent techniques for Se speciation analysis. One of the principal problems in interfacing LC with plasma detectors is the perturbation introduced by the solvent from the analyte. An alternative approach is the introduction of the sample in the gaseous state such as in the HG technique.

The coupling of LC with a helium alternating current plasma (ACP) detector give good results in the determination of As and Se [137]. Tetrabutylammonium phosphate is used as an ion-pairing reagent for chromatography. Sample introduction into the ACP was done by the HG method. Another combination interfacing LC with UV and direct current plasma emission (DCPE) detection was used successfully to determine Se species using C_{18} columns for separation. Separation of ionic Se(IV) and Se(VI) can be achieved using NH_4^+ ion-pairing reagents under aqueous–organic conditions. The two species are separated on the basis of their different retention times [145].

The application of LC–ICP-AES to $TMSe^+$, Se(IV) and Se(VI) speciation gave promising results. The eluent used in this system for anion exchange is ammonium citrate at pH 3.3. The sample is introduced into the ICP system by a thermospray vaporizer in the spray chamber [133].

LC–MS [146,147] has been used to separate Se amino acid species from serum glutathione peroxidase for the determination of selenocysteine. XAD-8 columns were used to retain these amino acid species. The separation of selenocysteine and selenomethionine from their sulphur analogues has been achieved previously by LC [148–150]. A derivatization step is necessary to stabilize the selenocysteine species before the separation step [149,151].

Detection

Detection in liquid state. Several types of detectors have been developed for the determination of Se species. Fluorimetric methods are based on the detection of the fluorescent complex formed between Se(IV) and the ligand 2,3-diaminonaphthalene [103]. The total inorganic Se content [Se(IV), Se(VI) and Se(0)] was determined by reducing Se(IV) and Se(VI) to Se(0) and coprecipitating the latter with Te in the presence of hydrazine sulphate, and further complexing it with 2,3-diaminonaphthalene to form the fluorescent complex [152–155]. In fluorimetric analysis the detection limit may be influenced by extractants, temperature and pH. The detection limit achieved with this method was 20 pmol l^{-1}.

Two different electrochemical techniques have been reported for natural water analysis: differential-pulse polarography (DPP) and differential-pulse cathodic stripping voltammetry (DPCSV). Both methods are based on the electrochemical determination of Se(IV). Two mechanisms using a mercury electrode were proposed: formation of Se(0) and further reduction to SeH_2 and formation of a HgSe film and subsequent dissolution and reduction [156–158].

Detection in gaseous state. The detection of volatile Se species (dimethyl selenide and dimethyl diselenide) is carried out after GC separation on the basis of their different retention times. A wide variety of detection methods have been used such as ETAAS, GFAAS [49,123,124] and MWPD (121,130).

The most commonly applied technique after hydride Se derivatization is atomic absorption spectrometric (AAS) detection using a quartz furnace or flame system. With this kind of detector, complete atomization of the analytes is critical to achieving good sensitivity [159–161]. These techniques are highly sensitive and yield detection limits in the ng l^{-1} range, and are not subject to major interferences or high background noise levels [114]. The sensitivity of the AAS system depends on the gas mixture delivered to the flame or quartz furnace, the atomization cell geometry and the carrier flow-rate. In the batch process mode, a high gas flow-rate gives higher and narrower peaks. In continuous-flow processes, a slow gas flow-rate may increase the sensitivity but it leads to a longer gas residence time in the AAS cuvette, which may increase the probability of

dimerization or condensation of the analyte [116,162–165]. A different detection mode has been reported using a hydride generation system coupled with a UV–visible molecular absorption spectrometer with diode-array detection (DAD). The detection limit obtained was 1.8 μg of Se(IV) in the analysis of a health-care product [166,167]. Selenomethionine and selenocysteine are determined by chelation on copper sulphate Chelex-100 columns [92] or XAD-8 columns [105,143,144] and subsequent elution or by LC coupled to HG-AAS detection [105]. These organic species are retained on the columns and separated from inorganic species; subsequent oxidation is required to convert Se(−II) species into Se(IV) to perform HG-AAS detection.

A coupling scheme has been developed for inorganic Se species that considerably improved the sensitivity: hydride generation–inductively coupled plasma atomic emission spectrometry (HG–ICP-AES). The Se hydrides generated are carried into the ICP system by a continuous flow of argon into the drain outlet of the spray chamber. The gas flow system to the plasma must be modified so that either a conventional nebulizer or the hydride generator can be used for sample introduction [168,169]. A further coupling method developed for total inorganic selenium determination in biological reference materials is hydride generation–inductively coupled plasma mass spectrometry (HG–ICP-MS) [170]. This method maximizes the analytical sensitivity with a detection limit of 6.4 pg of total Se. Cu is an important interferent in this method but it may be removed by forming a chloro complex.

In all of these techniques, Se(IV) is determined directly after derivatization process, whereas Se(VI) is determined by difference after reduction of the solution with HCl, and the organic selenium content is also determined by difference after destroying organic compounds by oxidation.

Isotope dilution mass spectrometry (IDMS) has been developed for the selective determination of Se species in natural waters [Se(IV), Se(VI) and organic species including trimethylselenonium

TABLE 2

Techniques used for the speciation analysis of organic and inorganic Se compounds

Species	Matrix	Analytical method	Detection limit	Ref.
Se(IV)	Water	DPP	10 $\mu g\ l^{-1}$	98
Se(VI)		DPCSV	0.04 $\mu g\ l^{-1}$	
Selenourea				
Se(IV), Se(VI)	Natural waters	MFS	5 $ng\ l^{-1}$	103
Se(IV), Se(VI)	Vegetable crops, soils	GLC–ECD	2 $ng\ l^{-1}$	132, 178
Se(IV)	Soil extracts	SCIC–LC–CD	110 $\mu g\ l^{-1}$	123
Se(VI)			60 $\mu g\ l^{-1}$	
Se(IV), Se(VI)	Natural waters	HG–QFAAS	3 $ng\ l^{-1}$	179
Se(IV), Se(VI)	Ground waters	IC–HG–AAS	10 $ng\ l^{-1}$	180
Se(IV), Se(VI)	Sediments, biogenic particles	HG–AAS	10 $ng\ l^{-1}$	112
Se(IV), Se(VI),	Milli-Q water	LC–ICP-AES	54 $ng\ l^{-1}$	133
$TMSe^+$			14 $ng\ l^{-1}$	
DMSe, DMDSe	Synthetic air samples	GC–AAS	0.2 $ng\ l^{-1}$	125
DMSe, DMDSe	Oil fuel	GC–MWPD	–	121
DMSe, DMDSe	Breath of mice	GC–GFAAS	0.2 $ng\ l^{-1}$	124
DMSe	Air	GC–GFAAS	0.1 $ng\ l^{-1}$	123
DMDSe			0.2 $ng\ l^{-1}$	
DESe			0.1 $ng\ l^{-1}$	
SeME, SeCYS	Erythrocytes	LC–MS	–	146, 147
Elemental Se(0)	Milli-Q water	HG–ICP-AES	0.6 $\mu g\ l^{-1}$	168
Total inorganic Se	Milli-Q water	HG–UV–DAD	1 $mg\ l^{-1}$	167
Total Se	Natural water	ID–MS	10 $ng\ l^{-1}$	141
Total Se	Biological material	HG–ICP-MS	1.3 $ng\ g^{-1}$	170

ion] [141]. Isotope dilution requires the formation of ionic compounds of Se by a negative thermal ionization (NTI) method to form negative thermal ions on the surface of a hot metal filament [142,171–174]. It is essential to prepare a spiked solution that contains all Se chemical forms to be determined or converted after separation [142, 175]. Total Se is determined by converting all Se species into the same chemical form and carrying out a comparison with the spiked solution. MS detection is used.

The detection limits for all of these techniques are presented in Table 2. A schematic diagram summarizing the different techniques used is presented in Fig. 3.

QUALITY CONTROL OF SELENIUM DETERMINATION

The growing international concern about environmental contamination by toxic trace elements has led to the implementation of monitoring programmes for controlling Se levels in terrestrial and aquatic environments. Reference analysis methods are those which have the smallest uncertainty for specified determinations among methods in more general use [176]. Thus a reference method gives optimum precision and accuracy only when used under carefully controlled conditions. Measurement compability can be ascertained only by the analysis of reference materials

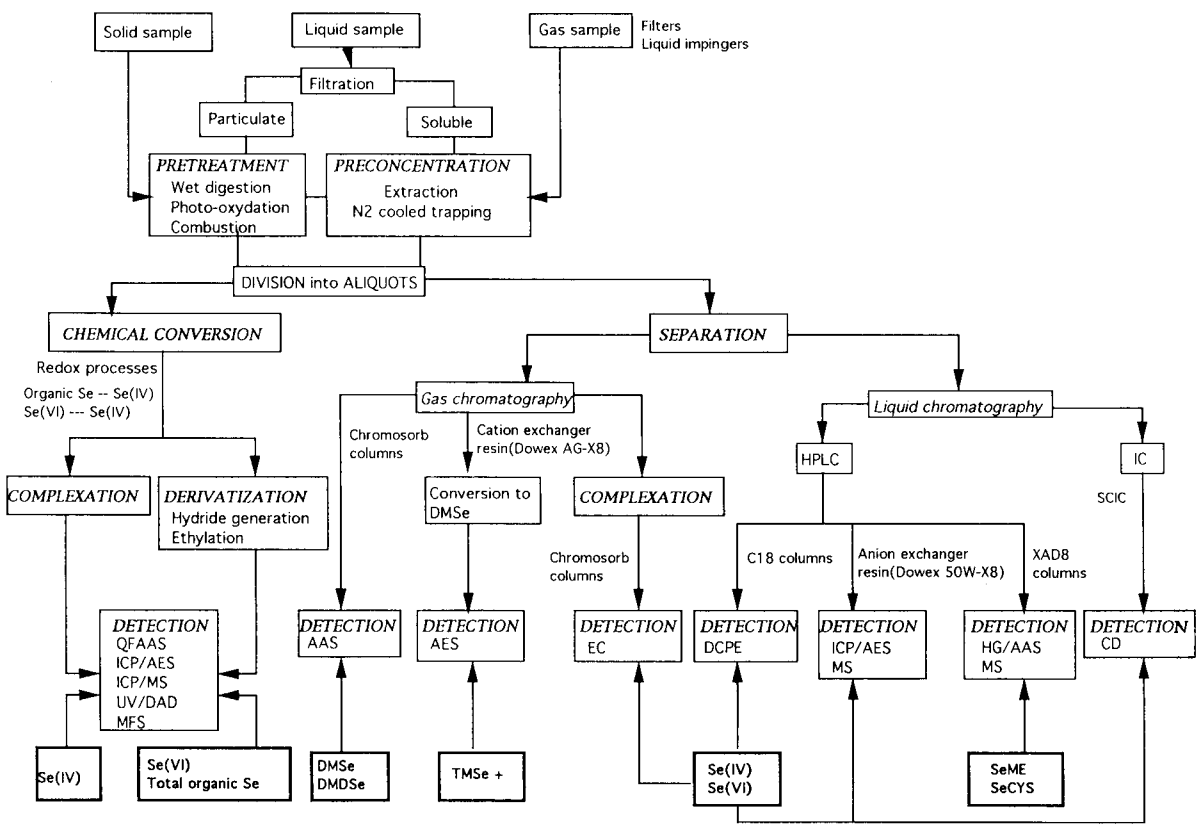

Fig. 3. Procedures used for the determination of Se species in a wide variety of matrices.

that have been analysed by reference methods. Such reference material must be virtually identical with the materials routinely analysed in order to reflect the actual analytical sources of variability [177].

The Commision of the European Communities has undertaken through the Community Bureau of Reference (BCR) the organization of intercomparisons involving 20–30 well trained laboratories using a wide variety of analytical techniques. The achievement is the production of a series of certified reference materials (CRMs) for environmental analysis of Se as given in Table 3. However, no CRMs are available for the quality control of Se species. A new project aimed at the production of CRMs certified for their content of, e.g., Se(IV), Se(VI) and methylated (dimethyl selenide, dimethyl diselenide) and organic (selenomethionine, selenocysteine) selenium species is in progress.

ABBREVIATIONS

AAS	atomic absorption spectrometry
ACP	helium alternating current plasma
AES	atomic emission spectrometry
BCR	Community Bureau of Reference
CD	conductimetric detection
CRM	certified reference material
DAD	diode-array detector
DCPE	direct current plasma emission
DPCSV	differential-pulse cathodic stripping voltammetry
DPP	differential-pulse polarography
ECD	electron-capture detection
ETAAS	electrothermal atomic absorption spectrometry
GC	gas chromatography
GFAAS	graphite furnace atomic absorption spectrometry
HG	hydride generation

TABLE 3

CRMs available for environmental analysis of Se

Matrix	Se level ($\mu g\ g^{-1}$)	Producer	Ref.
Marine sediment	0.43 ± 0.06	NRCC	BCSS-1 [181]
Marine sediment	0.34 ± 0.06	NRCC	MESS-1 [181]
Marine sediment	1.09 ± 0.11	NRCC	PACS-1 [181]
Estuarine sediment	2.04 ± 0.18	BCR	CRM 277 [182]
Lake sediment	0.68 ± 0.06	BCR	CRM 280 [182]
River sediment	0.214 ± 0.034	BCR	CRM 320 [182]
Sea lettuce	0.593 ± 0.032	BCR	CRM 279 [183]
Rye grass	0.028 ± 0.004	BCR	CRM 281 [184]
White clover	6.70 ± 0.25	BCR	CRM 402 [185]
Wholemeal flour	132 ± 10 $ng\ g^{-1}$	BCR	CRM 189 [177]
Dogfish liver	7.34 ± 0.42	NRCC	DOLT-1 [186]
Dogfish muscle	1.62 ± 0.12	NRCC	DORM-1 [186]
Oyster tissue	2.1 ± 0.5	NIST	SRM 1566a [187]
Lobster	6.88 ± 0.47	NRCC	TORT-1 [188]
Mussel tissue	1.66 ± 0.04	BCR	CRM 278 [189]
Plankton	1.75 ± 0.10	BCR	CRM 414 [190]
Cod muscle	1.63 ± 0.07	BCR	CRM 422 [191]
Bovine muscle	183 ± 12 $ng\ g^{-1}$	BCR	CRM 184 [177]
Bovine liver	446 ± 13 $ng\ g^{-1}$	BCR	CRM 185 [177]
Pig kidney	10.3 ± 0.5	BCR	CRM 186 [177]
Human hair	2.00 ± 0.07	BCR	CRM 397 [192]
Single-cell protein	1.03 ± 0.05	BCR	CRM 274 [177]
Open ocean water	0.024 ± 0.004 $\mu\ l^{-1}$	NRCC	NASS-2 [193]

IC	ion chromatography
ICP	inductively coupled plasma
ID	isotope dilution
LC	liquid chromatography
MFS	molecular fluorescence spectrometry
MS	mass spectrometry
MWES	microwave emission spectrometry
MWPD	microwave-induced helium plasma detection
NIES	National Institute for Environmental Studies (Japan)
NIST	National Institute of Standards and Technology (USA)
NRCC	National Research Council of Canada
NTI	negative thermal ionization
QFAAS	quartz furnace atomic absorption spectrometry
SCIC	single column ion chromatography

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Analytica Chimica Acta, 286 (1994) 371–379
Elsevier Science B.V., Amsterdam

Effect of physicochemical parameters on trace inorganic selenium stability

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(Received 17th February 1993)

Abstract

The stability of Se(IV) and Se(VI) species at 10 and 50 ng ml^{-1} concentrations, at different pH values, in the presence or absence of chloride and stored at different temperatures and in different containers was reported. Se(IV) was determined by hydride generation atomic absorption spectrometry (HG-AAS) and Se(VI) by reducing Se(VI) to Se(IV) and calculating the difference between total inorganic Se and Se(IV). Neutron activation analysis (NAA) was used for quality control. Complete loss of Se(IV) occurred after twelve months when samples were stored in polyethylene containers at pH 2 in the absence and in the presence of chloride. Se(VI) was stable under those conditions for the twelve months tested. The stability of Se(IV) increased at pH 6, with two months being the maximum storage time without risk of Se(IV) loss. The presence of Cl^- decreased the risk of losses in some cases. PTFE containers increased Se(IV) losses at pH 6, especially at 10 ng ml^{-1}. Both species were stable at −20°C for the twelve months tested and losses of Se(IV) and Se(VI) were lower at 40°C than at room temperature. A discussion of these results and data published in the literature are included in this paper.

Keywords: Atomic absorption spectrometry; Hydride generation; Neutron activation methods; Metal speciation; Selenium

Soluble selenite or selenate in natural waters may be released from particles containing bound selenium, which act as an "elemental reservoir". Adsorption/desorption and changes in Se chemical form in particulate matter have been extensively studied in recent years and it now seems to be clear that the principal factors that control these processes and Se concentration are: (i) the manner in which the element is associated with the particular matter (usually as ferric selenite); (ii) the oxidation state [which can be (−II), (0), (IV) or (VI)], and (iii) the water–sediment interface, redox potential and pH [1,2]. For example, in the selenium-contaminated sediments of Kesterson Reservoir, reducing conditions give low selenium solubility, but above 200 mV selenite is slowly oxidized to selenate and under oxidizing conditions at 450 mV selenate is the predominant dissolved species (95% at pH > 9 and 75% at pH 7.5–6.5). To determine all the parameters that

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SSDI 0003-2670(93)E0620-M

influence adsorptive capacity, it is of paramount importance to understand selenium biogeochemistry and bioavailability.

Many problems in Se speciation analysis are associated with: (i) the low concentration of each species to be determined, and (ii) the lack of knowledge of sampling and storage. In fresh water the usual range is 0.02–1 ng ml^{-1} [3,4] and in sea water 0.004 ng ml^{-1} at the surface and 0.06 ng ml^{-1} in the deep ocean in the form of selenite and 0.03 ng ml^{-1} at the surface and 0.12 ng ml^{-1} in deep water in the form of selenate [5]; but depending on geological factors, groundwater may reach much higher concentrations (up to 6000 ng ml^{-1}) [6].

The various factors which affect the sorption or loss of selenium, or interconversion of one species into another during sampling, sample storage and sample analysis may be classified into four categories according to Massee et al. [7].

(1) The analyte itself, especially because of its chemical form and concentration, demands simple procedures for high sensitivity determinations.

(2) Characteristics of the solution, such as the presence of acids (pH), dissolved material (e.g., salinity, hardness), complexing agents, dissolved gases (especially oxygen, which may influence oxidation state), suspended matter (competitor in the sorption process) and microorganisms (e.g., algae). Although the United States Environmental Protection Agency [8] has recommended nitric acid at $pH < 2$ for the preservation of Se in natural waters, the losses observed in polyethylene containers and the fact that nitrate interferes with the hydride generation method for selenium, led Cheam and Agemian [9] to investigate how the preservation of Se(IV) and Se(VI) species is affected by the water type, concentration, pH and temperature.

(3) Properties of the container, such as chemical composition, surface roughness, surface cleanliness and surface area/volume ratio. The history of the container, such as age, cleaning method, exposure to heat, etc. is important because it may directly influence the type and number of active sites for sorption.

(4) The influence of external factors, such as temperature, contact time, access of light and occurrence of agitation.

Although literature contains valuable data, the scant treatment of species preservation and some contradictory results led us to investigate certain aspects of species preservation which are yet not clear. Determination of inorganic selenium species has been carried out by several methods such as fluorimetric [10], GC–MS [11], IDMS [12], HG-AAS [13] and NAA [14]. We have used the last two methods for the determination of Se(IV) and Se(VI). The physicochemical parameters evaluated were container–pH relationship, effect of chloride, effect of light and the combined effect of temperature and chloride on short- and long-term stability of Se(IV) and Se(VI) each at 10 and 50 ng ml^{-1}.

EXPERIMENTAL

Containers

To study the effect of physicochemical parameters, the two container materials tested were polyethylene and PTFE. Container volumes were 500 and 100 ml for polyethylene and 1000 and 500 ml for PTFE. Stock and initial working solutions were prepared in 1000-ml and 5-l polyethylene containers, respectively. The cleaning procedure was as follows. New containers were filled to overflowing with nitric acid solution at pH 2, capped and stored for at least 2 days. Then, each container was thoroughly rinsed, filled with Milli-Q quality water and sealed until use.

Reagents

Sodium selenite (Aldrich, 99%) and sodium selenate (Aldrich, 98%), were used. A 0.5% solution of sodium tetrahydroborate (Aldrich) was stabilized with 1% sodium hydroxide (Merck). Other reagents such as HCl, HNO_3, ascorbic acid, active charcoal, etc. were analytical grade.

Design of storage study

Eight initial working solutions with a total volume of 10 l were prepared. Two concentrations of 10 and 50 ng ml^{-1} of each species, each of them at two different pH values (pH 2 by adding

H_2SO_4 and pH 6 by adding no extra reagent) with and without 100 mg l^{-1} of chloride (as NaCl) were prepared in polyethylene containers. Sample homogeneity was achieved by continuous pumping with a peristaltic pump and PTFE tubes for 5 h. Oxygen was removed from bottles by bubbling with N_2. These initial working solutions were placed in 1000-ml polyethylene and PTFE containers kept in the dark at −20, 20 and 40°C and in sunlight at 20°C. Solutions were stored and total selenium and Se(IV) were quantified after 1 day, two weeks, 1 month, 2 months, 6 months, 9 months and 1 year.

Determination procedure

Neutron activation analysis (NAA). Standards were prepared by 100-fold dilution of 1000 mg l^{-1} and 5000 mg ml^{-1} Se(IV) or Se(VI) solutions for the determination of selenite or total selenium, respectively. Then, 250 ml of 10 mg l^{-1} selenium solution or 50 ml of 50 mg l^{-1} selenium solution were directly pipetted (controlled weighing) into about 150 mg of active charcoal in the irradiation capsules. To evaluate recovery in the selenite and total selenium determinations, samples with known concentrations of selenite/selenate were analyzed by the same procedure.

The sample processing method was similar to that developed by Massee et al. [14] and can be summarized as follows: (i) reduction of Se(VI) to Se(IV) by refluxing after addition of concentrated HCl, (ii) reduction of Se(IV) to elemental Se by ascorbic acid, (iii) adsorption of elemental selenium by activated charcoal, and (iv) determination of selenium by NAA. The analytical procedure included 10 s neutron irradiation of each sample with a reactor (RPI) (thermal flux of 1.9×10^{12} n cm^{-2} s^{-1}), rapid transfer to the detection position, and measurement of the induced radioactivity for 10 s. Whenever possible, this sequence was repeated up to three times to enhance the sensibility of the measurements. The 161.9-keV gamma-ray, associated with the decay of the Se-77m radionuclide, was quantitatively measured with a high resolution HPGe detector coupled to a computer which synchronized the measurements with the sample movements. Spectral data were analysed by a gamma spectrum analysis program from Canberra (SAMPO-90) and by home-made software.

For the initial and final times, all the capsules corresponding to the same sample (including the replicates of the sample under study, recovery samples, standards, blanks and capsules with a standard reference material for quality control of the process) were irradiated and counted on the same day, after which they were processed following a preset schedule. The average number of capsules processed per day was around 70, and the whole process took around 2 h per set. The process was designed to distribute capsules of the same type over the total processing time so as to compensate for the potential lack of reproducibility of the processing conditions.

Hydride generation atomic absorption spectrometry (HG-AAS). Selenium was determined with a Perkin Elmer Model 2380 atomic absorption spectrometer equipped with a Se electrodeless discharge lamp operated by a 6-W power supply. A spectral bandwith of 0.7 nm was selected to isolate the 196 nm selenium line.

Prereduction of Se(VI) to Se(IV) was performed by boiling in 6 M HCl for 10 min, followed by determination of total Se by hydride generation using a continuous system hydride generator.

In both analytical procedures the concentration of Se(VI) was evaluated as the difference between total selenium and Se(IV).

Statistical treatment. Quality control of the eight initial working solutions was by analysis of variance (ANOVA) using the general linear model procedure [15]. The influence of physicochemical parameters was determined by statistical treatment of the data obtained. Stability could therefore be assessed by calculating the ratio (R_x) of the mean value (X_x) of measurements made in the different experiments to the mean value taken as reference ($X - 20$°C). The uncertainty U_x was obtained from the coefficient of variation (C.V.) of measurements obtained in different experiments:

$$U_x = \left(\text{C.V.}_x^2 + \text{C.V.}_{-20°\text{C}}^2\right)^{1/2} \cdot R_x/100$$

The stability was evaluated by plotting R_x versus time. In the case of ideal stability, R_x should

be 1. In practice some random variations are likely owing to the uncertainty of the measurement; R_x should, however, be between $1 - U_x$ and $1 + U_x$.

The quality control analysis of the eight 10- and 50-ng ml^{-1} working solutions at pH 2 and pH 6, with and without chloride, showed that pH and Cl^- do not affect Se(IV) and Se(VI) stability at the two concentrations tested. The F factor experimental values obtained were always lower than the theoretical ones.

RESULTS AND DISCUSSION

Stability of inorganic selenium solutions stored at −20°C in polyethylene containers

Eight initial working solutions were used to verify the stability of Se(IV) and Se(VI) solutions stored at −20°C at two pH values (2 and 6) with and without Cl^- at two concentrations (10 and 50 ng ml^{-1}). In all cases the result showed optimum preservation of both species stored at −20°C in polyethylene containers up to one year. Sample acidification was not necessary, which is an advantage if H_2SO_4 interferes with the analytical method. NAA quality control of samples taken randomly showed no significant differences at either species concentration at the evaluated time.

Effect of container and pH with and without chloride

A total of 12 storage containers, 8 polyethylene and 4 PTFE, were filled with the 8 initial working solutions and kept at ambient temperature in the dark for 1 year. The Se(IV) and total Se contents were measured by HG-AAS after 1 day, 1 week, 2 weeks, 2 months, 6 months, 9 months and 1 year. In a parallel study, 7 randomly taken solutions kept in polyethylene containers and 4 in PTFE were analyzed by NAA after three weeks and four months of storage.

Samples at pH 2 in polyethylene containers completely lost Se(IV) at the 10 and 50 ng ml^{-1} levels in one year. The losses started after about 1 month of storage. The same behavior was observed for samples containing chloride (as NaCl). Figure 1a shows this behavior for a 50 ng ml^{-1} Se(IV) solution without chloride. On the contrary, Se(VI) is stable for a whole year (Fig. 1b). Since algae cannot grow at pH 2 and partial transformation into Se(VI) is not clearly observed, the Se(IV) losses might be attributed to adsorption onto the container wall.

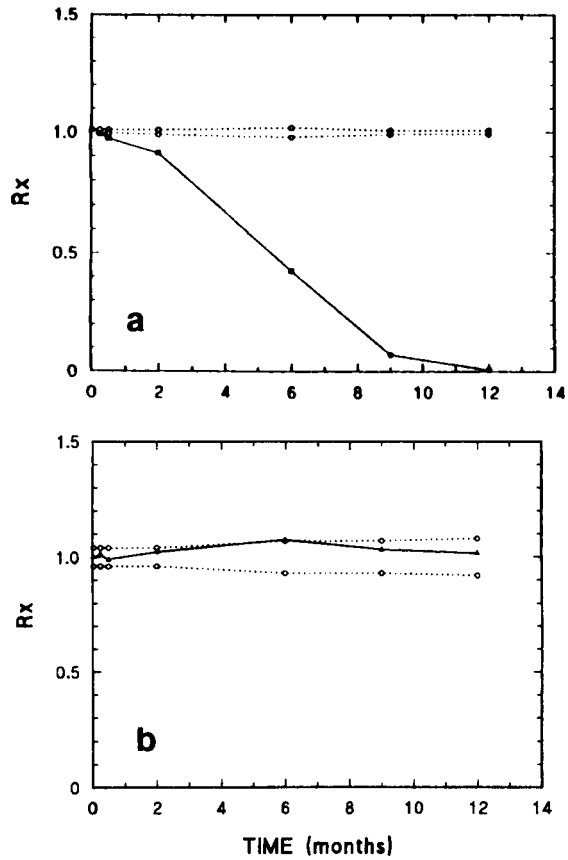

Fig. 1. Stability of selenium species at +20°C and pH 2 in polyethylene containers. (a) Solutions of 50 ng ml^{-1} Se(IV). (b) Solution of 50 ng ml^{-1} Se(VI).

The stability of 10 and 50 ng/ml Se(IV) at pH 6 seems to be higher than at pH 2. Nevertheless, the maximum storage time without risk of Se(IV) losses was two months. Figure 2 shows the results obtained for 10 ng ml^{-1} Se(IV) without and with chloride. Chloride seems to decrease the risk of Se(IV) loss (70% without Cl^- as opposed to 20% with Cl^- in one year), but total stabilization was not obtained. On the contrary, Se(VI) species were stable during the 12 months tested.

When a PTFE container was used, dramatic losses of Se(IV) were observed at pH 6, and were higher at 10 ng ml^{-1} than at 50 ng ml^{-1}. An increase in Se(VI) species in PTFE, at pH 6, for 10 and 50 ng ml^{-1}, was observed, which suggests that there was no loss of Se(VI) and perhaps, some transformation of Se(IV) into Se(VI) were at this pH at ambient temperature. In contrast, Se(IV) species were stable at pH 2 and, therefore, there was no increase in Se(VI) concentration at this pH. It was concluded that Se(IV) and Se(VI) are clearly stabilized at pH 2 (Fig. 3).

The high R_x uncertainty range for the determination of 10 ng ml^{-1} Se(VI) seen in the R_x vs. time plot in Fig. 3b is due to the indirect determination of this species. Furthermore, since total Se was determined using half the sample concentration used for Se(IV), the R.S.D. was increased by a factor of two.

The results of NAA quality control were in good agreement with those obtained by hydride generation atomic absorption (Tables 1 and 2).

The literature data are somewhat controversial: 4% losses of Se(IV) in 15 days from distilled water at pH 7 in flint glass and 8% in polyethylene container have been reported by Shendrikar and West [16]. Nevertheless, it has been shown that acidification reduces the sorption process. Cheam and Agemian [9] reported that the stability of inorganic 1 and 10 ng ml^{-1} Se(IV) and Se(VI) species in pH 1.5 sulphuric acid media in 500-ml pyrex or polyethylene bottles was satisfactory during the 125 days tested. Higher pH values proved unsatisfactory in all cases and pyrex was apparently a better container material than polyethylene. For 1 ng ml^{-1} Se(IV) at pH 5.4 losses occurred in both pyrex and polyethylene bottles, whereas complete recovery was obtained in 25-gallon barrels for distilled and natural (harbour) water. This indicates that the size of the container, or rather its surface area per unit volume, directly influences Se(IV) stability. The results at 10 ng ml^{-1} are the same as at 1 ng ml^{-1} but the ten-fold increase in Se(IV) concentration decreases losses at higher pH. The stability of Se(IV) is also dependent on bacterial activity but adjustment to pH 1.5 with sulphuric acid successfully prevents this effect. More pronounced stabilization is observed in natural water (Hamilton harbour, Canada), the reason being that apparently natural water with dissolved salts, is a better preservative for Se(IV) than distilled water because surface interaction is lower. With regard to Se(VI) preservation, the stability of this species is not affected for at least four months by the container (glass or polyethylene), pH, concentration or water type (natural or distilled). These authors also reported the effect of storage tem-

TABLE 1

Effect of container and pH

	PTFE		Polyethylene	
	Se(IV)	Se(VI)	Se(IV)	Se(VI)
pH 6				
Initial conc.	10 ng ml^{-1}	10 ng ml^{-1}	10 ng ml^{-1}	10 ng ml^{-1}
2 weeks	10.2 ± 0.4	7.1 ± 0.9	9.1 ± 0.1	11.0 ± 0.4
1 month	9.6 ± 0.3	9.6 ± 1.1	9.4 ± 0.1	10.0 ± 0.4
Initial conc.	50 ng ml^{-1}	50 ng ml^{-1}	50 ng ml^{-1}	50 ng ml^{-1}
1 month	48.6 ± 0.6	51.6 ± 1.1	49.6 ± 0.7	53.2 ± 1.9
3 months	41.8 ± 0.5	57.5 ± 1.3	50.6 ± 0.6	47.0 ± 1.8
pH 2				
Initial conc.	10 ng ml^{-1}	10 ng ml^{-1}	50 ng ml^{-1}	50 ng ml^{-1}
1 month	10.5 ± 0.2	7.4 ± 0.4	52.2 ± 0.5	42.2 ± 1.9
1.5 months	9.9 ± 0.2	9.4 ± 0.5	52.5 ± 0.4	56.3 ± 2.1
Initial conc.	50 ng ml^{-1}	50 ng ml^{-1}	10 ng ml^{-1}	10 ng ml^{-1}
1 month	48.9 ± 0.6	49.0 ± 1.3	9.5 ± 0.4	10.1 ± 0.6
3 months	49.7 ± 0.6	49.6 ± 2.0	9.7 ± 0.4	10.2 ± 0.6

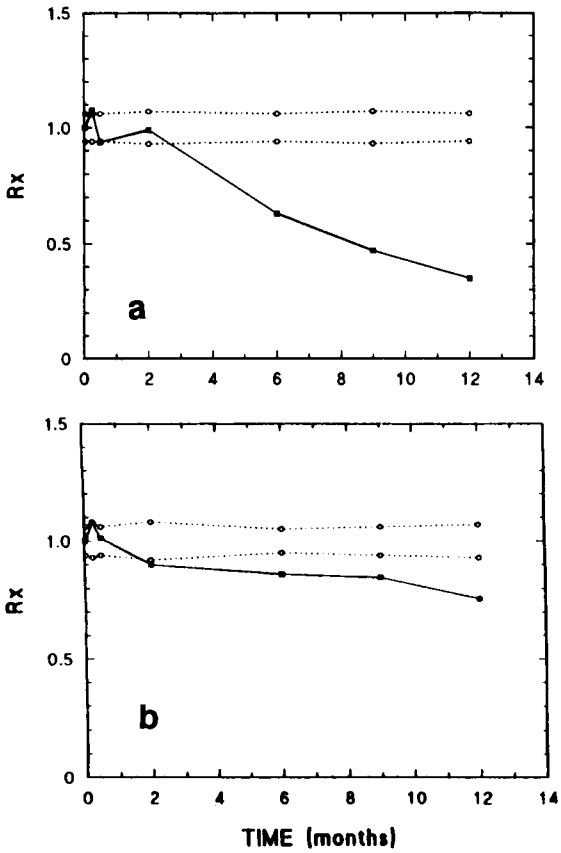

Fig. 2. Stability of selenium species at +20°C and pH 6 in polyethylene containers. (a) Solution of 10 ng ml^{-1} Se(IV). (b) Solution of 10 ng ml^{-1} Se(IV) with 100 mg l^{-1} chloride.

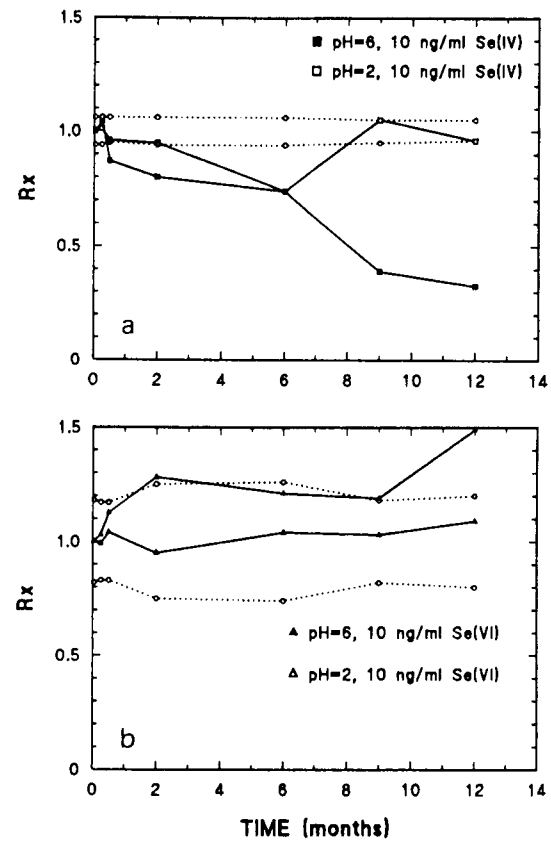

Fig. 3. Stability of selenium species at 20°C and pH 2 and 6 in PTFE containers. (a) Solution of 10 ng ml^{-1} Se(IV). (b) Solutions of 10 ng ml^{-1} Se(VI).

perature on the stability, and found that 4°C for about one month preserves Se(IV) in distilled water. Schutz and Tukekian [17] observed no loss of selenium from natural sea water and Measures and Burton [18] reported constant inorganic Se(IV) and Se(VI) content in sea water samples acidified to pH 2 with hydrochloric acid and stored in glass or polyethylene containers for 4.5 months. An interlaboratory quality control study [19] using sample acidification of 0.02% (v/v) sulphuric acid and storage at room temperature in polyethylene bottles found excellent recoveries over the 0–1000 $\mu g\ l^{-1}$ selenium concentration range and confirmed the effectiveness of the proposed preservation method. A high acid concentration, such as 32% HCl, enables the storage of natural water in polyethylene containers for several weeks without significant losses of selenite

TABLE 2

Effect of pH using polyethylene container (100 mg l^{-1} Cl^-)

	Se(IV)	Se(VI)
pH 6		
Initial conc.	50 ng ml^{-1}	50 ng ml^{-1}
2 months	50.6±0.8	53.7±2.3
2.5 months	50.7±0.7	55.0±2.3
Initial conc.	10 ng ml^{-1}	10 ng ml^{-1}
2 months	9.9±0.2	12.3±0.7
4 months	10.2±0.2	9.4±0.6
pH 2		
Initial conc.	10 ng ml^{-1}	10 ng ml^{-1}
3.5 months	10.2±0.2	10.2±0.6
4 months	9.9±0.2	8.9±0.7

and selenate [20]. May and Kane [21] found no apparent change in Se(IV) concentration after 50 days storage of 2 mg l^{-1} solution in polyethylene or borosilicate glass containers in 15% hydrochloric or 5% sulphuric or mixed $HCl-H_2SO_4$ acid. Massee et al. [7] showed that Se(IV) stability at the ng ml^{-1} level for 28 days was independent of pH (pH 1, 2, 4 or 8.5 with nitric acid or sodium hydroxide), container type (borosilicate glass, high-pressure polyethylene or polytetrafluoroethylene), surface area to sample volume ratio and sample matrix (distilled or artificial sea water). The reason for the observed stability of Se species may be that Se(IV) and Se(VI) form oxyacids which are partly dissociated, leading to negatively charged ions.

Cutter [22] studied speciation in biogenic particles and sediments and concluded that the stability of selenite and selenate during the storage of spiked samples (30-ml linear polyethylene bottles, 10 ml HCl at pH 1.6, 1.2 ng of Se per ml as selenite or selenate, refrigerated storage), prevented microbial growth and speciation changes for 3 weeks. Dunju et al. [23] showed that quartz containers are better than polyethylene (PE) and glass containers for preserving both species in acidified water samples. No concentration changes were observed for Se(IV) at 0.06–0.13 ng ml^{-1} and Se(VI) at 0.03–0.13 ng ml^{-1} in water samples at pH 1.1 containing up to 3.2 mg l^{-1} fulvic acids in polyethylene containers for 30 days.

Finally, May and Kane [21] studied the matrix-dependent instability of selenium(IV) stored in PTFE containers in different acidic matrices (HCl, $HCl + H_2SO_4$ and H_2SO_4) and found that the combination of Se(IV), PTFE and H_2SO_4 should be avoided when preparing samples for speciation purposes. These results clearly differ from ours, which showed a similar phenomenon but at pH 6.

Effect of pH and temperature stability in polyethylene containers

To study the effect of temperature (40°C) at pH 2 and 6 on stability in polyethylene containers, samples were stored in 100-ml vessels (instead of 500 ml as in the other experiments) and analyzed after they reached room temperature.

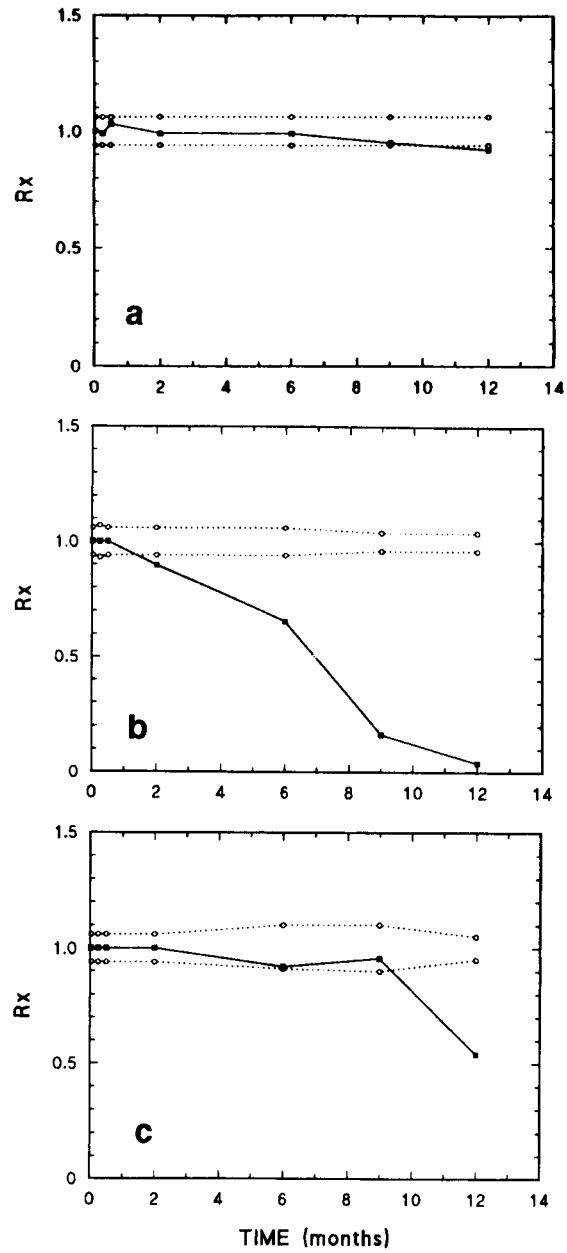

Fig. 4. Stability of selenium species at −20°C, +20°C, +40°C and pH 2 in polyethylene containers. (a) Solution of 10 ng ml^{-1} Se(IV) at −20°C. (b) Solution of 10 ng ml^{-1} Se(IV) at +20°C. (c) Solution of 10 ng ml^{-1} Se(IV) at +40°C.

At pH 2, the results at 40°C clearly differ from those at room temperature (Fig. 4). At 40°C no significant differences were observed for either of

the first nine months tested. Losses were about 50% lower than under the same conditions at room temperature. The presence of chloride did not affect the stability of either species. Se(VI) was stable for the first year tested.

At pH 6 and 40°C the loss of Se(IV) was much lower than at ambient temperature (Fig. 5) and the results were similar to those obtained at room temperature in the presence of 100 mg l^{-1} chloride.

The higher stability of both species at 40°C compared to 20°C is surprising. One of the reasons for this behaviour could be that high temperatures, such as 37–40°C, may increase the motion of solution molecules and thus their collisions with the wall, which might increase the rate of selenium species retention on the walls, but since the retention forces may be weak due to the anionic nature of selenium (oxoanions), the increased molecules motion could be expected to decrease retention on the walls.

The literature contains no data on the stability of inorganic Se species at 40°C.

For quality control five random samples were analysed by NAA after two weeks and two months at 40°C. The samples were analyzed at pH 2 and 6, in polyethylene containers in the dark without Cl^- at two concentrations (10 and 50 ng ml^{-1}) for each species. Not significant differences in Se(IV) concentration were found. Since 37.5–40°C is a good temperature range for algal growth, temperature seems to have no effect on sample preparation in distilled water.

Effect of light

The effect of light was studied in the solutions containing both species at 50 ng ml^{-1} and at pH 2 and 6 chosen. The results at pH 2 showed that 15 days is the maximum storage time without Se(IV) losses. After one month of storage, 10% loss of Se(IV) was detected. The presence of chloride increased Se(IV) stability up to one month. Se(VI) species were stable in the presence and absence of chloride for all the months tested.

Both species were stable at pH 6 with and without chloride for one month. The quality control analyses by NAA gave similar results. The results were in agreement with those obtained under the same conditions but in the dark, therefore we concluded that light had no significant effect on inorganic selenium species stability for the period tested.

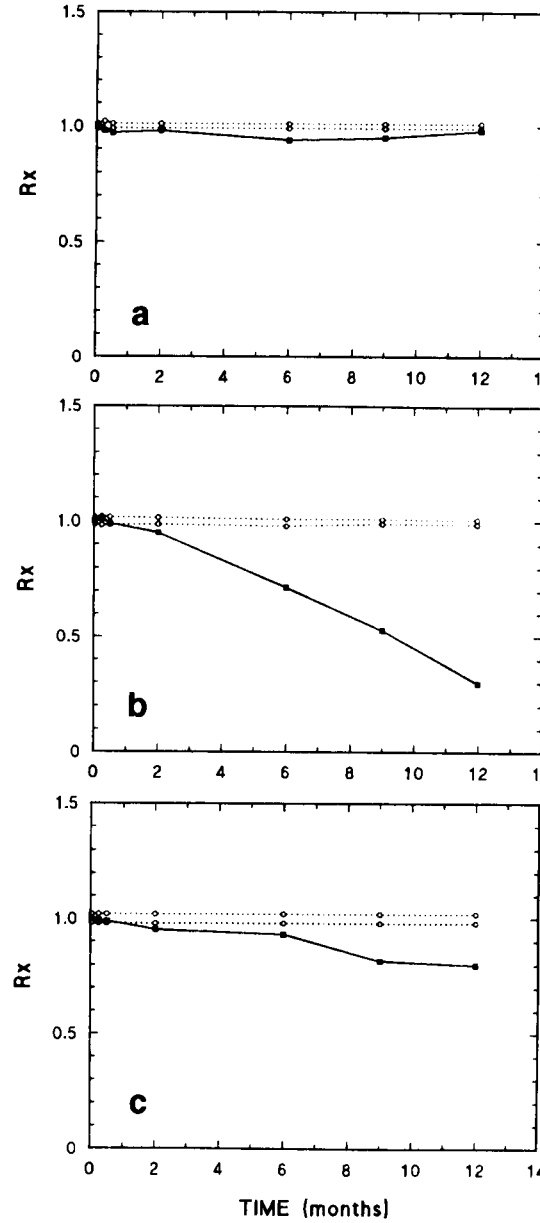

Fig. 5. Stability of selenium species at −20°C, +20°C, +40°C and pH 6 in polyethylene containers. (a) Solution of 50 ng ml^{-1} Se(IV) at −20°C. (b) Solution of 50 ng ml^{-1} Se(IV) at +20°C. (c) Solution of 50 ng ml^{-1} Se(IV) at +40°C.

Conclusions

The optimum temperature at which there is no significant risk of selenium losses at 10 and 50 ppb over the twelve months tested is −20°C. Samples stored at this temperature need not be acidified, which is an advantage if acids interfere with the speciation method.

Stability at room temperature and 40°C strongly depends on both pH and container type. Generally, both Se(IV) and Se(VI) stored in polyethylene containers at 40°C were more stable than at room temperature, particularly at pH 6. The presence of chloride tends to stabilize both species.

The maximum time of sample storage at room temperature is two months (pH 6) and 9 months (pH 6) in polyethylene and PTFE containers, respectively.

The authors thank the Spanish Ministry of Education and DGICYT (Project PB91-0376) for providing the financial support and Max Gormann for revising the manuscript.

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Analytica Chimica Acta, 286 (1994) 381–390
Elsevier Science B.V., Amsterdam

Review

Speciation analysis of organolead compounds by gas chromatography with atomic spectrometric detection

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(Received 17th February 1993; revised manuscript received 6th May 1993)

Abstract

The state of the art of hyphenated techniques involving gas chromatography applied to speciation analysis of organolead is presented. Recent developments in the field of injection techniques, gas chromatographic separation, interface design and detection are discussed. An overview of procedures applicable to the analysis of water samples, sediments and biological materials is given. Particular attention is given to the factors affecting the accuracy of analysis.

Keywords: Gas chromatography; Environmental analysis; Hyphenated techniques; Organolead; Speciation analysis

Despite increasingly more severe restrictions in many countries the use of organolead compounds as antiknock agents remains large and accounts for the presence of organolead in the environment [1–4]. Tetraethyllead and tetramethyllead are the principal compounds added to the gasoline but some mixed methylethyllead compounds may also be involved. During combustion they undergo a variety of degradation processes which continue in the troposphere under the influence of sunlight and some atmospheric constituents [1–4]. A degradation scheme showing the species involved is shown in Fig. 1. Natural production of organolead via biomethylation remains still a controversial issue.

Starting from the seventies, the growing concern about the contribution of organolead to the lead burden of the biosphere has stimulated the development of species selective analytical methodology. The currently accepted approaches are based almost exclusively on the coupling of a separation technique such as gas chromatography (GC) or liquid chromatography (LC) with an element selective and sensitive detector. The latter is usually an atomic absorption or an atomic emission spectrometer [4–6], but post-column chemical reaction [7,8] and electrochemical [9,10] detectors are also in use.

Recent advances in instrumentation have enabled the detection of organolead at the sub-pg level on a routine basis while severe limitations have still remained on the level of sample preparation. Most of existing procedures are cumbersome requiring a large amount of sample and several separation/preconcentration steps. Controversies exist over the recovery of analytes and the efficiency of their derivatization depending on the matrix type involved. Ultratrace analysis for organolead in the remote environment is likely to face blank value problems. No reports on the independent confirmation of the accuracy of the methods developed are available.

This paper is aimed to critically evaluate the state of the art of gas chromatography with atomic spectrometric detection for speciation analysis of organolead in different samples. Recent instru-

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SSDI 0003-2670(93)E0325-2

mental developments: *on-line* preconcentration, interfacing of a capillary column to quartz and graphite furnace and detection by microwave induced plasma atomic emission spectrometry (MIP-AES) are discussed. Particular attention is given to the factors affecting the accuracy of analysis such as: standards purity, risk of contamination and losses and stability of the analytes.

INSTRUMENTAL ADVANCES IN THE DETERMINATION OF ORGANOLEAD COMPOUNDS

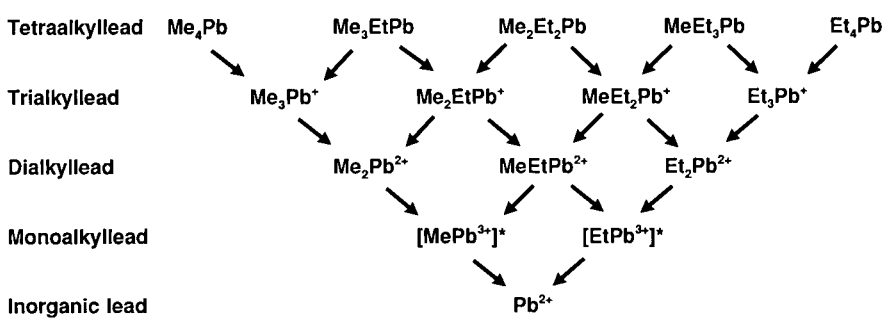
On-line preconcentration

Due to the limited capacity of a GC column only a tiny fraction of the derivatized extract is finally processed in the hyphenated system. The resulted loss in sensitivity of the overall method is the most pronounced when a capillary column is used (reduction factors 1:250 ÷ 1:5000). To circumvent this drawback precolumn trap enrichment was proposed as long as a decade ago for the preconcentration of butylated organolead species but operating the trap in *off-line* mode was inconvenient [11]. This approach was revisited in a recent work in *on-line* version [12]. The principle of preconcentration is based on the differences in volatility of the solvent and the analytes. Three consecutive processes taking place in the injection liner are involved: sample injection, solvent venting and transfer of the analytes onto the column. A solution of organolead species in a volatile solvent is injected onto a cool (0–10°C) Tenax packed liner. Then the temperature is slightly raised to increase the solvent vapour pressure and maintained for 1–5 min while a stream of the helium gas passes through the liner sweeping the solvent off the column. The less volatile analytes are kept in the liner until the volume of the solvent is reduced to 1 μl or less. Then the purge valve switches the carrier helium gas stream into the column while the temperature of the liner is raised to the effective injection temperature prompting the release of the analytes.

When coupled to a capillary column the system allows for effective separation of picograms of analytes from a ten orders of magnitude or more larger bulk of the solvent. Up to 25 μl of the derivatized extract can be processed at a time. Larger amounts can be handled by successive injections of 25-μl volumes at 1 min intervals but the blank starts to be a severe limitation [13].

Chromatographic separation

Non-polar phases have been recommended in the literature for the separation of tetraalkyllead species. Packed column usually filled with Chromosorb W containing a 3–10% loading of OV-101 or Carbowax which were used until recently [6] are being replaced by open-tubular megabore [14] or capillary columns [12,15,16] with polymethoxysilane coatings (DB-1, HP-1, RSL-150). Figure 2 shows examples of separation of butylated organolead compounds on packed, megabore and capillary column. Packed columns do



* very unstable, evidence for the presence only circumstantial

Fig. 1. Organolead compounds occurring in environmental samples.

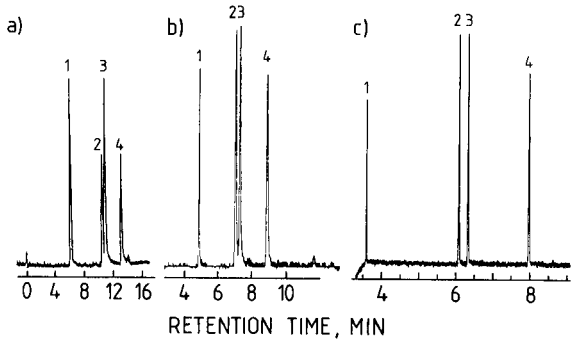


Fig. 2. Separation of butylated organolead cpmpounds: (a) packed column GC–QF-AAS, (b) megabore column GC–QF-AAS, (c) capillary column GC–MIP-AES. 1 = Me_3PbBu, 2 = Me_2PbBu_2, 3 = Et_3PbBu, 4 = Et_2PbBu_2.

not always allow for effective resolution between Me_2Pb^{2+} and Et_3Pb^+ when butylation is used as the derivatization technique. Propylated MeEtPb^{2+} may interfere in the determination of Et_3PbPr (with the same number of carbon atoms) but this problem is of minor importance and has never been investigated.

Atomic absorption spectrometry (AAS)

Quartz furnace (QF)-AAS has grown in maturity since the last review by Radojević [6]. Important novelties have concerned the development of an interface for the megabore [14] and capillary column [15]. The adaptation of the designs used earlier in the packed column GC (PGC)–QF-AAS coupling resulted in insufficient resolution (especially for Me_2Bu_2Pb and Et_3BuPb) and peak tailing [17]. The reason for these problems was probably the enhanced decomposition of analytes outside the atomization zone of the furnace due to a smaller linear velocity through the GC–AAS interface when a capillary column was used in place of a packed one. The extension of the interface heating zone to the edge of the detector casing in order to enhance the temperature gradient along the furnace side arm reduced most of these problems. A better understanding of the role of hydrogen radicals in the atomization mechanism of lead inside the quartz furnace enabled further improvements. They included addition of a heated quartz lined ceramic insert (acting as in situ hydride generator) to the interface and in consequence transferring the analytes as hydrides into the upper tube of quartz T-furnace [15].

For an optimized GC–QF-AAS system a detection limit of 1.6–2.3 pg was obtained which was a factor of 5 lower than ever reported before [18]. The megabore GC (MGC)–QF-AAS interface developed in our laboratory [14] showed an improvement in sensitivity by a factor of 2.5 over the PGC–QF-AAS interface [19]. Despite recent advances in coupling of the capillary column to the graphite furnace atomizer, GC–GF-AAS remains inferior to GC–QF-AAS both in terms of sensitivity and ease of operation. The detection limits were 8 pg (as Pb) for Me_4Pb and rapidly degraded for higher boiling species [20,21]. Problems associated with the long-term operation of graphite furnace at high temperatures make GC–GF-AAS inconvenient for routine analysis.

Though considerable improvements in the interface design all GC–AAS systems developed so far show a signal discrimination with the decreasing volatility of analytes.

Atomic emission spectrometry (AES)

Helium atmospheric pressure MIP had undergone considerable development in the last decade [22] which resulted in a commercially available atomic emission detector (AED) for gas chromatography [23,24]. Detection limits for lead usually quoted for laboratory-built instruments are in the sub-pg or low pg range [25,26], which is one order of magnitude lower than those of AAS. The commercial instrument offers many advantages over the earlier designs, particularly in terms of venting the solvent off the plasma, plasma stability and convenience of data handling. Furthermore, the use of a high performance monochromator with a photodiode array (PDA) spectrophotometer as a detector improved the sensitivity of the lead determination to the level of 0.03–0.1 pg depending on the compound [16]. Operating conditions are very critical especially with respect to the helium makeup gas and the hydrogen gas (acting as scavenger) flow rates. For example, reducing the makeup gas flow rate by 50% cuts the response by more than two orders of magnitude.

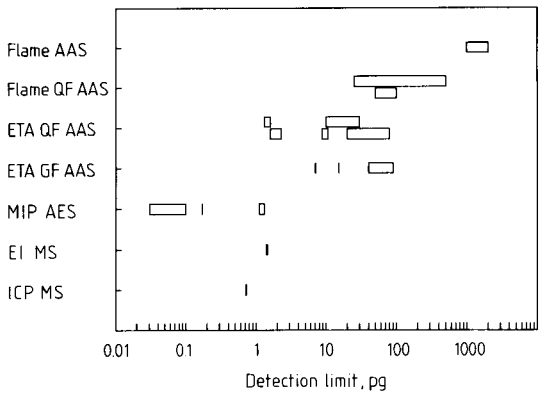


Fig. 3. Detection limits of various hyphenated techniques for speciation analysis of organolead.

Regarding the choice of the analytical wavelength the 283.306 nm line was found to be 5 times more sensitive than the 405.783 nm line in one work [25] while another [26] finds the latter wavelength the most sensitive. Apparently the relative sensitivity varies with the selectivity of the detector. The software controlling the data acquisition in the commercial instrument allows the measurement of lead emission at the 261.418 nm and 405.783 nm lines. The latter gives a response about three times as high as the former [16].

The PDA offers a possibility of confirmation of the elemental identity of the signals measured. This is done by recording the spectrum in the vicinity (± 20 nm) of the analytical line and comparing it with the pattern characteristic to a given element. This part of spectrum can be monitored continuously during the GC run indicating any spectral impurities when present.

Mass spectrometry

Reports on the use of mass spectrometry (MS) for detecting organolead in the GC effluent are

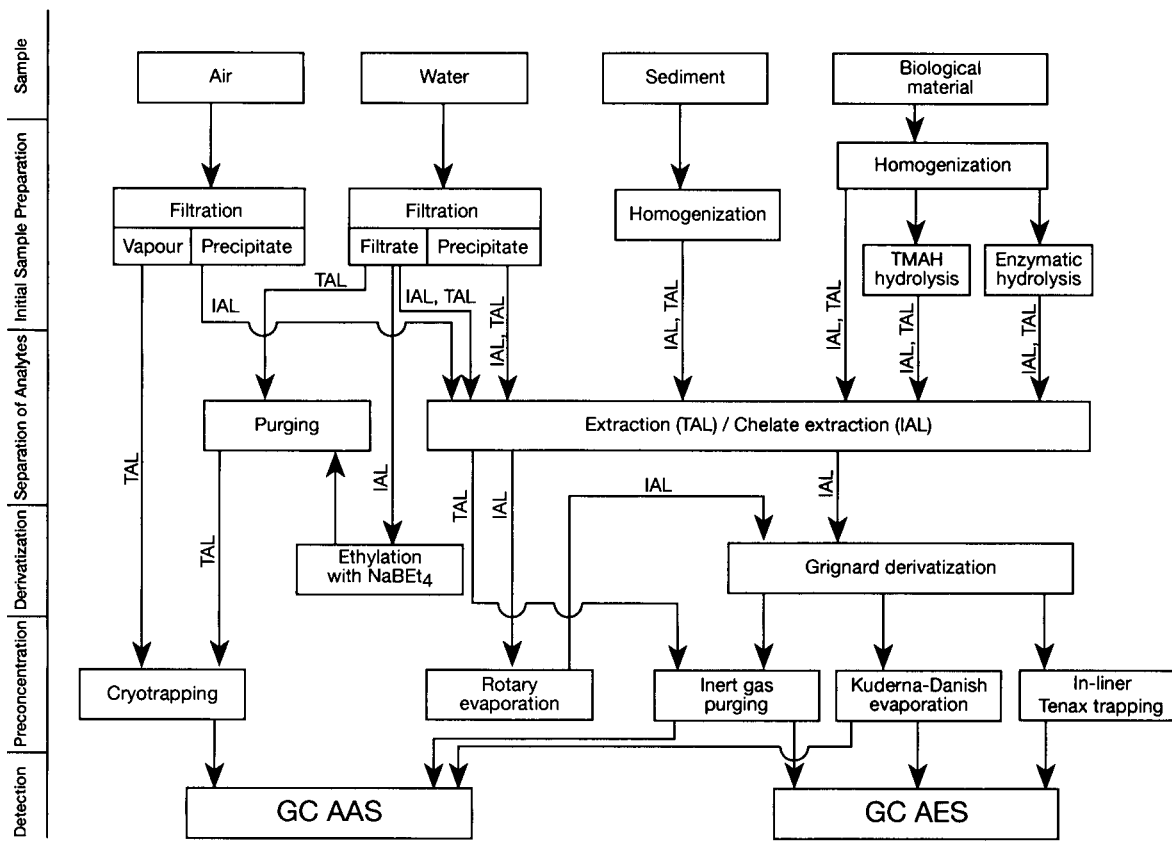


Fig. 4. Schematic layout of sample preparation for the speciation analysis of organolead.

very scanty despite potential attractiveness of this technique. Electron impact ionization mass spectrometry (EI-MS) in the single ion monitoring mode was reported to offer an absolute detection limit at the 1 pg level [27], which, together with large popularity of this technique in organic chemistry, makes it a valuable tool in speciation analysis. Similar detection characteristics are offered by CGC–ICP-MS, at the expense, however, of higher equipment and running costs and the necessity for a lab-made interface [28]. Sub-pg detection limits obtained with GC–MIP-MS for organotin [29] foreshadow a large potential of GC with plasma mass spectrometric detection also for lead.

ANALYTICAL METHODOLOGY. STATE OF THE ART

Determination of organolead compounds after GC separation

Gas chromatography compares favourably with LC in terms of the resolving power, ease of identification of unknown compounds and availability of sensitive detectors. Figure 3 shows a comparison of the absolute detection limits of GC-based hyphenated techniques proposed for lead speciation analysis.

Sample preparation

Speciation analysis for organolead requires an enrichment step due to very low concentrations of these pollutants in the biosphere which are not matched by the detection characteristics of even the state-of-the-art instrumentation. The complex nature of some types of samples, such as biological materials or sediments, makes the separation of the analytes from the sample matrix mandatory before a determination technique can be used. An additional step involving conversion of the analyte to a form amenable to GC (derivatization) is required for ionic organolead (IAL) compounds. A general layout of the sample preparation sequence is shown in Fig. 4.

Water samples. Tetraalkyllead (TAL) compounds may be quantitatively extracted into hexane [30] but have never been detected in real samples. IAL species are usually extracted as diethyldithiocarbamates [10,19,30,31] into an organic solvent and then propylated [12,16,30] or butylated [11,19,30,31] with a Grignard reagent. The amount of the sample taken for the analysis is dependent on the organolead concentration and the detection technique used. For the analysis of less polluted samples by AAS large volumes (up to 10 l) were reported [32] while a 100-ml sample is sufficient for GC–AED [12,16]. An enrichment step is needed unless a large volume injection technique [12] is used. Evaporation of the extract before derivatization is very effective for preconcentration of IAL at the expense, however, of the determination of TAL in the same run. Conditions for the enrichment of tetraalkyllead species by evaporation are very critical and losses for more volatile species can hardly be avoided. Recoveries are usually quantitative

TABLE 1

Speciation analysis for organolead in water [a]

Analyte	Separation/preconcentration	Derivatization	Detection technique	Experimental detection limit, ng l^{-1}	Application
IAL	DDTC extraction (pentane), rotary evaporation, dissolution in nonane	Butylation	QF-AAS	1.25– 2.5 [19]	Potable [32], rain [19,35], river and lake [35] water, snow [35]
IAL, TAL	DDTC extraction (benzene)	Butylation	QF-AAS	8	River water [36]
IAL, TAL	DDTC extraction (hexane)	Propylation [12,16,37–39], Butylation [16,33,34,39,40]	QF-AAS [33,34,37–40] MIP-AES [12,16]	2.8 –50 [33] 0.1 – 1.0 [38] 0.1 – 2 [12,16]	Drainage [34], surface [37], tap [12,16], potable [37] and rain [16,33,38–40] water, polar snow [12]

[a] IAL = Me_2Pb^{2+}, Et_2Pb^{2+}, Me_3Pb^+, Et_3Pb^+, $MeEtPb^+$, Me_2EtPb^+, $MeEt_2Pb^+$; TAL = $Me_nEt_{4-n}Pb$.

TABLE 2

Speciation analysis for organolead in dust, sediments, soil and aerosol [a]

Analyte	Separation/preconcentration	Derivatization	Detection technique	Experimental detection limit, ng g^{-1}	Application
IAL	DDTC extraction (pentane), rotary evaporation, dissolution in nonane	Butylation	QF-AAS		Soil [32], aerosol [43]
IAL, TAL	DDTC extraction (benzene), rotary evaporation	Butylation	QF-AAS	15 (wet weight)	River sediment [31,36]
IAL	DDTC, DMTC, APDC extraction (hexane), preconcentration under stream of N_2	Butylation, phenylation	QF-AAS		Soil, street dust [44]
IAL, TAL	DDTC extraction (hexane)	Butylation [33,41], propylation [38,41]	QF-AAS [33,38,41] Flame AAS [33,41]	0.6–10 (dust soil, sediments) [33], 1.3–35 (aerosol) [33]	Street and road dust, soil, sediments [31,33,41], aerosol [33,38], deposits [38]

[a] IAL = Me_2Pb^{2+}, Et_2Pb^{2+}, Me_3Pb^+, Et_3Pb^+, $MeEtPb^{2+}$, Me_2EtPb^+, $MeEt_2Pb^+$; TAL = $Me_nEt_{4-n}Pb$.

TABLE 3

Speciation analysis for organolead in biological materials [a]

Analyte	Sample preparation	Separation/ preconcentration	Derivatization	Detection technique	Experimental detection limit	Application [Ref.]
IAL, TAL	Homogenization, TMAH hydrolysis (60°C; 1–2 h)	DDTC extraction (benzene)	Butylation	QF-AAS	8 ng g^{-1} (wet weight)	Fish, clams, macrophytes [31,36]
Me_4Pb	Haemolysis by freezing (−20°C; 24 h)	Extraction (*n*-heptane)		GF-AAS	10 ng ml^{-1}	Blood [52]
Me_3Pb^+	Haemolysis by freezing	DDTC extraction (pentane)	Butylation	GF-AAS	3 ng ml^{-1}	Blood [21]
TAL, IAL	Homogenization, enzymatic (lipase VII, protease XIV) hydrolysis (37°C; 24–48 h)	Dithizone extraction (hexane)	Butylation	QF-AAS		Snails [51], birds organs and eggs [48–50] fish, shrimp, scallop [18]
IAL	Pulverization in liquid nitrogen by crushing in the mortar, TMAH hydrolysis (room temperature; 2 h)	DDTC extraction (pentane), rotary evaporation, dissolution in nonane	Butylation	QF-AAS		Grass, tree and shrub leaves [45]

[a] TMAH = tetramethylammonium hydroxide; IAL = Me_2Pb^{2+}, Et_2Pb^{2+}, Me_3Pb^+, Et_3Pb^+, $MeEtPb^{2+}$, Me_2EtPb^+, $MeEt_2Pb^+$; TAL = $Me_nEt_{4-n}Pb$.

though some authors observed lower extraction yields for dialkyllead species. Also for unfiltered samples adsorption losses on particulate matter may account for up to 20% of the tetraalkyllead present [33,34]. The principal procedures for organolead speciation analysis of water are summarized in Table 1.

Sediments, soil and particulate matter. Since alkyllead compounds are not involved in mineralogical processes the full decomposition of the matrix is not necessary. The separation methods of organolead from sediments and soils are exclusively based on solvent extraction.

Hexane is usually the solvent of choice due to its virtual insolubility in water, low boiling point and low viscosity, although some authors preferred benzene [31,36]. For extraction of IAL compounds a complexing agent, usually DDTC, is mandatory. EDTA is often added as an aid to disperse the sediment in a suspension and to facilitate the phase separation. Sodium benzoate was reported to increase the recoveries of the IAL species [31]. Extracts may contain suspended particles stabilized by the solvent [41] so centrifugation is recommended especially when they are further concentrated. Satisfactory yields are usually obtained for tetraalkyllead and trialkyllead species but not for dialkyllead. The reasons for losses are possible decomposition, irreversible adsorption or interactions with components of the sediment. Some soils contain sites capable of binding alkylleads (especially dialkylleads) to form hexane-soluble non-polar complexes resistant to the action of dilute acid [42]. Selected procedures for speciation analysis for organolead in sediment are summarized in Table 2.

Biological tissues. Earlier works applied direct extraction to release organolead compounds from tissue samples [31]. However, as organolead compounds may be incorporated in tissues of a living organism solubilization or decomposition of biological materials prior to separation of the analytes is necessary. A suitable digestion procedure should allow for complete destruction of the matrix while organolead species remain unchanged. Two principal approaches have so far been proposed. One involves digestion of the sample with tetramethylammonium hydroxide (TMAH) [31, 36,45] while the other is based on enzymatic hydrolysis with a mixture of lipase and protease [18,46–51]. Methods for the analysis of biological materials are summarized in Table 3.

ACCURACY OF THE ANALYSIS

Sampling and storage

Generally sampling and storage protocols recommended for the analysis of the particular type of sample for trace metals or volatile organic contaminants also apply for organolead. Some specific requirements are discussed below.

Water samples. The need for filtration prior to analysis is dependent on the level of particulate suspended matter. In unfiltered samples changes in the distribution of organolead may occur with time due to adsorption and desorption processes at the particles surface. Filtering ensures homogeneity of the analyzed sample but may lead to losses of volatile tetraalkylleads [31]. In general, filtration is recommended for samples with high particulate load like urban deposits, rivers, estuaries, shallow coastal waters and eutrophic lakes; it can be avoided for sea water, tap water and atmospheric deposits from remote environments.

Acidification of the samples is recommended to prevent losses by adsorption on the container walls. Unlike what occurs for inorganic lead this process does not seem to change the speciation of organolead. Preconditioning with the sample solution has to be avoided to prevent adsorption and accumulation of organolead compounds on the container surface [33,34]. Instead, having been emptied the container is treated with a portion of an organic solvent to release adsorbed organolead species which is then added to the extract.

Preservation of analytes in their authentic state before analysis may present difficulties. TAL compounds are likely to sorb onto glass walls [33,39], then decomposing on the active sites [53]. The IAL species are not adsorbed to an appreciable extent [39]. Organolead compounds are known to decompose in solution in a light-induced process promoted by microorganisms, suspended solids and various impurities [39,53,54]. TAL species are the least stable and degrade com-

pletely within few days [39]. Methyllead species are more stable than ethyllead and dialkyllead compounds are more stable than trialkyllead. Generally, no noticeable change of the IAL species in water samples stored in glass bottles at 4°C in the dark was observed for a period of up to 1–3 months [39,54]. It must be emphasized that decomposition rates dramatically depend on the origin of sample and very little is known on the stability of dilute solutions (below 10 ng l^{-1}).

Sediment and soil. Particulate matter suspended in natural waters can be collected by filtration (0.45 μm). For representative samples, however, very large volumes should be filtered bringing a risk of filter clogging so continuous-flow centrifugation is recommended.

Little is known about the stability of organolead in suspended matter and sediments during storage. Both chemically and microbially mediated degradation of Me_3PbCl was observed in sediment and soils from relatively pristine sites [48]. Freezing anoxic sediments was shown to cause very little change in the fractionation pattern for total lead analysis [55] and may be a useful storage procedure.

Biological materials. The need for rapid analysis or freezing the samples needs to be emphasized. Otherwise the enzymatic activity and natural proteolysis and autolysis processes will continue after sampling and can alter the speciation. The lipophilic character of organolead compounds favours their accumulation in particular tissues of living organisms so the dissection of the parts of interest and homogenization must precede the analysis. Blood samples were hemolyzed by freezing (−20°C) for at least 24 h [21]. Storage for extended periods under such conditions was found not to affect the stability of organolead initially present. Storage of samples in daylight should be avoided due to a hazard of considerable losses especially of ethyllead species [32].

Contamination risk

Precautions to avoid contamination from automotive sources need to be taken during sampling especially in the remote environment. Initial cleaning of containers is very important especially if the same containers are used for samples with different concentrations of the analytes.

At the level of laboratory contamination risks are generally not serious as organolead compounds usually occur in samples at concentrations well in excess of the atmospheric background. When ultratrace amounts, however, are determined, any work with concentrated standards in the same room is strongly discouraged; otherwise the persistent blanks remain for several days. Potential sources of contamination include the glassware and the electrodes used for pH measurements. Cleaning the glassware in hot concentrated HNO_3 proved to be efficient even after they were used to store concentrated organolead standards [13]. For the electrode it is recommended not to immerse it into solutions containing high levels of organolead.

Reagent impurities are removed by preextraction. A limiting factor is the purity of Grignard reagents which are not purifiable due to the low stability. PrMgCl was found to be the best choice in this respect [12]. Airborne contamination starts affecting the analysis at the levels below 0.1 pg g^{-1}. Analytical procedure blanks of 20–30 fg g^{-1} are common unless samples are not handled in clean bench cabinets [13]. While the efficiency of the HEPA filters used in clean rooms in removing particle adsorbed lead and organolead is beyond doubt, the degree of removal of the gas phase tetraalkyllead is unknown.

Losses

Due to their high vapour pressure tetraalkyllead compounds may be volatilized from samples. Hence, samples should be stored frozen if analysis cannot be done on the day of sampling. Whenever normalizing the concentration for weight is important, water content should be determined in a separate measurement as drying the sample prior to analysis will result in losses of organolead compounds. To avoid adsorption of TAL compounds onto the walls of sampling bottles and decomposition immediate extraction with hexane in the sampling bottles is recommended.

Standards

Only few organolead compounds: Me_3PbCl, Et_3PbCl, Me_4Pb and Et_4Pb are commercially

available to be used as standards. The synthesis of Me_2PbCl_2 and Et_2PbCl_2 was described in the literature [31,46,56]. The use of mixed methylethyl ionic standards has not been reported so far. These compounds were identified in some samples by prediction of retention times of the derivatized species using the Kovat's retention index [46] or on the basis of retention times of tetraalkyllead standards [57]. Actual retention times of the mixed compounds can be confirmed using transalkylation mixtures. Quantification of the mixed compounds is made by interpolation using the available standards. Discrimination of the response with the boiling point of the analyte must be taken into account.

Aqueous TAL standards should be prepared in ultrapure water just before use. Concentrated solutions of trialkyllead and dialkyllead can be stored in the dark for months without major deterioration. Working solutions should be prepared daily as dilute solutions demonstrate a slow degradation. After prolonged storage, redistribution reactions may give rise to the formation of mixed compounds if methyl- and ethyllead species are kept together. The rate of decomposition is increased by solar radiation and depends on the water quality; it is 20–50 times faster in distilled than in deionized water [54]. Since IAL salts decompose with time quantification is often done using derivatized standards.

The preparation of propylated and butylated di- and trialkyllead standards is described elsewhere [16,19]. The standards are quantified by converting the organolead to inorganic lead with a mixture of concentrated HNO_3 and $HClO_4$ and determining lead by flame AAS. The purity of the standards is controlled by GC–AAS or GC–AED by checking on the presence of other organolead peaks.

Interferences

Thanks to element selective detection following the chromatographic separation interferences in the speciation analysis are less frequent than in the analysis for total metals. However, extracts of samples rich in organic matter (proteins, lipids) usually contain coextractives which may affect the chromatographic separation and frequently lead to column clogging. Post derivatization cleanup (e.g. using alumina columns) is then necessary. An alternative is back extraction of the ionic compounds into the aqueous phase while the organic matter remains in the organic phase [10,42].

The side effect of Grignard derivatization is the formation of artefacts resulting in a number of peaks in the chromatogram not related to the determined species. However, when phenylation is used as the derivatization technique formation of artefacts corresponding to some analytes was reported [44]. The negative effect of artefacts present increase with the increasing sensitivity of analysis.

Validation of the results

In more than 90% of studies published till now organolead was determined by GC–AAS after chelate extraction followed by the Grignard derivatization. Neither the use of standard addition nor the reliance on synthetic standards can be regarded as the ultimate proof of accuracy as they do not always account for interactions between the analyte and the matrix. There is an urgent need for independent sample preparation procedures and comparison studies to throw more light on the accuracy of the published methods. On the level of sample preparation for gas chromatography hydride generation (restricted so far to trialkyl species) [58], ethylation (restricted to methyllead compounds) [59] and in situ butylation may offer alternative approaches. Recent developments in LC and wider availability of ICP-MS are expected to result in alternative methodologies. Issuing reliable standards is of paramount importance.

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Analytica Chimica Acta, 286 (1994) 391–399
Elsevier Science B.V., Amsterdam

Development of a coupled liquid chromatography–isotope dilution inductively coupled plasma mass spectrometry method for lead speciation

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(Received 9th February 1993; revised manuscript received 9th June 1993)

Abstract

A method has been developed for lead speciation using liquid chromatography with isotope dilution inductively coupled plasma mass spectrometry (LC–ID-ICP-MS). Inorganic lead (Pb^{2+}), trimethyllead (Me_3Pb^+) and triethyllead (Et_3Pb^+) were separated on a 5-μm Hypersil ODS column using a programmed gradient of 10:90 to 30:70 methanol–buffer eluent. The buffer was prepared by mixing equal amounts of 0.1 mol l^{-1} sodium acetate and acetic acid each containing 4 mmol l^{-1} sodium pentanesulfonic acid. To minimise the dead volume of the interface a single pass 40 ml volume spray chamber with a concentric glass nebuliser was used to coupled the LC and ICP-MS. Best precision isotope ratio measurements were obtained with peak jumping and short dwell times (80 μs). The overall detection limit (3σ) for trimethyllead ions was 0.48 ng g^{-1} as lead.

Keywords: Liquid chromatography; Inductively coupled plasma mass spectrometry; Lead; Isotope dilution

The toxicity of lead has long been recognised, and indeed is well documented, particularly for cases of accumulation in man [1]. However, it is now widely recognised that the form or "species" of an element is of the utmost importance when levels of that element in a sample are assessed for toxicological purposes. In the case of lead, organolead compounds are known to be more toxic than inorganic forms, and in addition, that the presence of these forms in environmental samples are predominantly due to anthropogenic sources. Of particular concern are the two main additives to petrol, tetramethyllead (TML) and tetraethyllead (TEL) which are known to accumulate along busy roadways. A knowledge of speciation is also important when assessing the mobility of lead in the environment. The Bureau of Community Reference (BCR) has therefore, as part of its programme to provide certified reference materials, undertaken to investigate the possibility of using isotope dilution mass spectrometry (IDMS) to enhance the accuracy of the determination of methylated and ethylated forms of lead. The ultimate aim of this study is to develop a method suitable for the determination of trimethyl- and triethyl-Pb in two candidate reference materials (artificial rainwater and urban road dust).

In order to speciate successfully and to quantify a mixture of lead species in a sample by elemental analytical techniques such as atomic spectrometry, a separation process must be employed prior to detection. One of the most widely used approaches is to couple the separative power of chromatography to the element specific detec-

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SSDI 0003-2670(93)E0371-D

tion offered by atomic spectrometry [2,3]. For lead, there is a large literature on the use of gas chromatography (GC) for separation followed by detection using flame or electrothermal atomization atomic absorption spectrometry (AAS), microwave induced plasma atomic emission spectrometry (MIP-AES), or inductively coupled plasma atomic emission spectrometry (ICP-AES) [4]. These GC techniques are to be recommended for tetraalkyllead species, however sample derivatisation (usually butylation) is required for the trialkyllead species of interest in this study. The use of liquid chromatography (LC) has also been reported for the separation of lead species, and again often coupled to AAS and ICP-AES for detection, although the sensitivity of both approaches is disappointing.

One of the more exciting advances in atomic spectrometry over the last ten years has been the development of commercial instruments using an inductively coupled plasma as an ion source for mass spectrometry [5–7]. Inductively coupled plasma mass spectrometry (ICP-MS) has most of the advantages of conventional ICP-AES instruments but with the additional advantages of better sensitivity, up to three orders of magnitude better for some elements. In addition the technique also offers the possibility of isotope ratio measurements and the opportunity to incorporate these measurements into an isotope dilution (ID) quantization procedure. The theory of isotope dilution analysis is outside the scope of this text, although the topic has been extensively covered elsewhere [8–10].

This paper reports on the development of a LC–ID-ICP-MS method suitable for trialkyllead species. The initial LC separation of the trialkyllead species was developed using AAS and then later transferred to ICP-MS as detector.

EXPERIMENTAL

Instrumentation

The initial chromatographic work was carried out using an isocratic HPLC pump (Model 3200, LDC Analytical, Riviera Beach, FL), an injection valve (Model U6K, Waters Assoc., Milford, MA) and an atomic absorption spectrometer (Model SP9, Philips Analytical, Cambridge). The eluent from the column (stainless steel, 25 cm × 4.2 mm i.d. packed with 5-μm Hypersil ODS, Jones Chromatography, Mid Glamorgan) was directly connected to the nebuliser of the spray chamber by a 15-cm length of capillary tubing. An air–acetylene, fuel lean flame was used. In these initial experiments the aim was to establish the "characterisation" of a separation system to separate inorganic lead (Pb^{2+}), trimethyllead (Me_3Pb^+) and triethyllead (Et_3Pb^+) chloride. The injection volume used was 100 μl and the concentrations studied were at the 50 μg ml^{-1} level. The separation was based on original work by Al-Rashdan et al. [11,12].

For the ICP-MS studies, a VG PlasmaQuad 2 instrument was used (Fisons Instruments, Cheshire). The operating conditions included a forward power of 1.5 kW, reflected power of < 5 kW, argon outer gas flow of 15 l min^{-1}, auxiliary gas flow of 0.7 l min^{-1} and a nebuliser gas flow of 0.73 l min^{-1}. A nickel sampler and skimmer of 1 mm orifice was used. Initially a high solids "V-groove" nebuliser was used with a standard Scott double-pass spray chamber however, these were later replaced with a standard Meinhard nebuliser and a single-pass 40-ml spray chamber of in-house construction. The spray chambers were cooled to at least -15°C using a refrigerated bath (Techne Refrigerated Bath RB5, Cambridge) circulating propan-2-ol. The tubing between the column and the nebuliser was reduced in internal diameter (0.2 mm) and length (30 cm). The ICP-MS system was optimised using 100 ng ml^{-1} Pb^{2+} solution in the presence of the acetate buffer LC mobile phase with monitoring at m/z 208. On a typical day the counts for this solution could be optimised to about 100–130 K by varying the various lenses on the mass spectrometer and torch sampling positions. In order to obtain quantitative results during a chromatographic run the commercial "single-ion monitoring" software was used on the ICP-MS instrument. The dwell time was set to 655 360 μs and the number of channels were varied depending upon the length of the chromatographic run time. Run times as long as 45 min could be facilitated under these conditions.

In the final optimised method an inert gradient LC pump (Varian 9010, Warrington) was used to obtain the most suitable conditions for the LC separation and quantitation by ICP-MS of the Me_3Pb^+ species. The sample volume was increased to 200 μl.

Reagents

All reagents used were of the highest purity available. The LC mobile phase composition was optimised experimentally and consisted of an acetate buffer containing 4 mmol l^{-1} sodium pentanesulfonic acid as an ion-pairing reagent (Eastman Kodak, UK, HPLC grade), and methanol (Rathburn Chemicals, UK, HPLC grade). The optimum acetate buffer strength was 0.1 mol l^{-1} and this solution was prepared by mixing equal amounts of solutions containing 0.1 mol l^{-1} sodium acetate and 0.1 mol l^{-1} acetic acid (Merck, Poole) each containing 4 mmol l^{-1} sodium pentanesulfonic acid. The pH of this buffer was 4.6. For the chromatographic development work, the final mobile phase composition was varied in methanol concentration to establish optimum separation of lead species as discussed later.

All acids used in cleaning glassware were of Aristar grade (Merck) and double-deionised 18 MΩ water was used throughout. Trimethyllead chloride and triethyllead chloride were purchased from Alfa Products (Johnson Matthey, Royston).

Preparation of enriched lead isotope standards

The standards required for the ID procedure were Me_3PbCl and Et_3PbCl. The most suitable isotope for enrichment in the ID procedure for Pb is ^{206}Pb. Standard reference materials were purchased from NIST (USA) for lead metal containing "normal" isotopic abundance (SRM 981) and two enriched ^{206}Pb SRMs (982 and 983). These were used as the starting material to prepare the organolead compounds.

The basic method involved the preparation of $PbCl_2$ from the Pb metal, the preparation of MeMgI or EtMgI (Grignard reagent) and reaction of both to obtain the methyl- or ethyllead compound. The compounds were purified by further recrystallisation from hot ethyl acetate to produce a white crystalline solid. Purity of the compounds was determined by comparing the nuclear magnetic resonance (NMR) spectra (270 MHz ^1H, ^{13}C and ^{207}Pb) of the prepared compounds to that of the commercially purchased alkyllead chloride compounds. No significant differences were noted even with a 100-fold scale expansion on the baseline.

A significant finding was that after three months storage in the dark, but at room temperature, the Et_3PbCl compound showed signs of decomposition. Initially this was observed as a slow colour change from pure white to an "off-white" colour. After six months the colour was brown. The brown component was insoluble in water and is suspected to be lead oxide. The NMR spectra of the water soluble component of the mixture showed strong Et_3PbCl components but also other carbon containing compounds. A further finding was that a quantity of Et_3PbCl prepared at the same time, but kept in a fridge, in the dark, showed no signs of decomposition. This is being investigated further.

Chromatography

Alkyllead compounds have been separated with reasonable success using LC by Al-Rashdan et al. [11] and Ebdon et al. [13]. In the former case the main problem encountered was the separation of the Me_3Pb^+ ion from the inorganic lead ion. In an attempt to optimise this separation both reversed-phase and ion-exchange separations were investigated [11]. Of the two, the reversed-phase separation using pentanesulfonic acid as an ion-pairing reagent was more suitable. However, the separation was still deemed to offer poor resolution. This work was carried out using a gradient LC separation with ICP-MS detection and it was concluded that the method could not easily distinguish between Pb^{2+} and other organolead species that eluted at or near the void volume. In real samples it is more likely that the vast majority of lead present in the sample is inorganic lead. Therefore, one of the main aims in this work was to optimise the separation of Pb^{2+} from Me_3Pb^+.

Signal processing for LC–ID-ICP-MS

At present no commercial software package is available for the complete LC–ID-ICP-MS pro-

cedure. However, it was possible to investigate the effects of certain instrumental parameters in order to maximise the precision of the isotope ratio measurement. The parameters studied included dwell times, number of measurements and the choice of peak jump or peak scanning modes of measurement.

RESULTS AND DISCUSSION

Data acquisition for LC–ID-ICP-MS

When a solution was aspirated continuously into the ICP-MS instrument used for this work, there were two basic measurement modes available to obtain isotopic data. These are peak jumping and peak scanning. The times of the dwell period and the number of peak jumps or scans can be varied. The results obtained in this study indicated that, in general, the best precision values were obtained with short dwell times (80 μs) with a high number of jumps or scans. Precisions of 0.1–0.2% were obtained in both modes of measurements however, the peak jump mode produced slightly better accuracy on a NIST SRM 981 standard solution.

In the complete LC–ID-ICP-MS procedure it is necessary to follow the chromatogram which can take up to 40 min, and also to measure the two lead isotopes (^{206}Pb, ^{208}Pb) continuously. Due to certain timing restrictions in the "transient" software of the instrument it is only practical to obtain data in the peak jumping mode of data acquisition so at least from this investigation of signal processing it has been established that these conditions are near optimum.

LC separation of inorganic lead from trialkyllead species

As discussed above some difficulties have been reported in the literature regarding the LC separation of Me_3Pb^+ and Pb^{2+}. The chromatographic development work in this study was carried out using an atomic absorption spectrometer since the performance of this detector is well characterised [13]. The eluent from the column was connected directly to the nebuliser by a short length of capillary tubing. It is important to note that the rise time of the sample liquid in an AAS spray chamber is about 6 s, that is, it takes that time for a solution to pass through the spray chamber to the flame to give a stable signal. So, although the volume of the spray chamber is relatively high (100 ml) the transport velocity of the aerosol through the spray chamber is also high (10 l min^{-1}).

The initial studies were performed using different mixtures of the aqueous buffer solutions (as described previously) and methanol to obtain the most suitable conditions for a separation under isocratic chromatographic conditions. Test solutions contained mixtures of Pb^{2+} and Me_3Pb^+ at concentrations of 50–100 μg ml^{-1}. The results can be summarised as follows:

(i) a methanol–buffer (70:30) mixture produced only one peak close to the solvent front;

(ii) a 50:50 mixture resulted in two unresolved peaks, the leading peak being inorganic Pb which was close to the solvent front;

(iii) a methanol–buffer (30:70) mixture produced two baseline-resolved peaks in 10 min but with the Me_3Pb^+ peak showing substantial tailing. A better separation was obtained using a 40:60 mixture as shown in Fig. 1A. The Et_3Pb^+ was then added into the chromatographic separation and Fig. 1B shows a chromatogram obtained with a 60:40 mobile phase composition. At lower methanol concentrations it was found that the Et_3Pb^+ took longer to elute and at higher concentrations merged with the Me_3Pb^+. Thus, at this stage the chromatography was "characterised" for Pb^{2+}, Me_3Pb^+ and Et_3Pb^+ using the AAS detector system. The optimum conditions for the chromatography indicated using a methanol–buffer (40:60) mixture for the Pb^{2+} and Me_3Pb^+ separation, and then a step to 60:40 to elute the Et_3Pb^+.

The expectation was that the chromatographic separation developed for LC–AAS would transfer, with only minor modifications, to LC–ICP-MS. Initially the eluent from the column was simply attached to a high solids "V" groove nebuliser fitted to a standard Scott double-pass glass spray chamber. The liquid flow-rate was 1.0 ml min^{-1}. A typical separation for Pb^{2+} and Me_3Pb^+ using methanol–buffer (40:60) is shown

in Fig. 2A. It can be seen that the baseline resolution previously achieved using the AAS system had been lost presumably due to the dead volume associated with the sample introduction system of the ICP-MS instrument. A separation could be achieved with a methanol–buffer (10:90) mobile phase, although it took up to 40 min for the Me_3Pb^+ to elute fully and the peak was 15 min wide with excessive tailing. The separation was not suitable for quantitation purposes.

The interface system was then investigated in order to reduce the dead volume between the end of the chromatographic column and the plasma. Close examination of the high solids nebuliser revealed a small dead volume between the capillary tubing "screw-in" end-cap and the nebuliser exit port. This was therefore replaced with a glass concentric nebuliser (Meinhard, Santa Ana, CA). The tubing from the end of the column to the nebuliser was reduced to 0.2 mm internal diameter and halved in length (35 cm). Although these were deemed to be positive steps, it was clear that most of the dead volume associated with the sample introduction system was due to

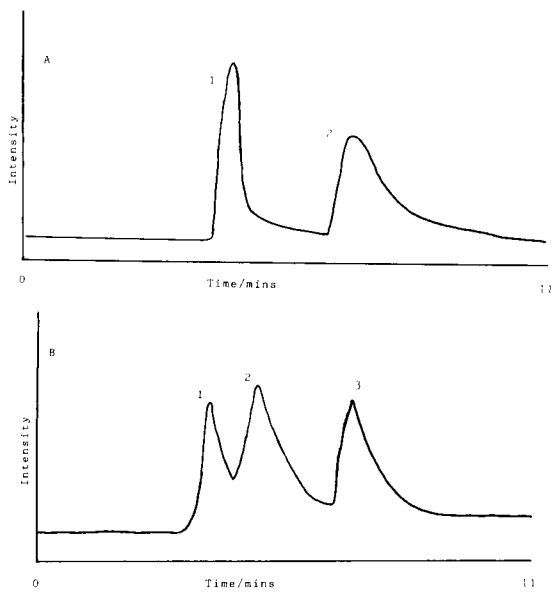

Fig. 1. LC–AAS using isocratic conditions of (A) methanol–buffer (40:60) and (B) methanol–buffer (60:40). Peaks 1, 2 and 3 correspond to Pb^{2+}, Me_3Pb^+ and Et_3Pb^+, respectively.

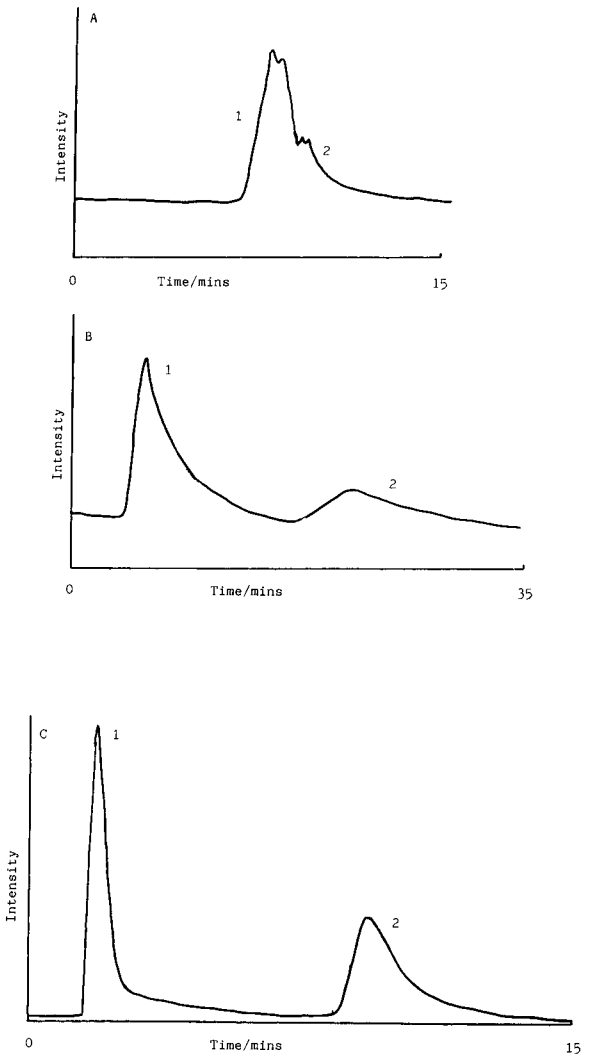

Fig. 2. LC–ICP-MS for (1) Pb^{2+} and (2) Me_3Pb^+. (A) Isocratic methanol to buffer ratio of 40:60, (B) isocratic methanol to buffer ratio of 17.5:82.5 and (C) gradient elution chromatography at 1 μg ml^{-1} concentrations.

the Scott double-pass spray chamber which had an internal volume of 120 ml. This was replaced with a single-pass glass spray chamber which had an internal volume of 40 ml. With the original system it was found to take 4–5 min for the signal to stabilise under conventional nebulisation conditions. This is significantly longer than would normally be expected, however the modified sample interface produced stable signals within 60 s.

Although this is clearly superior to the original system, it is still 8–10 times longer than the AAS system used in the early development work. This suggests that in terms of the chromatography, the use of ICP-MS would necessitate using compromise conditions to achieve baseline resolution and for further investigation of the sample introduction system.

Figure 2B shows a chromatogram run under isocratic conditions with a methanol to buffer ratio of 17.5 to 82.5 using the improved interface system. The separation of Pb^{2+} and Me_3Pb^+ took about 30 min and could be used for quantitative purposes although the Me_3Pb^+ peak still showed signs of tailing. These results demonstrate the improvement in the LC–ICP-MS separation possible following reduction of the dead volume in the interface system.

In order to refine the chromatography, the separation shown in Fig. 2B would clearly benefit from a gradient elution system. To this end a gradient LC system was employed and the result of a separation of 1 μg ml^{-1} Pb^{2+} and Me_3Pb^+ is shown in Fig. 2C. The separation starts with a methanol–buffer (10:90) mobile phase mixture for 2 min and after 10 min the mixture is 30:70 for a further 5 min. The peaks can be seen to be well resolved with a separation time of only 15 min. The gradient system used was investigated further.

Assessment of the blank in gradient elution LC–ICP-MS

From the chromatograms shown in Fig. 2A and B it is apparent that there is a significant blank value which appears as an elevated baseline. Figure 2C does not show a significant baseline shift only because the concentration of Pb was so high (1 μg ml^{-1}). At low levels (ng ml^{-1}) the blank would be problematical and was investigated. It was concluded that the elevated baseline was due to Pb since the signals observed (about 2000–4000 counts at m/z 208) showed the expected isotopic ratio values for Pb at m/z 204, 206, 207 and 208. The components of the mobile phase were individually analyzed by ICP-MS at the concentrations used (0.1 mol l^{-1} sodium acetate and acetic acid and 4 mmol l^{-1} sodium pentanesulfonic acid). The results showed that the acetic acid contained less than 0.25 ng ml^{-1} Pb but that the sodium acetate contained 1.7 ng ml^{-1} and the sodium pentanesulfonic acid contained 1.9 ng ml^{-1} of Pb.

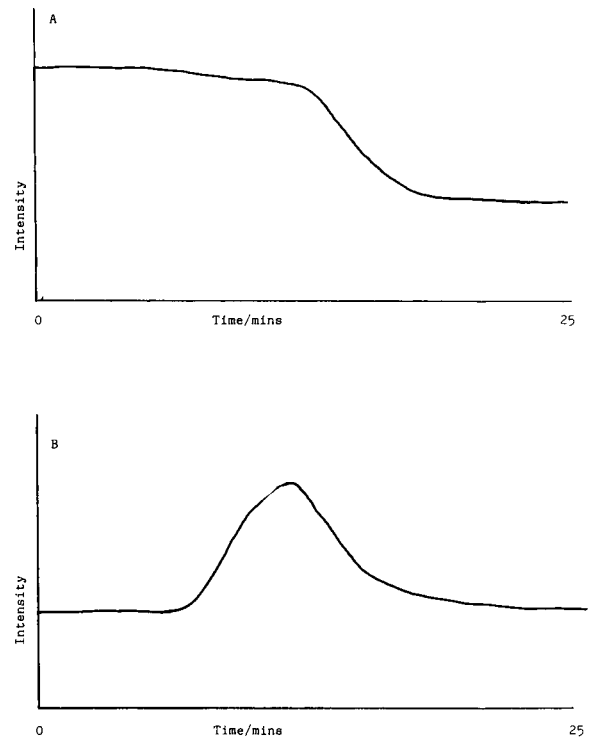

Fig. 3. Effect of methanol gradient on the baseline response in LC–ICP-MS. (A) ICP-MS optimised on an aqueous buffer containing 100 ng ml^{-1} Pb, (B) ICP-MS optimised on a solution containing 100 ng ml^{-1} Pb, 20% methanol and 80% buffer.

Figure 3A shows a chromatogram using only the LC pump to run a gradient. In this case the gradient was run as 0 to 3 min, methanol–buffer (10:90) and 3 to 10 min, methanol–buffer (30:70). The total run time was 1803 s. There was no sample added and no valve switching involved. The ICP-MS was optimised in this case, with an aqueous buffer solution containing 100 ng ml^{-1} Pb and then left for about 30 min to wash out the spray chamber. In an isocratic chromatographic run the baseline remains constant throughout the run. In the gradient elution case (Fig. 3A), the baseline remains constant until the start of the

methanol gradient, then decreases steadily until the end of the gradient, where it remains constant at this decreased sensitivity until the end of the run.

In order to explain this, individual solutions containing different concentrations of methanol (0–30%) and 50 ng ml^{-1} lead were investigated. The trend measured was that of a steady decrease in signal intensity of 0–65% in 0–30% methanol. In this first experiment the signal was optimised with an aqueous solution. Further experiments were carried out, but in each case the lens settings on the ICP-MS instrument were optimised for 0, 10, 20 or 30% methanol solutions, and then the series of 0–30% methanol solutions run. The results basically showed that the effect of methanol concentration on lead intensity in ICP-MS varied depending upon the lens settings of the ICP-MS instrument. As an example, the maximum signal obtained from a solution of 50 ng ml^{-1} lead in 20% methanol was obtained with different lens settings, especially the extraction lens, compared to an aqueous solution. A situation was reached that when the optimised conditions for the 20% methanol solution were used to run a gradient in LC, the signal at the beginning of the chromatographic run was suppressed. This situation is shown in Fig. 3B. In this blank gradient run, the baseline remains constant for a time then rises to a particular value, then follows the depressive response observed in the case shown in Fig. 3A.

The effect of methanol on lead is dependent upon lens settings, especially the extraction lens, which tends to suggest that the methanol changes the spatial position within the plasma where maximum lead ion concentration is found. Further experiments were carried out on this phenomena and the situation is far more complex than it first appeared. The effects vary depending on position of plasma, nebuliser gas flow and lens settings and further investigations into this area of research are continuing. In LC terms the baseline fluctuations are due to the effect of methanol on lead present as a contaminant in the buffer solutions and therefore can be minimised by employing a buffer "clean-up" stage. Further, the effects are only apparent when measuring near the detection limit for lead (i.e., at parts per billion levels). At high lead concentrations the effects are masked by the decreased electronic gains employed.

Final development of the proposed LC–ICP-MS separation of Me_3Pb^+ from Pb^{2+}

The chromatogram shown in Fig. 2C was obtained with Pb concentrations of 1 $\mu g\ ml^{-1}$ which resulted in counts in the region of 8×10^6. Due to this fact, the baseline problems are not apparent. At the levels of interest in environmental samples (ng ml^{-1}), the baseline variations and blank level are potential problems. The gradient

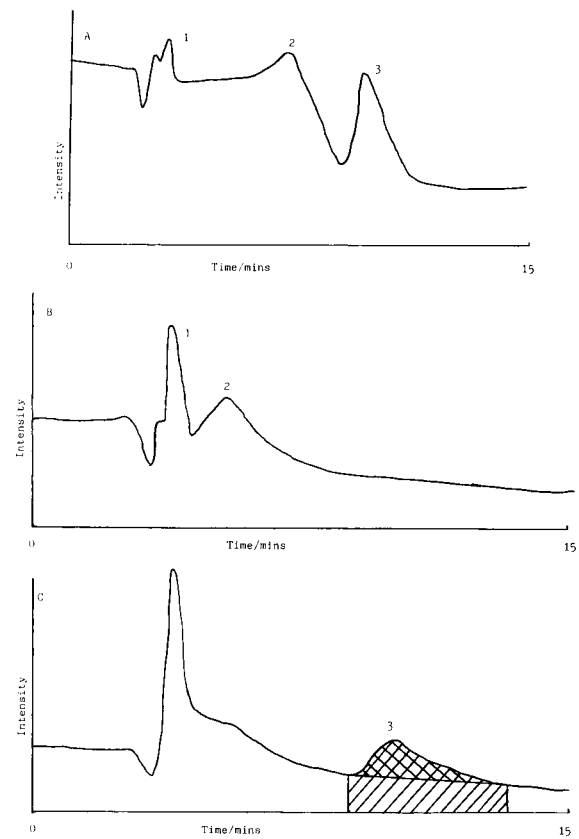

Fig. 4. LC–ICP-MS using gradient elution. Points 1, 2 and 3 show "system peak", methanol gradient, and Me_3Pb^+ peak, respectively. Chromatogram A highlights the need for a linear baseline during elution of the Me_3Pb^+ species, (B) sample blank under optimised gradient and (C) rainwater sample analyzed to assess accuracy of proposed procedure.

elution programme must be constructed in such a way that when the Me_3Pb^+ elutes, the baseline must be at least linear during the peak elution time for quantitative purposes. Figure 4A shows a situation where the baseline under the Me_3Pb^+ peak is not linear and highlights this problem. The gradient programme in this case was from 10:90 to 30:70 ratio of methanol to buffer between 4 to 7 min. The sample contained only 50 ng ml^{-1} Pb as Me_3Pb^+ (point 3 in Fig. 4A). Two other points, 1 and 2, shown in Fig. 4A are reproducible between different chromatograms when samples were loaded onto the LC column. The baseline fluctuation at point 1 corresponds to the sample injection time and was due to a "system peak". The small pressure pulse due to the sample switching valve possibly changes the plasma stability for a period producing this effect on the baseline. At point 2 in Fig. 4A the gradient is beginning to effect the methanol content in the plasma and hence effect the baseline. With respect to the separation and determination of Me_3Pb^+ from Pb^{2+} these baseline fluctuations are irrelevant.

Figure 4B and C shows the results of two chromatograms using a compromise gradient elution programme (Table 1). The aim was to achieve a fast separation between Pb^{2+} and Me_3Pb^+ but a linear baseline during the elution time of the Me_3Pb^+. Figure 4B shows the blank chromatogram with the "system peak", gradient curve and, towards the elution time of the Me_3Pb^+ peak (see Fig. 4C), a long linear baseline. The chromatogram in Fig. 4C was obtained with a sample containing approximately 50 ng ml^{-1} Pb as Me_3Pb^+ in the presence of an excess of Pb^{2+}. The determination of Me_3Pb^+ in this sample is discussed in the next section.

TABLE 1

Gradient elution programme in final method

Time (s)	Description	Methanol–buffer ratio
0–2	Isocratic	10:90
2–2	Step to	20:80
2–4	Isocratic	20:80
4–4	Step to	25:75
4–10	Gradient to	30:70
10–15	Isocratic	30:70
15	End	–

TABLE 2

Results for the rainwater sample for trimethyllead ion

Sample No.	Mean net signal/counts	Element content (ng g^{-1})	Precision/ R.S.D. (%) ($n = 6$)
1	285173	46.5	6.2
2	292008	47.0	4.7
3	303035	45.6	2.0
4	274914	43.0	2.8
5	259676	42.9	4.9

Assessment of accuracy of the LC–ICP-MS method

In order to assess the accuracy of the method, an artificial rainwater sample which had been spiked with an excess of Pb^{2+} and a known quantity of Me_3Pb^+ was analyzed. This sample was used in an interlaboratory exercise throughout Europe [14] to assess the accuracy of several different analytical techniques for Me_3Pb^+ determination. After 1000-fold dilution, the sample was said to contain "of the order of" 50 ng ml^{-1} Pb as Me_3Pb^+ in the presence of an excess of Pb^{2+}.

The analysis was carried out using the chromatographic conditions developed and the gradient programme shown in Table 1. The ICP-MS instrument conditions were optimised on an aqueous buffer containing 100 ng ml^{-1} Pb^{2+}. The sample volume used was 200 μl A blank and three standards containing 0, 28.1, 55.5 and 76.7 ng g^{-1} of Pb, as Me_3Pb Cl, were run in duplicate for calibration. Five separate aliquots of the sample were analyzed in duplicate, interspersed between either a blank or standard 2 (55.5 ng g^{-1}). The results obtained are shown in Table 2. The mean result obtained for the five separate sample aliquots was 45.0 ± 1.94 ng g^{-1} which compares favourably with values found by other laboratories [14] of 42.7 ± 4.8 ng g^{-1}. The blank produced a total background count of 5596 with a standard deviation of 1867 counts ($n = 6$). The results for standard 2 (55.5 ng g^{-1} Pb as Me_3Pb^+) gave a

mean signal of 346 645 counts with a percentage relative standard deviation of 0.68% ($n = 6$). The detection limit based on 3 times the standard deviation of the blank was 0.48 ng g^{-1} Pb (total) as Me_3Pb^+.

Conclusions

Several problems in the speciation of lead by LC–ID-ICP-MS have been identified and solutions found. The standards, often the biggest problem in IDA, have been prepared and purity verified, although the long term stability of the Et_3PbCl compound is questionable. The data acquisition procedure in the ICP-MS instrument has been investigated and the most suitable measurement criteria found. The problems reported for gradient elution chromatographic separations involving organic solvents are well known. If the separation can be performed using an isocratic mobile phase then that is to be recommended, however, as long as a stable baseline can be obtained during the elution time of the target species, a gradient can be used to sharpen the peaks and reduce separation time.

In the current study the only problem left unsolved is due to a lack of 'customised' software to process the measured data in LC–ID-ICP-MS. At present the software for the ICP-MS instrument was not designed to do LC-IDA. The mechanism to solve this problem has been identified and includes the exporting of the measured data to another computer, a format change to ASCII, and incorporation into a scientific graphics package (Fig. P, Biosoft, Cambridge).

One of the authors (AAB) would like to thank the Commission of the European Communities (Bureau Community Reference) for financial support of this work under BCR grant number 5486/1/9/348/91/06-BCR-UK(10). The authors would also like to thank Prof. J. Wardell and G. Spencer, University of Aberdeen, for synthesising the alkyllead compounds.

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Analytica Chimica Acta, 286 (1994) 401–409
Elsevier Science B.V., Amsterdam

Method comparison for the determination of labile aluminium species in natural waters

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(Received 17th February 1993; revised manuscript received 4th June 1993)

Abstract

An inter-laboratory comparison project to evaluate the portability of a standard method for the determination of aluminium species in waters and to investigate the probability of producing reference waters for aluminium speciation analysis is described. High-density polyethylene containers were found to be appropriate for the storage of water samples, intended for speciation analysis, after leaching with 10% (v/v) nitric acid for 48 h. A quality control programme for total aluminium in water samples was completed by all participating laboratories, showing the value of such a parallel programme, especially for aluminium with its inherent problems with contamination. Water samples (lake and tap) proved to be stable for up to 30 days, after which increasing pH caused aluminium hydroxy species to precipitate. A defined Driscoll–Pyrocatechol Violet fractionation method was found to be robust enough to be fully portable, the participating laboratories achieving relative standard deviations of 15% for the toxic "labile monomeric" aluminium fraction in the more stable water samples.

Keywords: Atomic absorption spectrometry; Flow injection; Aluminium; Quality control; Speciation; Waters

It is now well established that various forms of aluminium are toxic to a wide variety of living organisms. In humans, aluminium has been directly linked to the dialysis dementia syndrome [1] and implicated in various disorders suffered by patients undergoing regular haemodialysis [2]. Aluminium accumulation in the body has also been controversially linked to Alzheimer's disease [3]. High concentrations of aluminium have been shown to inhibit plant growth in acidic soils, resulting in low yields of certain commercial crops [4]. In the aquatic environment, with acid rain mobilizing aluminium from poorly buffered soils into water sources, much interest has been aroused in the effect of increased aluminium concentrations on natural water biota and fish populations [5,6].

It is now thought that the aluminium hydroxy species $Al(OH)_2^+$ [7,8] and $Al(OH)^{2+}$ [9] are the

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SSDI 0003-2670(93)E0370-M

toxic aluminium species with regard to fish populations. These species are thought to have two toxicological mechanisms. The first, at high concentrations and adequate pH, is a simple clogging of the gills by colloidal aluminium hydroxy species [9,10]. The second mechanism, at more modest aluminium levels ($< 200\ \mu g\ l^{-1}$), in waters with a low calcium content, increases the passive permeability of the branchial epithelium in the fish gills, hence increasing Na^+, K^+ and Cl^- efflux, causing fish to have difficulty in maintaining their osmoregulatory balance [10].

As no specific, direct method for the measurement of these toxic aluminium species exists, a number of fractionation procedures have been developed to distinguish between broad groups of aqueous aluminium species. All of the developed procedures usually measure operationally defined aluminium fractions which are not always coincident and thus makes direct comparison between published methods difficult. Up to now, proposed methods for measuring the toxic (or "labile monomeric") aluminium fraction in waters have been validated by a comparison of methods, but mostly by means of in-house laboratory trials. There have been attempts to standardize or validate different methodologies by inter-laboratory comparison exercises, but little or no information from these programmes has been published [11].

This paper presents the results and conclusions from an inter-laboratory project between three laboratories for the measurement of the "labile monomeric" aluminium fraction in waters. The four major aspects of the project were as follows: selection of appropriate sample storage containers; definition of an adequate cleaning regime of such containers for the storage of sample waters (destined for speciation analysis) for up to 1 year; elaboration of a quality control programme for total aluminium which was performed by all the participating laboratories; and analysis of six different sample waters for the "labile monomeric" aluminium fraction. This last analysis was performed by each laboratory using a defined "standard" Driscoll–Pyrocatechol Violet (PCV) fractionation method and an alternative method developed in-house by each participant.

EXPERIMENTAL

Reagents

All reagents and water samples were stored in high-density polyethylene containers, previously leached with 10% (v/v) nitric acid for 48 h, and filled with high-purity water until use.

All reagents and standards were prepared with high-purity water (18 MΩ) obtained with a Milli-Q system (Millipore) (or equivalent). An aluminium stock standard solution was prepared by dissolving 1.00 g of aluminium foil in 20 ml of H_2SO_4 (1 + 1). The resulting solution was diluted to 1 l with 50 ml of concentrated HNO_3 and high-purity water. Working standard solutions of $100\ \mu g\ l^{-1}$ were prepared by diluting 10 ml of this solution to 100 ml with HNO_3 (1 + 9).

All reagents used by all the participating laboratories were of analytical-reagent grade or better.

Description of samples

Two types of water samples were collected and divided into three subsamples each. Lake water (3×10 l) was collected from Lake Enol, Covadonga National Park, Asturias, Spain, and tap water (3×10 l) from the domestic water supply of the city of Oviedo, Asturias, Spain. All the samples were collected in precleaned, high-density polyethylene bottles, filtered through a 0.45-μm filter (Lida, Kenosha, WI, USA) and stored in the dark at ambient temperature.

The six 10-l subsamples (three lake water and three tap water) were then prepared as shown in Table 1. When required, additional aluminium was added (aluminium sulphate solution) and some solutions were acidified to pH 4.5–5.0 with H_2SO_4 to prevent precipitation and to mimic the pH found in natural acidic waters. From each 10-l subsample, 1-l aliquots were taken and stored in 1-l polyethylene containers, two of which from each subsample were sent to each participating laboratory for subsequent aluminium speciation analyses.

Homogeneity of total aluminium concentration of the 1-l samples was verified by electrothermal atomic absorption spectrometry (ETAAS) analysis [12].

TABLE 1

Description of subsamples used for the intercomparison exercise for the determination of aluminium species

Sample	Water type	Approximate Al concentration ($\mu g\ l^{-1}$) (as initially prepared)	Initial pH (as initially) prepared
A	Lake	25	6.6
B	Lake	100	6.6
C	Lake	370	4.5
D	Tap	75	7.0
E	Tap	240	7.0
F	Tap	1000	5.2

Driscoll–Pyrocatechol Violet (PCV) method

This was selected as the "reference method" and was developed from several different procedures already published, and has been published in full elsewhere [13]. The Driscoll–PCV method directly measures three operationally defined aluminium fractions in waters: acid reactive aluminium (Al_r), total monomeric aluminium (Al_{tm}) and non-labile monomeric aluminium (Al_{nl}). The Al_r fraction is the fraction as determined by PCV detection after acidification of the sample to pH 1.0 for 1 h. Al_{tm} is the fraction determined by PCV detection without prior acidification of the sample. Al_{nl} is the fraction determined by PCV detection without prior acidification after being passed through a column containing Amberlite IR-120 cation-exchange resin. The "labile monomeric" aluminium fraction (Al_{lm}) of interest in this study is taken as the difference between the Al_{tm} and Al_{nl} fractions.

The method finally adopted for al the participating laboratories included modifications such as a 10-min PCV reaction time [14], small (3.5-ml) samples [15], a 1-ml volume column of Amberlite IR-120 cation-exchange resin [14] and a split calibration procedure (0–100 and 100–400 μg Al l^{-1}) [16]. Another modification from the original Driscoll method [17] is that the analytical column for fractionation was conditioned with respect to its ionic composition from the Na^+ form and not from the H^+ form. This considerably reduced the time required for the whole procedure [13].

FIA–8-hydroxyquinoline-5-sulphonic acid (8-HQS) method (laboratory A)

This method is based on the Driscoll fractionation mechanism, but using 8-HQS in a micellar medium for the fluorimetric determination of the aluminium fractions. This method has been described in full elsewhere [13].

The main characteristics of the system, which can be seen in Fig. 1A, when compared with the Driscoll–PCV method are the use of a smaller analytical column of Amberlite IR-120 cation-exchange resin (shorter conditioning time), a smaller sample size (100 μl), a short reaction time (15 s), a lower detection limit (0.9 $\mu g\ l^{-1}$) and better precision (1.4% at the 50 μg Al l^{-1} level).

Liquid chromatographic (LC) method (laboratory B)

This method has already been published in full [18,19], and is shown here diagrammatically in Fig. 1B. A short (50-mm), low-capacity cation-exchange resin column is used (Dionex CG2), with 0.1 M K_2SO_4 adjusted to pH 3.0 as the eluent. The detection system involves postcolumn derivatization with 8-HQS and fluorescence detection. However, this technique separates the aluminium fluoride species from the other inorganic aluminium species, the significance of which will be discussed further under Results and Discussion.

Flow injection Driscoll–PCV method (laboratory C)

This alternative method is based on the automation of the Driscoll–PCV method by the development of a flow injection system (Fig. 1C). This has been achieved successfully by several workers [16,20], and the method used for the present investigation is based on this earlier work. The advantages are the use of relatively small sample volumes (μl), which in turn allow the use of smaller analytical columns with the subsequent advantages in conditioning time. The automation of the PCV detection method also allows an improvement in precision and thus lower detection limits and a more precise calculation of the Al_{lm} fraction is achievable.

RESULTS AND DISCUSSION

Selection of container material

Three types of container materials were chosen to be tested for their suitability to hold water samples for trace aluminium speciation. The cleaning of all containers was performed according to the recommendations of Laxen and Harrison [21], i.e., that a 48-h soak with 10% (v/v) HNO_3 was adequate for both the preliminary cleaning of new bottles and for the removal of trace metal contamination. Therefore, the procedure adopted for this programme was as follows: rinsing with distilled water to remove solid particles; leaching with 10% (v/v) HNO_3 for 48 h; rinsing with Milli-Q-purified water (or equivalent) and filling with ultra-pure water until use.

The aluminium content of the three plastics was determined by dissolution using concentrated H_2SO_4–H_2O_2 (30%, v/v) [22,23], followed by inductively coupled plasma atomic emission spectrometric detection. The results obtained were 4.8 ± 0.5, 18.5 ± 1.2 and 3.7 ± 0.4 μg Al g^{-1} for polyethylene, polypropylene and polystyrene, respectively. All of the values fell within the range of reported aluminium concentrations in plastics over the last 20 years [24,25]. It can be seen that polypropylene plastic containers contain a higher concentration of aluminium in the solid.

However, information on the possible leaching of traces of aluminium from these materials into any prepared solutions was also required. For this investigation, six solutions were prepared: three in 0.1 M HNO_3 (containing 0 and ca. 10 and 50 μg Al l^{-1}) and three in 0.1 M citric acid, containing the same concentrations of aluminium as for the HNO_3 samples. Nitric acid was chosen to test each plastic's susceptibility to leach aluminium under acidic conditions, and citric acid to test the ability of an organic ligand, in a less acidic environment, to prevent adsorption of aluminium on the container walls. The solutions were stored at ambient temperature in the dark and analysed by ETAAS [12] routinely over a period of time. Typical results for a solution of 0.1 M HNO_3 (in this instance containing 0.0 μg Al l^{-1}) for all three plastics are given in Table 2. It was found that both polystyrene and polypropylene leached aluminium in both the HNO_3 and citric acid media by the end of 30 days. However, the aluminium concentration of solutions in the high-density polyethylene material proved to be stable over the test period and over an extended period of 1 year. Therefore, high-density polyethylene was chosen as the container material for all subsequent aluminium-related work.

Quality control of total aluminium analyses

Because of the difficulty in determining trace amounts of aluminium (especially due to contamination problems), a quality control programme for total aluminium analysis was initiated to run in parallel with the speciation analysis programme, as a control of the participating laboratories' ability to determine trace amounts of aluminium.

Two water samples were prepared for the quality control programme, one containing ca. 11 μg Al l^{-1} (sample X) and the other ca. 55 μg Al l^{-1} (sample Z). These samples were prepared in 0.01 M HNO_3 and were stored in previously cleaned 0.5-l, high-density polyethylene containers. Each of the three laboratories analysed each

TABLE 2

Aluminium leaching data using 0.1 M HNO_3 for polyethylene, polypropylene and polystyrene (initial aluminium concentration 0.0 μg l^{-1}) [a,b]

Plastic	Day							
	0	1	2	15	30	90	210	360
Polyethylene	0.8 ± 2.2	-0.2 ± 0.3	0.2 ± 0.4	-1.0 ± 0.2	0.1 ± 0.2	-0.3 ± 0.6	-0.4 ± 0.5	0.0 ± 0.7
Polypropylene	1.1 ± 1.0	-1.3 ± 1.0	0.3 ± 1.3	2.3 ± 0.4	10.5 ± 1.0	–	–	–
Polystyrene	0.7 ± 1.6	-1.2 ± 3.6	0.6 ± 3.3	0.9 ± 2.2	5.7 ± 1.5	–	–	–

[a] Results are means ± standard deviations ($n = 3$) in μg l^{-1}. [b] Clearly the results for polyethylene are below the detection limit as are the results for polypropylene until day 15 and polystyrene until day 30.

TABLE 3

Quality control data: final means ± standard deviations as obtained form the participating laboratories

Laboratory	Al concentration ($\mu g\ l^{-1}$)	
	Sample X	Sample Z
A ($n = 10$)	10.5 ± 0.8	54.9 ± 0.9
B ($n = 8$)	10.3 ± 0.8	55.8 ± 4.1
C ($n = 12$)	11.2 ± 1.3	57.9 ± 1.7

sample periodically over the period of study (4 months) and the final quality control data are given in Table 3.

Using the Grubbs test for outliers, as recommended by Taylor [26], all of the results obtained from all the laboratories for both samples were considered to be of that data set at the 95% confidence level. Grand averages [26] were also calculated for both data sets and these were 10.6 ± 0.2 μg Al l^{-1} for sample X and 55.7 ± 0.2 μg Al l^{-1} for sample Z. From these results it can be concluded that all three laboratories were able to determine trace concentrations of aluminium correct without major problems due to contamination or poor precision.

Stability of total aluminium in sample waters A–F

The stability of the total aluminium content of each of the six sample waters (A–F) over a period of 1 year was evaluated by ETAAS [12]. The data obtained are given in Table 4. As can be seen, for all of the samples the concentration of Al, as determined by ETAAS, remained virtually constant for up to 30 days. After this period, with varying degrees of severeness, a decrease in the measurable aluminium was experienced. This decrease was matched by an increase in pH (7.5–8.0) for all the solutions. With this rise in sample pH over time, the aluminium loss was probably due to the precipitation of aluminium hydroxy species which were then not sampled for analysis by the ETAAS technique.

Stability of aluminium species in sample waters A–F

The stability of the aluminium species present in samples A–F, and of the portability of the standardized Driscoll–PCV speciation method, was determined by a four-round analysis programme. Each round of analysis, carried out in each of the three laboratories, consisted of six replicate analyses each of the sample waters for the Al_r (acid reactive) and Al_{lm} ("labile monomeric") aluminium fractions by the common Driscoll–PCV method and the Al_{lm} fraction by one of the other speciation methods developed and described earlier. The Al_{lm} fractions were calculated following the procedures recommended by Taylor [26].

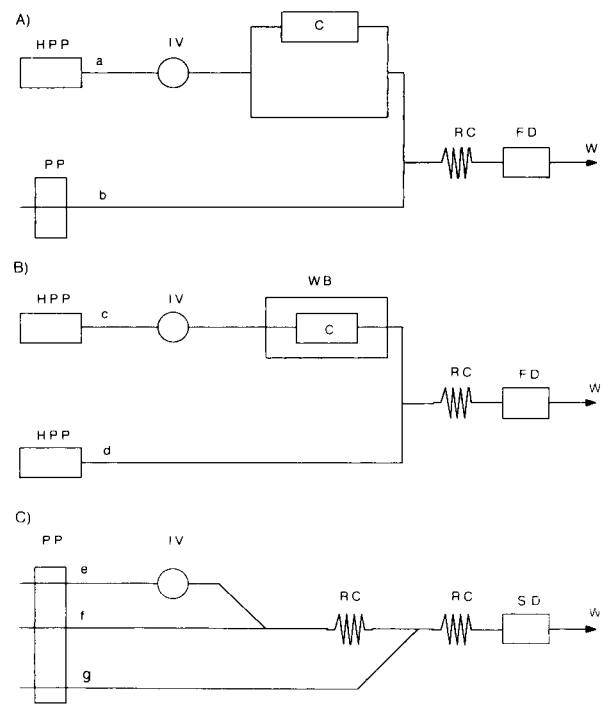

Fig. 1. Schematic diagrams of the three alternative aluminium speciation methods. (A) Flow injection–8-hydroxyquinoline-5-sulphonic acid (8-HQS); (B) HPLC; (C) flow injection–Driscoll–PCV (as set up for Al_{tm} determination). HPP = high-pressure pump; IV = injection valve; RC = reaction coil; FD = fluorescence detector; SD = spectrophotometric detector; W = waste; PP = peristaltic pump; C = column; a = NaCl; b = 8-HQS in cetyltrimethylammonium bromide and 1,10-phenanthroline solution; c = K_2SO_4 (pH 3.0); d = 8-HQS; e = 1,10-phenanthroline + hydroxylammonium chloride solution; f = PCV solution; g = hexamethylenetetramine buffer.

TABLE 4

Stability of total aluminium in sample waters A–F [a]

Sample	Day								
	0	1	2	15	30	60	120	240	360
A	25.8 ± 1.6	21.0 ± 2.1	23.4 ± 4.0	23.0 ± 0.9	25.1 ± 1.3	17.8 ± 1.4	18.3 ± 1.3	15.8 ± 0.3	14.9 ± 0.3
B	107 ± 0.7	108 ± 3.5	82.4 ± 0.3	83.9 ± 6.0	94.3 ± 2.1	64.7 ± 2.1	66.6 ± 4.8	66.7 ± 4.3	63.7 ± 2.2
C	370 ± 7.8	351 ± 8.5	357 ± 7.1	374 ± 23	472 ± 19	404 ± 9.2	344 ± 16	326 ± 9.2	302 ± 11
D	73.3 ± 3.1	73.4 ± 1.6	76.7 ± 0.6	82.2 ± 5.1	64.9 ± 3.6	84.9 ± 0.8	53.9 ± 0.9	37.4 ± 2.9	30.5 ± 1.1
E	240 ± 5.7	240 ± 4.9	249 ± 13	255 ± 1.4	240 ± 0.7	238 ± 0.6	217 ± 4.2	215 ± 13	209 ± 2.1
F	1026 ± 31	1114 ± 48	1097 ± 10	1065 ± 16	967 ± 86	943 ± 61	757 ± 61	443 ± 41	359 ± 9.2

[a] Results are mean Al concentrations ± standard deviation ($n = 3$) in $\mu g\ l^{-1}$.

Samples A and D

These two samples consisted of the original lake (25 μg Al l^{-1}) and tap (75 μg Al l^{-1}) waters without any additional aluminium spike. From the data submitted by the laboratories, it is clear that the PCV detection method was inadequate for these two samples, as many results for the Al_{nl} fraction could only be stated to be below the detection limit of the method. This was due to the initial low aluminium concentration and the lack of organic ligands to produce a measurable non-labile monomeric aluminium fraction in both samples. However, means of the values for the Al_r fraction of samples A and D were 13.5 μg Al $l^{-1} \pm 19\%$ ($n = 12$) and 10.5 μg Al $l^{-1} \pm 24\%$ ($n = 10$), respectively. These results are considered to be very good when taking into account that the concentrations of aluminium measured were extremely close to the detection limits of the PCV method.

Sample B

This proved to be one of the most promising samples, giving the most consistent results for all of the aluminium fractions as measured by the standard Driscoll–PCV method, as given in Table 5. The average Al_r fraction was 60.0 μg Al $l^{-1} \pm 11\%$. This sample also provided a good correlation between the Driscoll–PCV-calculated Al_{lm} fraction and the values obtained from the alternative methods. Disregarding the Driscoll–PCV result 1 from laboratory B (high) and Driscoll–PCV result 2 form laboratory C (low), the mean for all the Driscoll–PCV Al_{lm} measurements was 39.9 μg Al $l^{-1} \pm 14\%$ and that for the alternative Al_{lm} values was 48.7 μg Al $l^{-1} \pm 11\%$ (excluding result 4 from laboratory C). These two data sets therefore give an overall mean of 44.5 μg Al_{lm} $l^{-1} \pm 15\%$. These values are considered to be very acceptable in the light of the already described instability of the samples.

TABLE 5

Aluminium speciation results for sample B, all laboratories, using Driscoll–PCV and alternative methods

Laboratory	Analysis round	Aluminium fractions (μg l^{-1}) [a]		
		Driscoll–PCV technique		Alternative technique:
		Al_r	Al_{lm}	Al_{lm}
A	1	59.0 ± 4.0%	38.8 ± 5.2%	47.2 ± 1.1%
	2	54.3 ± 4.6%	40.2 ± 7.2%	47.7 ± 2.0%
	3	58.4 ± 3.7%	36.4 ± 5.9%	45.4 ± 1.8%
	4	60.5 ± 3.2%	34.6 ± 7.9%	46.3 ± 3.9%
B	1	79.4 ± 2.1%	63.8 ± 3.3%	51.2 ± 5.0%
	2	60.1 ± 1.0%	45.2 ± 6.4%	52.9 ± 7.0%
	3	62.4 ± 0.4%	51.6 ± 2.3%	41.5 ± 6.4%
	4	65.2 ± 0.9%	43.3 ± 3.3%	47.5 ± 8.3%
C	1	58.4 ± 4.7%	31.5 ± 10%	52.2 ± 1.0%
	2	56.1 ± 4.9%	26.5 ± 11%	59.5 ± 1.1%
	3	54.2 ± 3.4%	39.3 ± 8.2%	44.5 ± 0.9%
	4	59.7 ± 6.3%	38.2 ± 6.2%	17.3 ± 1.8%

[a] Mean results ± relative standard deviations ($n = 6$).

Samples C and F

These two samples received a far greater addition of aluminium than the other samples and thus experienced far worse aluminium hydroxy species precipitation problems. Even after careful coordination of the sampling method and fast analysis for these two samples (for rounds 3 and 4), few useful data were obtained by the three laboratories.

Sample E

Together with sample B, this sample provided some of the best results as regards the Al_{lm} fraction, as given in Table 6. The Driscoll–PCV method for the Al_{lm} fraction gave a mean of 84.4 μg Al $l^{-1} \pm 3.0\%$ (excluding result 1 from laboratory A) and the alternative methods gave a mean of 75.6 μg Al $l^{-1} \pm 17\%$. An overall mean of 78.9 μg Al_{lm} $l^{-1} \pm 14\%$ was obtained from the two data sets. These results could be improved upon if it is taken into consideration that the LC alternative method of laboratory B separates the aluminium fluoride species from the other inorganic aluminium species and therefore it gave consistently lower results than the other two alternative methods. As sample E was a tap water, it is reasonable to assume that it originally contained a significant proportion of aluminium fluoride (which was indeed reported by laboratory B). When these results are excluded from the mean of the Al_{lm} fraction, a value of 83.3 μg Al $l^{-1} \pm 6.8\%$ is obtained.

TABLE 6

Aluminium speciation results for sample E, all laboratories, using Driscoll–PCV and alternative methods

Laboratory	Analysis round	Aluminium fraction ($\mu g\ l^{-1}$) [a]		
		Driscoll–PCV technique		Alternative technique:
		Al_r	Al_{lm}	Al_{lm}
A	1	146 ±2.8%	18.6±27%	86.6± 0.7%
	2	140 ±0.4%	83.2± 3.1%	90.1± 1.3%
	3	145 ±2.0	82.7± 4.3%	82.1± 1.0%
	4	153 ±5.0%	87.4± 8.0%	75.9± 1.0%
B	1	104 ±3.6%	87.9± 2.1%	76.6±11%
	2	101 ±1.6%	84.8± 3.4%	63.3± 9.9%
	3	98.0±0.6%	80.8± 1.4%	51.1± 5.5%
	4	106 ±1.9%	84.3± 2.8%	57.2± 6.3
C	1	126 ±2.1%	NC [b]	85.3± 0.8%
	2	117 ±3.4%	NC	91.7± 0.5%
	3	78.1±4.7%	NC	70.1± 0.6%
	4	96.0±5.9%	NC	77.2± 0.8%

[a] Mean results ± relative standard deviations ($n = 6$)
[b] NC = non-calculable.

Conclusions

High-density polyethylene containers, cleaned using the procedure described, are adequate for the long-term storage (12 months) of adequately stabilized water samples collected for trace aluminium analysis. Stabilization and long-term storage of natural water samples such as those intended for aluminium speciation analysis are fraught with difficulties and may be unachievable in practice without the addition of chemical stabilizers. Samples containing 100–200 $\mu g\ l^{-1}$ of total Al provided the best speciation results, both for the standardized Driscoll–PCV method and for the alternative aluminium speciation methods used. A parallel quality control programme to determine each participating laboratory's ability to analyse water samples for their total aluminium content should always be undertaken in any intercomparison exercise such as this. Moreover, in the case of aluminium, it is strongly recommended that participating laboratories should all use an atomic spectrometric technique, preferably ETAAS, because many molecular techniques may fail to measure all the aluminium species present in any quality control sample [27]. This would especially be the case if the sample was to be used for both purposes. The Driscoll–PCV aluminium speciation method is portable and, although not ideal, is suitable as an initial standard aluminium speciation procedure. Its performance would have been better here if the samples had contained a significant fraction of non-labile monomeric aluminium (Al_{nl}), the samples had been more stable with time and a better specification of equipment and methodology had been followed in the different laboratories, e.g., if all the laboratories had been supplied with identical analytical columns.

B.F. acknowledges the Community Bureau of Reference (BCR, Brussels) for the provision of a post-doctoral grant, and financial assistance from CICYT, Spain (project PM88-0183-C02-01) is also acknowledged. The authors also thank Professors L. Ebdon (University of Plymouth) and M. Valcárcel (University of Córdoba) for their initial input into the project.

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Analytica Chimica Acta, 286 (1994) 411–421
Elsevier Science B.V., Amsterdam

Progress towards an x-ray emission method for the speciation of sulphur in coal using S K_β emission

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(Received 17th February 1993; revised manuscript received 9th June 1993)

Abstract

This article summarises the progress that has been made in the use of x-ray fluorescence spectroscopy to determine the chemical state of sulphur in coal. A set of sulphur K_β emission lines were recorded for a series of compounds of mineral and organic nature using a modified commercial wavelength dispersive spectrometer. Some of these emission lines will form the future base for the speciation of sulphur in coal.

Keywords: X-Ray fluorescence spectrometry; Coal; Sulphur speciation

Sulphur occurs in coal under variable proportions and various forms. When coal is burnt for power generation, the sulphur produces sulphur dioxide. These emissions cause extra maintenance cost to the industry and can contribute to acid rain. In order to reduce such emissions, either coal with a low concentration of sulphur ("low sulphur" coal) must be used or the sulphur should be removed from the coal before combustion, or from the waste gases after combustion. Much interest has been devoted to desulphurisation, combustion and post-combustion treatments in the prospect of utilising the huge resources of high-sulphur coal [1]. There is a growing need for a fast and reproducible technique to assess the sulphur distribution (or speciate) sulphur in coal.

Nature of sulphur in coal and its analysis

Coal is an organic sedimentary rock. It originates from plant material that accumulated under swamp conditions to form peat. The organic matter in peat undergoes decomposition by bacteria and chemical transformation (diagenesis). Sulphates can also be present if the swamp has been flooded by sea water. The bacteria reduce sulphur containing molecules to hydrogen sulphide and complex sulphides are also formed. These can be incorporated to organic matter or, if iron is available, precipitate with it to form pyrite and marcasite (FeS_2), and other sulphides in lower amounts. During the coalification process, the peat is buried and transformed by the action of moderate temperatures and high pressures over periods of millions of years. The rank, that is to say the calorific value of the coal, increases and the matrix becomes more aromatic. Coal macerals loose oxygen and hydrogen and nature of the organic sulphur changes.

The chemical forms of sulphur in coal can be broadly classified as pyritic and organic, the latter being sometimes divided into aromatic and aliphatic forms. Sulphates and elemental sulphur are also found. Some techniques to determine the chemical state of sulphur in coal are briefly described below.

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SSDI 0003-2670(93)E0369-I

The American Society for Testing and Materials (ASTM) technique [2] uses various acids to dissolve selectively the various forms of sulphur in coal, allowing the quantification of sulphur in terms of sulphate, pyritic and organic types. However errors increase with the presence of pyrite and non-pyritic iron [3].

Buchanan and Warfel [4] and Vorres [5] characterised the elemental and sulphate sulphur distribution in coal by solvent extraction and gas chromatography. They found that elemental sulphur and sulphates only occur in weathered coals.

Attar and Dupuis [6], using a thermokinetic reduction technique and comparing their results to those of model polymers, found thiols and thiolates in low rank coals. Whatever the rank, the majority of the sulphur found was to be of thiophenic type. A constant occurrence of aliphatic sulphides was found, suggesting that sulphides might have acted as an intermediate state between thiolic and thiophenic sulphur.

Rasa coal has a high organic sulphur content and hardly any pyrite (less than 1%). Since pyrite is often an interference in the analysis, this renders it the ideal starting point for the study of sulphur speciation [7–11].

White et al. [7] used a pyridine/toluene azeotrope for extraction of organic sulphur and analysed the extracts by high-resolution mass spectrometry. He found a wide range of aromatic sulphides, disulphides and thiophenic sulphur. White also found analogue compounds that underwent oxidation or molecules containing nitrogen atoms as well as sulphur. It is interesting to note that no thiols and aliphatic sulphur containing molecules were observed. Although these techniques use very sensitive modes of detection, the question must be asked: do the forms of sulphur interconvert during the analysis? Hence [3], the need for direct analysis.

Spectroscopic techniques provide various non destructive methods to achieve sulphur speciation. The analysis is effectuated in situ and the properties of the complex matrix are not altered. The bonding in molecules causes spectral shifts associated with the changes in the chemical environment. The distribution of sulphur in coal is assessed by comparing spectra from coal and model compounds with a similar type of bonding.

X-Ray photoelectron spectroscopy was used by Kelemen et al. [9] to speciate sulphur in coal using the large shift in the S2p ionisation energy (e.g., 5.3 eV between cysteine and sulphone). This technique is very sensitive to surface modifications and the analysis is made difficult by the low counting rate of sulphur 2p line and charging effects. Pyrite also hampers the analysis.

X-Ray absorption fine structure (XAFS) probes the transition from core electronic levels (1s, 2p in the case of sulphur) to non-occupied bonding electronic, or continuum states. An XAFS spectrum can be divided into two regions: the near edge fine structures or XANES up to about 30 eV above the absorption edge and the Extended region, or EXAFS covering about 300 eV above the absorption edge. EXAFS allows the atomic distances around the probed element to be determined whilst XANES [8–16] yields information concerning the bonding state of the atoms. Gorbaty and co-workers [8,10] used the sulphur *K* absorption edge to characterise organic sulphur in Rasa coal as either of sulphidic or thiophenic sulphur. They confirmed a majority of thiophene-like sulphur (70%), the rest of the sulphur being of sulphidic type [7]. Waldo et al. [12] found an increasing percentage of thiophenic sulphur when the rank increases. These conclusions confirmed those of Huffman and co-workers [13–15] who showed that thiophenic sulphur always predominates whatever the rank. However the authors always referred to benzyl sulfides to describe organic sulphide contents [8,14], justifying their assumption on EXAFS results [13]. In a more recent paper, Brown et al. [11], using sulphur *L* absorption edge in XANES technique, suggested that aryl sulphides would be present in Rasa Coal rather than alkyl sulphides. Sulphones, sulphoxides were found also in maceral separates and treated coals [12,15].

To date, the XAFS techniques are the most promising ways to assess the organic sulphur distribution in coal. For the moment, discord remains on the type of sulphides present in coal. In the future, these techniques will allow to the creation of a valuable pool of information that will compensate for the great variability of coal

and the lack of standards in coal technology, and will render coal banks [17–20] of even greater use.

Although XAFS spectroscopy is by far the most powerful technique, it must be admitted that it is not directly applicable to industry analysis since it requires a synchrotron as a source of radiation. X-Ray emission spectroscopy (XES) is a technique used widely for quantitative analysis on a routine basis. The implementation of high resolution could allow a conventional spectrometer to perform elemental speciation. Previous XES work in the characterisation of sulphur in coal is reported in the next section.

X-Ray fluorescence analysis

When a core level of an atom in a a molecule is ionised, the system can be stabilised by an electronic rearrangement from the outer levels to the core hole. The excess of energy can be released as an x-ray quantum of energy equal to the energy difference of the two levels involved. Not only this process is atom specific, it also obeys the electric dipole selection rule, that the angular momentum number cannot vary by anything else than one:

$$\Delta l = \pm 1 \quad (1)$$

and also

$$\Delta j = \pm 1, 0 \quad (2)$$

Hence, s vacancies attract only electrons from p levels. In turn, p vacancies will only be filled by electrons coming from s or d orbitals. All possible transitions that conform to these selection rules for sulphur are shown in Fig. 1. Both core and valence levels have been shown as split by j–j coupling. However, for the valence levels of sulphur ($n = 3$) this will be irrelevant, since the participation of the 3s and 3p (and possibly 3d) orbitals in molecular orbitals will be of much greater importance.

Valence or molecular orbitals (MOs) can be described in terms of LCAO combinations of atomic orbitals (AOs) of the atoms involved in the bonding. Each compound will be characterised by its own set of molecular orbitals, which vary in number, energy and AO composition as

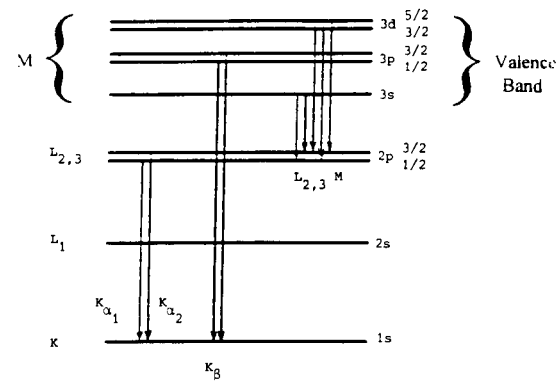

Fig. 1. Allowed x-ray transitions from sulphur atoms, assuming j–j coupling for all p and d levels (binding energy increases from top to bottom).

the bonding varies. It will be convenient to distinguish x-rays that arise from electronic relaxation from valence shell orbitals to core orbitals as valence x-rays (VXR).

The energy of any one VXR will be the difference in ionisation energies between the core orbital and a specific molecular orbital. Similarly the intensity of a VXR will depend upon the amount of the x-ray active atomic orbital present in that molecular orbital. It can be shown [21] that the VXR intensities are directly proportional to the squares of the AO coefficients in the LCAO representation of molecular orbitals. The extensive fine structure shown by any one VXR spectrum is therefore a direct reflection of the composition (in terms of one particular atomic orbital) of the molecular orbitals present in a molecule, and of their energies. At the simplest level, VXR spectra can be used as fingerprints to identify particular molecules or atomic environments. At a more sophisticated level they can be used to investigate the electronic structure of molecules. By contrast the x-rays that arise from core-core transitions (e.g., $K_{\alpha 1,2}$ for sulphur 2p $\rightarrow$ 1s) show only very small changes in either their energies or their peak profiles in response to changes in the chemical state of the emitting atom. This is because changes in valency or ligand environment, which alter the effective charge at the atom, cause changes in ionisation energy that are very nearly the same for all core orbitals.

In order to achieve the high resolution necessary to observe the VXR fine structure, or the very small energy shifts in other x-ray peaks, it is necessary that the x-ray beam should strike the diffracting crystal at as high an angle as possible. This is because the best wavelength dispersion is achieved at large values of θ (θ is the angle of reflection for an x-ray of wavelength λ incident upon a crystal with a periodicity d; n is the order of reflection).

$$\text{Bragg's Law, } n\lambda = 2d \sin \theta \tag{3}$$

therefore,

$$\text{dispersion} = \frac{d\theta}{d\lambda} = \frac{n}{2d \cos \theta} = \frac{\tan \theta}{\lambda} \tag{4}$$

Previous use of XES for sulphur speciation

The electronic configuration of the ground electronic state of the sulphur atom is $1s^2\ 2s^2\ 2p^6\ 3s^2\ 3p^4\ 3d^0$. The presence of free unoccupied 3d AOs allows sulphur to expand up to a valence state of +6. Dramatic changes occur in the electron population densities associated with sulphur 3s, 3p or 3d character, in the molecular orbitals of different sulphur containing molecules. And these changes are reflected in the sulphur x-ray emission spectra.

In the case of the S K_α line, generated by transitions from between the core orbitals 2p and 1s, only small chemical shifts can be observed, as discussed above. Even so, Pinkerton et al. [22] found shifts of 1.33 eV between inorganic sulfides and sulphate sulphur K_α emissions. He has used these displacements successfully to speciate sulphur in plant material.

The $L_{2,3}M$ emission line probes the S 3s and S 3d participation in the valence band orbitals after a transition of these electrons to the S 2p electronic level. Due to the low energy of the emission line (150 eV) it requires the use of a different excitation x-ray tube, is far more sensitive to surface effects, and so will not be considered further in this paper.

Finally, S K_β arises as the result of an electronic transition between the 1s molecular level of sulphur and the highest occupied molecular orbitals of σ, π-bonding and of non-bonding type containing S 3p character. Over the years, much work has been done to determine S 3p contribution to bonding in various inorganic and organic compounds [21,23–27]. If x-ray lines present low energy satellite(s) due to molecular orbitals of mainly ligand character, these lines are called $K_{\beta'}$. These spectral changes enabled Dolenko to identify many different sulphur compounds by their K_β emission profiles [23,24].

Hurley and White [28] used x-ray intensities at set positions corresponding to half maximum of reference peaks to speciate sulphur in coal in terms of organic and pyritic sulphur. The $K_\beta/K_{\beta'}$ ratio is then used to calculate the amount of sulphur present as sulphate. Mixtures of standards were used to establish a relationship between intensities and compositions. It is surprising that Hurley's total sulphur correlated so well with wet chemical results since measurements were taken at only seven wavelengths, and two of those were for background determination. However, attempts at speciation, the percentage of sulphur present as pyrite for example, were less successful, despite the very small particle size used (less than 44 μm). These results should be regarded as tentative in the light of the complexity of sulphur distribution in coal established by XAFS and this work.

Horn [27] used S K_β emission line to speciate sulphur in a coal of fairly large particle size. It was assumed that the observed S K_β spectrum was composed of just four components with sulphur being classified as sulphate, pyrite, organic-aliphatic and organic-aromatic. It was found that the intensity of S K_β emission had to be corrected by an empirical factor depending upon the chemical form of the sulphur. These factors were calculated by linear regression against the results from standard coals of known composition. Relative to organic sulphur the pyrite concentration obtained by XES was underestimated by a factor 2.4, and the sulphate concentration overestimated by a factor 1.7. Although in this work, advantage was taken of the whole spectrum for curve fitting the authors failed to consider that sulphoxides or sulphones might also be present in the coal sample.

This paper will present S K_β lines from various simple compounds, some of which will be used as fingerprints of the sulphur constituents in coal. It is hoped that these lines will identify the different types of sulphur present in coal and that these results will form the basis of an XRF method for the quantitative analysis of sulphur in coal.

EXPERIMENTAL

The XES spectrometer was a PW1410 manufactured by Philips operated at 10^{-1} Torr. The excitation was performed using a scandium anode x-ray tube (40 mA, 50 kV). X-Rays emitted from the sample were collimated into a flat beam (divergence 0.15° 2Θ), and, after diffraction, were detected with a gas flow proportional counter (90% argon–10% methane). The output from the counter was amplified and analysed on a Harwell 2000 series electronics before being counted on a CBM PET computer.

It is clearly not possible to reproduce exactly the chemical forms of organic sulphur in coal, but it is the immediate environment of sulphur that has the greatest effect upon the VXR spectrum. A series of compounds was therefore chosen for study which would represent sulphur in combinations such as R–SH, R–S–R′, R–S–S–R′ etc., where R and R′ are organic groups which could be either aliphatic or aromatic. Such model compounds were selected to represent the many different chemical environments of sulphur in coal, and are listed in Table 1. The organic samples were purchased from commercial suppliers (Aldrich and BDH) and were used without further purification. The mineral samples were made available by The British Museum – Natural History, London. The samples were mounted on a sulphur free substrate when solid at temperature of the analysis, but for liquid compounds a CF 100 Oxford Instruments cryostat was used so that they could be examined in the solid state [29]. For the analysis of the minerals, a fresh fracture was analysed. In the case of organic compounds, no sample deterioration could be detected by observing the shape of the emission lines of successive spectra.

As the purpose of this work was to measure S K_β spectra in high resolution, it was first necessary to ensure that no distortion in peak shape was introduced by the spectrometer and then to select the crystal which gave the best resolution. To effect these tests lithium sulphate was chosen. The sulphate ion is highly symmetric (tetrahedral, point group T_d) and the main S K_β peak arises from one energy level, a triply degenerate set of molecular orbitals (t_2). This peak should therefore be symmetric, and have a breadth due only to the following: (i) 1s core hole lifetime broadening, (ii) vibrational broadening of the final state, and (iii) spectrometer factors, viz. (a) divergence of the x-ray beam in passage through the collimator, and (b) imperfection in the crystal.

At high angles and with the "fine" collimation setting (giving a peak width $\pm 0.07°$ 2θ) it has been shown that horizontal divergence creates a "low-energy" tail which can be removed by the insertion of an auxiliary collimator [30]. The ef-

TABLE 1

Samples analysed in the course of this work

Mineral samples		Organic samples	
Pyrite	Li_2SO_4	Cystine	Dithiane [a]
Marcasite	Elemental sulphur	Thiophene [a]	Methionine
Pyrrhotite		Dibenzothiophene	Thioanisole [a]
Chalcopyrite		Di-*tert.*-butyl thiane [a]	Octane thiol [a]
Galena		Diphenyl sulphide [a]	Thiophenol [a]
Sphalerite		Polyphenylene sulphide	Diphenyl sulphone
		Di-*tert.*-butyl sulphide [a]	Diphenyl sulphoxide [a]

[a] Analysed using the cryostat at 100 K.

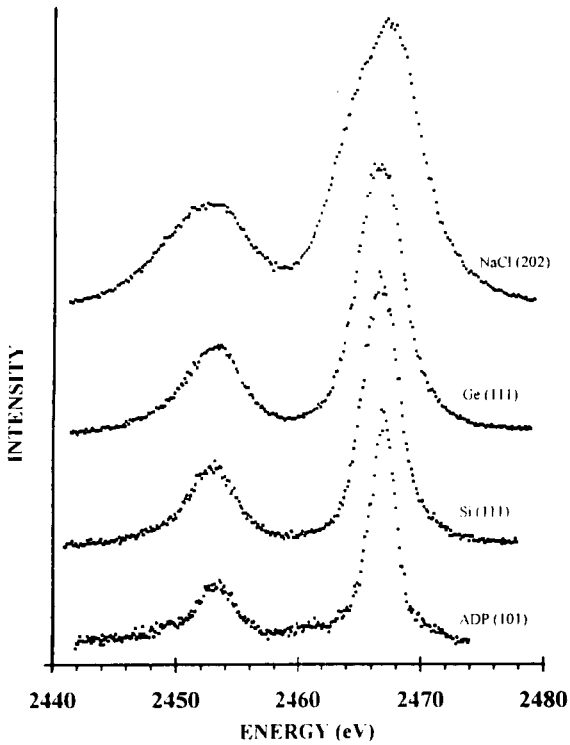

Fig. 2. S K_β emission from Li_2SO_4 diffracted on various crystals (peak intensity above the background for NaCl (202): 230 cps, Ge(III): 260 cps, Si(III): 160 cps, ADP (101), 2nd order: 40 cps).

fect of using this extra collimator is shown in Fig. 2. Whilst it has the desired effect of removing peak distortion it unfortunately also has the effect of reducing peak intensity by about two-thirds.

Four crystals, listed in Table 2, were tested and the results are shown in Fig. 2. As can be seen, the smallest "full-width at half-height"

TABLE 2

Characteristics of the crystals for S K_β diffraction

Crystal	d (nm)	Order	Angle (° 2Θ)	$E/\Delta E$	Possible contamination lines
NaCl (202)	0.2821	1	125.95	350	
Ge (111)	0.3266	1	106.51	590	
Si (111)	0.3135	1	100.98	710	Pb $M_{\alpha 1,2}$
ADP (101)	0.532	2	141.66	1000	Ca K_α (3rd order)

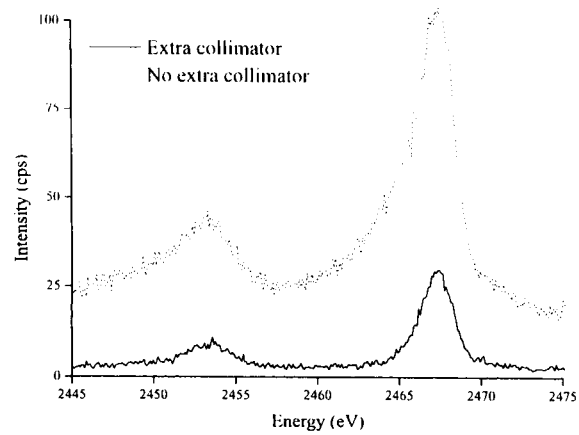

Fig. 3. Effect of the collimator on S K_β emission spectrum recorded an a ADP (101) crystal.

(FWHH) was achieved with the ammonium dihydrogen phosphate (ADP 101, [31]) crystal in the 2nd order but with low intensity. Sodium chloride gave a broad peak of only modest intensity. The most intense signals were obtained with silicon 111 [31] and germanium 111 [31]. As a slightly narrower peak width was observed with Si 111 it was chosen for the work reported below.

RESULTS AND DISCUSSION

As indicated above, the S K_β spectrum from lithium sulphate was used as a standard, and was calibrated against the spectrum reported by Kosuch et al. [25] S K_β 2467.4 eV. (The value observed by Hurley and White [28, Fig. 1] of 2440.0 eV, must be the result of a computational error, as all other determinations of the sulphur K_β energy lie in the range 2465 ± 3 eV whatever the chemical state of sulphur.)

The main peak in the sulphate S K_β spectrum is due to sulphur 3p character in the t_2 σ bonds formed by the overlap between S 3p and O 2p orbitals. The satellite (S $K_{\beta'}$) at 2453.4 eV reflects the presence of S 3p character in more tightly bound molecular orbitals that are mostly O 2s in character. This $K_{\beta'}$ peak can be used analytically as an indicator of the presence of S–O bonds [32] and the S K_β–$K_{\beta'}$ profile from sulphate to establish the presence of sulphate.

Mineral (sulphide) samples (Fig. 4a–d)

Whilst the use of Si-111 gives S K_β peaks of reasonable intensity, so that statistically significant spectra can be collected in a few minutes, the resolution is not the best that can be observed, even with a flat crystal spectrometer (Table 2). Despite this limitation, differences in peak profile can be discerned which reflect the underlying electronic structure in the sulphur anions.

Pyrite is found in coal in various textural forms [1,33,34]. Three pyrite samples with different morphologies (a pyrite cube, euhedral randomly-orientated crystallites and anhedral pyrite) and marcasite (Fig. 4a) were examined. All gave identical S K_β spectra. The sulphur–sulphur bond in the S_2^{2-} unit has filled σ, π and π^* orbitals, transitions from which all contribute to the S K_β peak leading to its breadth.

Whilst the other sulphide minerals that were studied, chalcopyrite (Fig. 4b), galena (Fig. 4c) and sphalerite (Fig. 4d) have structures with isolated sulphide anions (S^{2-}), broad S K_β peaks are still observed. In chalcopyrite the sulphur 3p orbitals interact with the 3d orbitals of both copper and iron which have different ionisation energies. The complexity of this interaction is reflected in the peak shape (main peak 2464.5 eV, shoulder 2468.5 eV). The S K_β from galena was difficult to record being superimposed on the high-energy trail of a lead M_α peak. Despite the problem, a simple symmetric peak is observed at 2466.5 eV. For sphalerite the S K_β peak is displaced to high energies (2467.5 eV) and is reasonably symmetric. The sloping background at low energies may be due to lead impurity [35]. In both samples the peak shape presumably reflects a simple bonding situation of coordination by a single type of cation (Pb^{2+} and Zn^{2+}, respectively).

Elemental sulphur (Fig. 4e)

The sulphur K_β spectrum for elemental sulphur is very broad (FWHH, 7.2 eV) due to the presence of sulphur 3p character in S–S bonds and also in the sulphur lone pair. The spectrum measured by Aksela et al. [36] using a grating spectrometer showed peaks separated from the highest energy K_β feature by 2.8 and 5.2 eV, indicating that the observed peak width is real and not an instrumental artifact.

Organic compounds (Fig. 4f–p)

In order to cover the wide variety of possible bonding environments that sulphur may adopt in coal, many different types of organo-sulphur compounds were investigated. For the most part, samples were chosen in which sulphur was present as S(II), although single examples of S(IV) (sulphoxide) and S(VI) (sulphone) were included. As the S K_β spectrum is generated by transitions from molecular orbitals with S 3p character, it is necessary to consider the participation of these orbitals in bond formation in order to be able to rationalise the shape, position and fine structure of the S K_β peak. The simplest picture of the bonding roles of S(II) 3p orbitals is that two of them contribute significantly to the formation of two σ bonds and that the third will house a lone pair of electrons perpendicular to the plane of the σ bonds. When S(II) is bound to an alkyl group and either hydrogen (octanethiol, Fig. 4n), sulphur (cystine, Fig. 4f) or another alkyl group (di-*tert.*-butyl sulphide, Fig. 4k) a broad (FWHH, ca. 6 eV) symmetric and featureless peak centred at about 2468 eV is observed. This may be rationalised as due to transitions from the least tightly bound lone pair orbital (2e) and from two rather more tightly bound (circa 3 eV) σ orbitals each with about 50% S 3p character.

A very different peak profile is observed if one of the groups bound to sulphur is aromatic. The peak itself is situated at about 2466 eV and has, at about 2468 eV, a high energy shoulder, the intensity of which, relative to the main peak, varies from compound to compound. The shoulder is at its most intense in diphenyl sulphide (Fig. 4j) and is also prominent in thiophenyl (Fig. 4m) and thioanisole (Fig. 4l). When sulphur is part of, rather than joined to, an aromatic system, the high energy shoulder is rather less intense, as in thiophene (Fig. 4g) and dibenzothiophene (Fig. 4h). Despite these changes in relative intensity, the presence of this high energy feature is clearly diagnostic of sulphur conjugation to an aromatic grouping and enables sulphur in such an environment to be distinguished from sulphur bound to

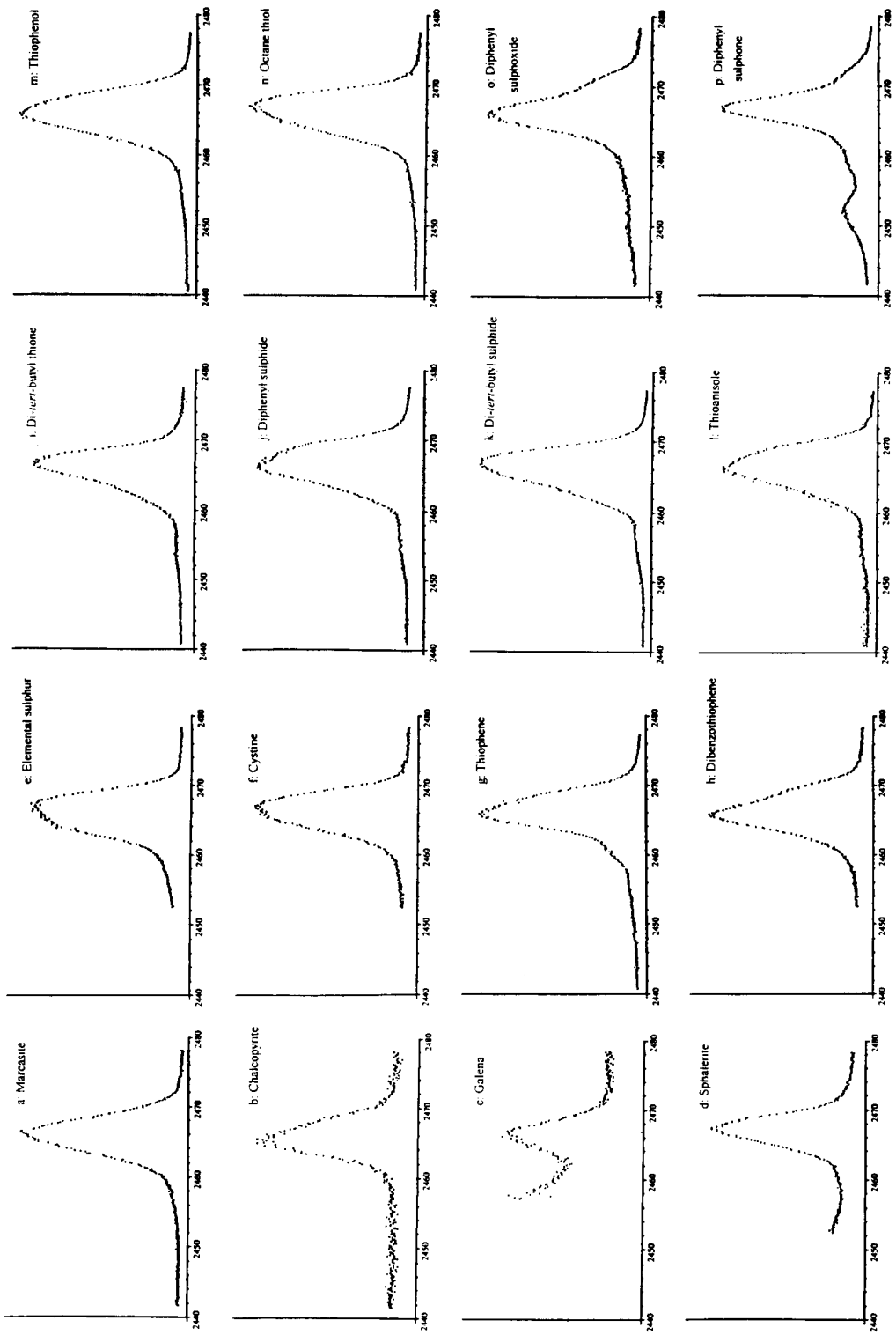

Fig. 4. S K_β emission from selected compounds. Horizontal axis: energy in eV; vertical axis: intensity.

alkyl groups. The changes that are observed in the relative intensity of the high energy peak are presumably due to different degrees of lone pair delocalisation around the aromatic ring system. It is interesting to note that this delocalisation would appear to be more efficient when sulphur is part of five membered ring as in thiophene and thiophene derivatives, than when it is simply bound to a benzene ring.

The contrast between sulphur bound in or to an aromatic ring and just forming a double bond to carbon is shown in the S K_β spectrum for di-*tert.*-butylthione (Fig. 4i). Here the main peak (FWHH, ca. 5 eV) is generated by transitions from both the lone pair and the π orbital. The peak width shows that the π orbital is more tightly bound than the lone pair by not more than about 2 eV. The remainder of the peak, between 2458 and 2464, may be understood as arising from S 3p character in the single C–S σ bond and to a lesser extent in S 3p participation in C–C bonds.

With an increase in formal valency, quite different S K_β peak profiles are observed. In diphenyl sulphoxide (Fig. 4o) sulphur retains one pair of "non-bonding" electrons but the low intensity of the high energy feature (now displaced to 2470), when contrasted with diphenyl sulphide, clearly shows the effect of forming the additional bond to oxygen, profoundly alters the nature of the "lone pair". Indeed, the persistence of a high energy feature in diphenyl sulphone – now at 2472 eV – where no lone pairs are to be ascribed to sulphur, suggests that the shoulder at 2470 in diphenyl sulphoxide might be due to other electronic interactions than the presence of a lone pair.

The increase in formal valency increases the tendency for "low-energy" satellites to be observed. They are most prominent in Fig. 4p and are due to the participation of S 3p in molecular orbitals that are mostly O 2s (2452 eV), and mostly C 2s (2459 eV) in character. Indeed, as has already been proposed [32], the existence of $K_{\beta'}$ satellites can be used analytically to establish the presence of bonds to specific ligands. It is interesting to note that in diphenyl sulphoxide, where the formal valency of sulphur is only four, the relative intensities of the $K_{\beta'}$ features are less than for diphenyl sulphone [S(VI)]. In part, for the "O 2s" peak at 2450–2452 eV, this is because in the sulphone, sulphur is bound to two oxygens whereas in the sulphoxide, only to one. But a reduction in intensity is also observed for the "C 2s" $K_{\beta'}$ peak at 2457–2459. It is also significant that the comparable peak in, S(II) spectra, i.e., at some 8–10 eV less than the main K_β peak, can only be observed as a featureless low energy tail, just above background (2456 $\pm$ 2 eV, Fig. 4g, i and n). As bond lengths generally decrease with increasing valency, it seems reasonable to suggest that the ability of S 3p orbitals to overlap with ligand valence shell s orbitals would also increase with valency thus enhancing the contribution that 3p character could make to those orbitals. There is also an overall shift to higher energies of the main S K_β peak, with an increase in formal valency [S(II), S(IV), S(VI)]. This is due to the increase in the S 1s ionisation energy. From x-ray photoelectron experiments it has been shown that the S 2p ionisation energy increases as follows: S $2p_{1/2,3/2}$ (average values), S(II) 162 eV, S(IV) 167 eV, and S(VI) 169 eV, and since the S K_α emission energy changes by no more than a volt with valence state, it follows that the S 1s level changes by about 6 eV in going from S(II) to S(VI). This change will result in the S K_β x-ray energy increasing by a similar amount as the valency of sulphur increases.

Prospects for coal analysis

The changes observed in the S K_β peak profile, even using a Si-111 crystal, are quite sufficient for the speciation of sulphur, at least into specific types of chemical combination; sulphate, sulphone, pyrite, mineral sulphide, sulphur, aliphatic-sulphur and aromatic-sulphur (i.e., sulphur in, or bound to an aromatic ring). The error associated with the measurement of each type of S K_β peak profile can be seen from the spread of data points in each spectrum of Fig. 4. A detailed analysis of the types of sulphur present in coal would require the measurement of S K_β spectra of at least comparable quality. Using compounds selected from Table 1 it is hoped to be able to establish a collection of basic S K_β profiles which

can be used to analyse observed S K_β spectra from coal using curve fitting procedures. For the next step it may then be possible to reduce the number measurements from a whole spectrum to a dozen or so readings at specific wavelengths.

Whilst this seems to be an achievable aim, it must be admitted that greater problems for in situ sulphur speciation by XRF lie in the heterogeneous nature of the material to be analysed. Coal is a mixture of minerals and macerels of various types. And whilst chemical bonding affects VXR emission, the nature of the particle from which the x-ray must emerge and the matrix through which it will subsequently pass can both alter the observed S K_β profile, and overall K_β intensity. These alterations can be due to variations in scattering power and specific absorption processes which will be different for a carbon-rich matrix and a pyrite particle.

These effects should be minimised if very small particle size is used and the samples are thoroughly homogenised. Huffman, using the XANES K absorption K edge [14] has claimed that particle size effects can be eliminated if samples are ground to a particle size of less than 40 μm. This observation is a good guide for S K_β emission analysis.

A further problem might be introduced by small concentrations of impurities which may have absorption edges at somewhat lower energies than S K_β. The detection and estimation of such impurities should, however, pose no problem for XRF analysis.

Conclusions

X-Ray emission of the valence band of sulphur containing compounds constitutes a fingerprint of sulphur in a particular bonding state. On a commercial XRF spectrometer, high-resolution analysis of S K_β emission lines can be achieved by diffracting the emitted x-rays at high angle. However, instrumental peak distortion occurs and an extra collimator has to be used to correct for lateral divergence, reducing then the intensity of the signal.

Of all the crystals tested, ADP-101 had the best resolving power but was not suitable for the speciation of sulphur in coal (present in concentrations of the order of 1–10%) due to its low reflectivity. The Si-111 crystal is a compromise between resolution and reflected intensity. Compounds that represent the various types of sulphur present in coal were examined for their S K_β emission; the spectral differences that were found were attributed to the bonding with adjacent atoms.

In the case of mixtures of various types of sulphur as in of coal, a reconstruction of S K_β emission peak using model compounds will be attempted and the emitted intensities will be related to relative compositions. Several assumptions will have to be made and precautions taken. The choice of the model compounds will have to be made with scrutiny with the help of in situ techniques such as XAFS. The relation between (relative) concentration and emitted intensity will have to be assessed and efficiencies established. These might depend on factors such as particle size distribution, mineralogy, nature or the matrix, or impurities. The influence of these parameters will have to be assessed and interferences catered for.

The authors would like to express their thanks to the Community Bureau of Reference which founded this project (Grant No. B/90000937) and to The Natural History Museum for the gift of mineral samples.

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Analytica Chimica Acta, 286 (1994) 423–429
Elsevier Science B.V., Amsterdam

Three-stage sequential extraction procedure for the determination of metals in river sediments

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(Received 17th February 1993; revised manuscript received 27th July 1993)

Abstract

A three-stage sequential extraction procedure, following a protocol proposed by a European working group coordinated and supported by the Community Bureau of Reference (BCR), has been applied to two river sediments from an industrial region of East Catalonia, Spain. The extractant solutions were: Step One, acetic acid (0.11 mol l^{-1}); Step Two, hydroxylammonium chloride (0.1 mol l^{-1}, pH 2); Step Three, hydrogen peroxide (8.8 mol l^{-1}) oxidation followed by extraction with ammonium acetate (1 mol l^{-1}, pH 2). The six elements determined in the extracts were Cd, Cr, Cu, Ni, Pb and Zn. Analysis of the extracts was carried out by flame atomic absorption spectrometry (FAAS). There were slight differences in analyte sensitivity between calibrants prepared in the extractant matrices and acidified aqueous calibrants. No significant matrix interferences were found except for Cr in the acetic acid and hydroxylammonium chloride extracts, which required determination by the standard additions method. The other elements (plus all the elements in the ammonium acetate extracts) were determined by direct calibration against calibrant solutions prepared in the same matrices as the samples.

Keywords: Atomic absorption spectrometry; Sequential extraction; river sediments

The ability to determine the chemical forms of heavy metals in marine and freshwater sediments is becoming increasingly important. It is also necessary to assess the potential of the sediments as either a sink or a source of heavy metals in the aquatic environment [1]. The determination of total concentrations in sediments does not give sufficient information about the mobility of metals, hence there is a need to characterise element availability.

Methods for determining the different forms of metals in sediments include sequential extraction, whereby a series of single reagents is used to extract operationally defined phases from the sediment in a defined sequence [2]. Many of the sequential extraction schemes employed are based on the five-stage Tessier [3] protocol or modifications thereof [4]. Other strategies that can be employed involve single extraction with or without progressive acidification [1]. Progressive acidification is a means of assessing the possibility of metal mobilisation from sediments brought about by pH changes in the environment. Possible causes of pH changes include industrial inputs and acid rain. Single extraction methods aim to remove metals from specific solid phases (e.g., exchangeable, carbonate, reducible, organic) in the sediment.

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SSDI 0003-2670(93)E0441-9

TABLE 1

List of extractants used at each stage in the sequential extraction scheme and the sediment phases extracted

Extraction step	Reagent/concentration/pH	Sediment phase
First	Acetic acid CH_3COOH (0.11 mol l^{-1})	Acid soluble (e.g., carbonates)
Second	Hydroxylammonium chloride $NH_2OH \cdot HCl$ (0.1 mol l^{-1}) pH 2	Reducible (e.g., iron/manganese oxides)
Third	Hydrogen peroxide H_2O_2 (8.8 mol l^{-1}) followed by extraction with ammonium acetate NH_4OOCCH_3 (1 mol l^{-1}) pH 2	Oxidisable (e.g., organic matter and sulfides)

The work described in this paper is a study of the amounts of the metals extracted at each stage in a sequential extraction scheme. The three-stage extraction procedure followed the protocol proposed by a European working group coordinated and supported by the Community Bureau of Reference (BCR) at the workshop on sequential extraction in soil and sediments (SESS) held in Sitges, Spain on 29 March to 1 April 1992 [5]. The extraction scheme is an operationally defined procedure [6] in which the reagent used at each stage is intended to release metals associated with particular sediment phases (Table 1). The sediment extracts were analysed by flame atomic absorption spectrometry (FAAS). The occurrence of interferences in the flame due to the extractant and sediment matrices was also investigated.

EXPERIMENTAL

Instrumentation

A Perkin-Elmer 1100B flame atomic absorption spectrophotometer with deuterium background correction and Perkin-Elmer hollow cathode lamps were used for the analysis of the sediment extracts. The operational parameters, recommended by the manufacturer, are shown in Table 2. A fuel-lean air–acetylene flame was used for all the analytes except Cr, which required stoichiometric flame gas conditions [7].

Reagents

All reagents were Merck analytical grade or Suprapur grade. Stock solutions containing 1000 mg l^{-1} of the analyte were prepared in the laboratory. Solutions of Cd, Ni and Pb were prepared from nitrate salts, Cr from $K_2Cr_2O_7$, Cu from CuO and Zn was prepared from the metal. All calibrants and reagent solutions were stored in polyethylene containers. Double-deionised water (Culligan Ultrapure GS 18.3 Mohm cm^{-1} resistivity) was used for all dilutions.

Calibrant solutions

Multielement calibrant solutions were prepared in the appropriate extractant as indicated in Table 3. When Cr was included in the multielement calibrant solutions in nitric acid, acetic

TABLE 2

Flame and spectrometer parameters used for the analysis of extractant matrices

	Element					
	Cd	Cr	Cu	Ni	Pb	Zn
Wavelength (nm)	228.8	357.9	324.8	232.0	283.3	213.9
Bandpass (nm)	0.7	0.7	0.7	0.2	0.7	0.7
Air flow-rate (l/min)	8.0	8.0	8.0	8.0	8.0	8.0
Acetylene flow-rate (l/min)	2.5	4.4	2.5	2.5	2.5	2.5
Burner height (mm)	3	3	3	3	3	3

TABLE 3
Composition of multielement and single element standards

Calibrant solution	Multielement calibrants Element concentration (μg/ml)					Cr calibrants concentration (μg/ml)
	Cd	Cu	Ni	Pb	Zn	
1	0	0	0	0	0	0
2	0.08	0.20	0.20	0.80	0.04	0.20
3	0.16	0.40	0.40	1.60	0.08	0.40
4	0.40	1.00	1.00	4.00	0.20	1.00
5	0.80	2.00	2.00	8.00	0.40	2.00
6	1.20	3.00	3.00	12.00	0.60	3.00
7	1.60	4.00	4.00	16.00	0.80	4.00
8	2.00	5.00	5.00	20.00	1.00	5.00

acid and hydroxylammonium chloride the Cr AAS signal became increasingly irreproducible with time (> 24 h). This was overcome by preparing a Cr-only stock solution and calibrant solutions (Table 3).

Clean laboratory

Samples and calibrants were prepared in a clean laboratory with a Class-100 air work-bench. All glassware and plasticware were soaked in 2.7 mol l^{-1} nitric acid for at least 24 h and rinsed with double-deionised water before use.

Sample collection and pre-treatment

Sediment samples were collected from two rivers in an industrial region of East Catalonia, Spain. The sample collection sites are separated by a distance of approximately 500 m. Sediment 1 was collected from the river Tenes and sediment 2 was collected from the river Besòs, the river Tenes is a tributary of the river Besòs. Both rivers are typically Mediterranean with irregular flow-rates, heavily dependent on rainfall. The Tenes Basin receives effluent from the tanning industry.

Previous analysis by XRFS had indicated that the percentage concentrations of SiO_2, CaO, Al_2O_3, TiO_2, Fe_2O_3 and MgO in sediments 1 and 2 were 30.3 and 27.9, 16.9 and 22.3, 8.5 and 7.8, 0.9 and 0.4, 10.1 and 3.4, and 2.4 and 2.3, respectively.

The sediments were prepared by air drying. Particles less than 63 μm were separated with a nylon fibre sieve. The sediments were extracted as described below.

BCR three-stage extraction scheme

Apparatus. We used Nalgene PTFE centrifuge tubes, a Heraeus Christ Model Labofuge II centrifuge and an SBS horizontal rotary shaker (supplied by Afora S.A.).

Step one. Twenty ml of acetic acid (0.11 mol l^{-1}) was added to 0.5 g of dry sediment in a 50 ml PTFE centrifuge tube. The tube was then shaken for 16 h (overnight) at ambient temperature on a horizontal rotary mechanical shaker at a speed of 30 rpm. The extract was separated from the solid residue by centrifugation at 3700 rpm (corresponding to approx. 1500 *g*) and decantation of the supernatant liquid into a polyethylene container. The container was stoppered and the extract analysed immediately or stored at 4°C.

The residue was washed by adding 10 ml of double-deionised water, shaking for 15 min and centrifuging. The supernatant liquid was discarded, taking care not to discard any of the solid residue, which was retained for step two.

Step two. Twenty ml of hydroxylammonium chloride (0.1 mol l^{-1}, adjusted to pH 2 with nitric acid) was added to the residue in the centrifuge tube. The extraction procedure was then continued as described in step one.

The supernatant liquid obtained was discarded, taking care not to discard any of the solid residue, which was retained for step three.

Step three. Five ml of hydrogen peroxide (8.8 mol l^{-1}) was carefully added, in small aliquots, to the residue in the centrifuge tube. The tube was covered with a watch glass and digested at room temperature for one hour with occasional manual shaking. The procedure was continued for one hour at 85°C and the volume reduced to a few ml by further heating of the uncovered tube in a sand bath.

A further aliquot of 5 ml of hydrogen peroxide (8.8 mol l^{-1}) was added to the residue. The tube was covered again, heated at 85°C for one hour. The cover was removed and the volume reduced almost to dryness.

Twenty-five ml of ammonium acetate (1 mol l^{-1} adjusted to pH 2 with nitric acid) was added to the cool, moist residue. The extraction procedure was then continued as described in steps one and two.

RESULTS AND DISCUSSION

Calibration curves for the multielement and Cr calibrant solutions in the extractants were compared with graphs obtained for multielement and Cr calibrant solutions in nitric acid (0.27 mol l^{-1}). Correlation coefficients of 0.999 or better were obtained for all graphs. There were no significant differences (i.e., $< \pm 4\%$) in the slopes of the calibration curves obtained with solutions prepared in HNO_3 (0.27 mol l^{-1}), CH_3COOH (0.11 mol l^{-1}) or $NH_2OH \cdot HCl$ (0.1 mol l^{-1}). However, with CH_3COONH_4 (0.1 mol l^{-1}) (Table 4), the slopes of the calibration graphs were 6–12% greater than those obtained with calibrants prepared in HNO_3 (0.27 mol l^{-1}), indicating a slight improvement in sensitivity for each element. The precision of the slopes of the calibration graphs for standards prepared in HNO_3 (0.27 mol l^{-1}), calculated as the percentage R.S.D. values of results obtained on three non-consecutive days, was 2–6%.

Values of characteristic concentrations (based on an absorbance of 0.0044) [8] for the analytes in nitric acid and the extractant matrices and the corresponding detection limits (based on three times the standard deviation of the appropriate blank), on a dry sediment basis, are given in Table 5.

TABLE 4

Comparison of calibration slopes of analyte elements in nitric acid and ammonium acetate multielement and Cr single element standard solutions

Element	Slopes *		% Change in slope
	HNO_3 (8.8 mol l^{-1})	CH_3COONH_4 (1 mol l^{-1}, pH 2)	
Cd	0.1510 ± 0.0020	0.1674 ± 0.0023	+10.9
Cr	0.0686 ± 0.0083	0.0728 ± 0.0006	+6.1
Cu	0.0625 ± 0.0003	0.0702 ± 0.0005	+12.3
Ni	0.0318 ± 0.0003	0.0350 ± 0.0003	+10.0
Pb	0.0091 ± 0.00007	0.0101 ± 0.00009	+11.2
Zn	0.2671 ± 0.0019	0.2910 ± 0.0022	+8.9

[a] Method of least squares [13].

Standard additions calibration graphs for the extracts of sediment 1 were compared with direct calibration graphs obtained with calibrants prepared in the corresponding extractant solutions (Table 6). Multielement additions were made for all the elements except Cr. When the errors in the slopes are considered, only Cr in acetic acid and hydroxylammonium chloride seems to be affected, in the form of a suppressive interference, by components extracted from the sediment matrix. Among those elements reported to cause a suppressive interference on Cr in FAAS are Cu and Ni [9]. Tables 7 and 8 indicate that Ni was present in the acetic acid extracts and sometimes

TABLE 5

Comparison of characteristic concentrations and detection limits for flame AAS analysis of sediment extracts

Element	Characteristic concentration (mg/l)			Detection limit [a] (μg/g)	
	Nitric acid	Extracts 1 and 2	Extract 3	Extracts 1 and 2	Extract 3
Cd	0.04	0.04	0.03	0.5	0.6
Cr	0.11	0.11	0.10	1.6	1.8
Cu	0.10	0.10	0.09	1.3	1.5
Ni	0.18	0.18	0.16	2.4	2.7
Pb	0.70	0.70	0.63	9.5	10.7
Zn	0.02	0.02	0.02	0.3	0.4

[a] $c_L = 3\sigma_{blank}/S$ where σ_{blank} is the standard deviation of the appropriate blank and S is the slope of the calibration graph. Values calculated on a dry sediment basis.

TABLE 6

Comparison of direct calibration and standard additions graphs of analyte elements

Element	Slopes *		% Change in slope
	Calibration graph	Standard additions	
Acetic acid			
Cd	0.1806 ± 0.0010	0.1808 ± 0.0015	+0.1
Cr	0.0667 ± 0.0083	0.0553 ± 0.0004	−17.1
Cu	0.0601 ± 0.0009	0.0556 ± 0.0010	−7.4
Ni	0.0335 ± 0.0005	0.0314 ± 0.0006	−6.2
Pb	0.0068 ± 0.0005	0.0068 ± 0.0004	0
Zn	0.2504 ± 0.0042	0.2585 ± 0.0054	+3.2
Hydroxylammonium chloride			
Cd	0.1640 ± 0.0073	0.1746 ± 0.0171	+6.5
Cr	0.0655 ± 0.0006	0.0359 ± 0.0019	−45.2
Cu	0.0612 ± 0.0002	0.0616 ± 0.0007	+0.7
Ni	0.0370 ± 0.0003	0.0354 ± 0.0006	−4.3
Pb	0.0072 ± 0.0004	0.0071 ± 0.0005	−1.3
Zn	0.2498 ± 0.0019	0.2440 ± 0.0039	−2.3
Ammonium acetate			
Cd	0.1709 ± 0.0030	0.1694 ± 0.0033	−0.8
Cr	0.0773 ± 0.0027	0.0762 ± 0.0032	−1.4
Cu	0.0664 ± 0.0008	0.0683 ± 0.0008	+2.9
Ni	0.0357 ± 0.0004	0.0355 ± 0.0006	−0.5
Pb	0.0101 ± 0.0001	0.0102 ± 0.0002	+1.0
Zn	0.2774 ± 0.0030	0.2799 ± 0.0035	+0.9

[a] Method of least squares [13].

in the hydroxylammonium chloride extracts of the sediments analysed and so could cause interference. It is likely, however, that other, more abundant elements (e.g., Ca, Fe, Mg, Mn [10,11]) are also responsible for the effects observed. The suppressive effect on Cr was most severe in the hydroxylammonium chloride extracts. A significant amount of Fe could be extracted at this stage which could cause suppression of the Cr signal. The other analytes did not exhibit significant interferences, which was not unexpected as they are known to be less affected by interelement effects than Cr. Also, the fuel-lean and hence, hotter conditions used for Cd, Cu, Ni, Pb and Zn probably assisted thermal decomposition and atomisation of analyte-containing particles in the flame [12].

No interferences were observed for any of the analytes in the ammonium acetate extracts. The lack of any suppressive effects on Cr could have been due to the higher concentration of Cr compared with the other elements in this extract. However, several ammonium compounds, including ammonium chloride [9,14], ammonium bifluoride [15] and dodecylamine hydrochloride [16] are known to reduce the effects of interelement interferences on Cr and other elements. These compounds produce, by explosive decomposition, smaller particles that are more easily atomised in the flame. Ammonium acetate is likely to have a similar effect as it is vaporised completely at 120–180°C, whereas ammonium chloride vaporises at 300–380°C [17]. It is interesting that hydroxylammonium chloride, which decomposes above 151°C [18] is not as useful a releasing agent for Cr as ammonium acetate. The reasons for this are unclear and merit further study. Another useful releasing agent is 8-hydroxyquinoline [19] which has a boiling point of 267°C.

The three-stage extraction scheme was applied to two river sediments (1 and 2). Separate specimens of each sediment were extracted in triplicate. The results are shown in Tables 7 and 8.

TABLE 7

Amounts of metals extracted from sediment 1

Element	Mean concentration ($n = 3$) ± 1 s (μg/g)			Σ(1 + 2 + 3) (μg/g)
	Step 1	Step 2	Step 3	
Cd	(1.20 ± 0.08) [a]	(0.55 ± 0.13)	(1.49 ± 0.08)	(3.24 ± 0.08)
Cr	< 2	< 2	1596 ± 65	1596 ± 65
Cu	(7.40 ± 0.80)	(1.74 ± 0.10)	1000 ± 1	1009 ± 1
Ni	54 ± 2	21 ± 4	221 ± 24	296 ± 24
Pb	< 10	< 10	624 ± 32	624 ± 32
Zn	429 ± 33	199 ± 5	348 ± 4	976 ± 34

[a] () Indicates concentration near the detection limit of FAAS.

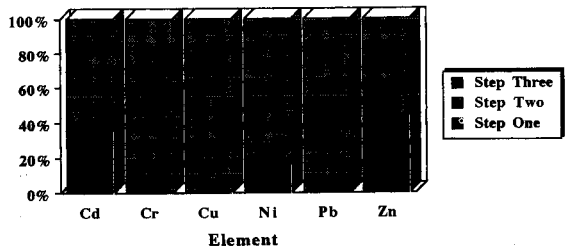

Fig. 1. Partitioning of metals in sediment 1; concentrations in each extractant expressed as a percentage of the total of each element.

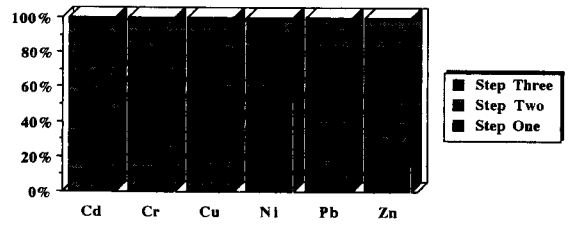

Fig. 2. Partitioning of metals in sediment 2; concentrations in each extractant expressed as a percentage of the total of each element.

The relative amounts of metals extracted at each stage are shown in Fig. 1 and 2.

Both of the sediments studied show similar partitioning of three of the six elements. Significant amounts of Cr, Cu and Pb are found mainly in the third extraction step (organic plus sulfide phase), which is not unexpected, as all three elements form stable organic complexes and resistant sulfides. The partitioning of Ni and Zn is different in each sediment which could indicate differences in the source of the elements. In sediment 1, 70–80% of the total concentration of Ni, is found in step 3, probably associated with the sulfide component, whereas in sediment 2, most of the Ni is partitioned equally between the acid soluble phase (step 1) and the organic plus sulfide phase (step 3). Zn is fairly evenly partitioned between all three phases in sediment 1, but in sediment 2 approximately 75% of Zn is found in step 3 with the remainder split between the other two extractants. The results for Cd are considered too low to be interpreted accurately.

Conclusions

Of the three extractant solutions considered, only ammonium acetate had a small influence on the sensitivities of the elements. Significant inter-element interferences were observed only for Cr in acetic acid and hydroxylammonium chloride extracts of the sediments, which required analysis by standard additions. Direct calibration with calibrants prepared in the appropriate extractant solutions is suitable for the other elements in the acetic acid and hydroxylammonium chloride extracts and for all the elements in ammonium acetate, which acts as a releasing agent for Cr. It is possible that addition of ammonium acetate or another releasing agent, such as 8-hydroxyquinoline, to the extracts obtained in steps 1 and 2 may reduce or remove the interferences on Cr and hence avoid the need for analysis by standard additions. Also, the use of fuel-lean

TABLE 8

Amounts of metals extracted from sediment 2

Element	Mean concentration ($n = 3$) ± 1 s (μg/g)			Σ(1 + 2 + 3)
	Step 1	Step 2	Step 3	
Cd	< 0.5	< 0.5	(1.43 ± 0.02) [a]	(1.43 ± 0.02)
Cr	5.79 ± 0.07	(1.95 ± 0.08)	734 ± 4	742 ± 4
Cu	< 1	< 1	94 ± 1	94 ± 1
Ni	14.5 ± 0.2	< 2	13.4 ± 0.2	27.9 ± 0.3
Pb	< 10	< 10	82.2 ± 1.0	82.2 ± 1.0
Zn	135 ± 4	101 ± 6	658 ± 6	894 ± 9

[a] () Indicates concentration near the detection limit of FAAS.

flame conditions could reduce the interferences on Cr, but at the expense of sensitivity [10]. The slight suppressive interferences observed for Cu (−7%) and Ni (−6%) in the acetic acid extract might merit analysis by standard additions or the addition of an appropriate releasing agent, in the opinion of some analysts.

The results obtained for two river sediments show that the sequential extraction scheme gave acceptably repeatable values for five of the six elements, with the exception of Cd, which was not at high enough concentrations in either sediment to be determined accurately. Due to the limited sensitivity of FAAS for most of the elements studied, except Zn, a more sensitive technique such as electrothermal atomic absorption spectrometry (ETAAS) would be required for the analysis of some of the extracts, particularly the determination of Cr, Cu and Pb in steps one and two.

Due to the variation in extraction schemes between different laboratories, it is not possible to compare their results or validate their procedures. The use of a common sequential extraction scheme, together with the future availability of reference materials certified for their trace metal extractable contents, should allow the data produced by different laboratories to be compared worldwide.

Rhodri P. Thomas would like to acknowledge the financial support of a Catherine and Richard S. Mackie Scholarship awarded by the University of Strathclyde.

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Analytica Chimica Acta, 286 (1994) 431–449
Elsevier Science B.V., Amsterdam

Determination of chlorobiphenyls, with the separation of non-*ortho*, mono-*ortho* and di-*ortho* chloro congeners in fish and sea mammals

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(Received 17th February 1993; revised manuscript received 7th July 1993)

Abstract

A method for the preparation, clean-up and separation of chlorobiphenyls (CBs) into fractions containing non-*ortho* CBs, mono-*ortho* CBs and di–tri-*ortho* CBs is given. Methods of clean-up by gel permeation, saponification and sulphuric acid impregnated silica gel are compared. Highly chlorinated CBs were shown to decompose under alkaline hydrolytic conditions. The cleaned-up samples were fractionated using a 250 mm pyrenyl liquid chromatographic column and determined by GC-ECD and GC–NICIMS. The limit of determination was 10–50 pg kg^{-1} (wet weight) for a 10-g sample and the overall long-term variance for the method was $\leqslant 15\%$.

Keywords: Gas chromatography; Chlorobiphenyls; Environmental analysis; Fish; Sea mammals

Most environmental monitoring and research programmes now stipulate that individual chlorobiphenyls should be determined in place of the earlier measurements of total polychlorinated biphenyls (PCBs) [1,2]. Measurement of the major individual congeners for monitoring purposes is now common place, but, in addition, there is a requirement to determine individual, toxic CBs which exhibit specific biological effects in fish and sea mammals, particularly the induction of liver microsomal enzyme activities [3–6]. The CBs which are the most toxic and induce both aryl hydrocarbon hydrolase (AHH) and ethoxy resorufin-*O*-deethylase (EROD) and bind with high affinity to the cytosolic receptor protein are approximately isoelectric with 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) [7,8]. Both the toxic mechanism and the enzyme induction involve an initial binding of the CB to the same arylhydrocarbon, Ah, receptor [9], and one of the common spatial features between the TCDD and these toxic CB molecules is the possible planar configuration for those congeners having unsubstituted *ortho* positions on the biphenyl molecule. A high correlation has been found between affinity to the receptor and AHH/EROD induction, and between induction and toxicity (weight loss, thymic atrophy) [10,11].

The chlorobiphenyls studied in the Laboratory's Marine Environmental Programme are given in Table 1 which gives the selected CBs in relation to their substitution patterns. The choice

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SSDI 0003-2670(93)E0421-3

was based on abundance, persistence and toxicity, and includes the non-*ortho* CBs which exhibit pure 3-methylcholanthrene (3-MC) type induction of the P450-IA sub-family, along with the mono-*ortho* CBs, which exhibit a mixed function induction (P450 IA/IIB) [10,12,13]. These specific CBs were included in addition to the seven CBs selected for monitoring purposes [14] (Table 1).

Accurate measurement of these toxic CBs is essential if identifiable correlations between cause-effect relationships are to be established not only in laboratory experiments, but also along gradients of chemical concentration in the natural populations of fish and sea mammals. De Voogt et al. [3] reviewed the measurement and distribution of CBs including non-*ortho* and mono-*ortho* CBs in the environment. Creaser et al. [15] and Wells [16,17] have recently reviewed the current state-of-the-art and future developments in the determination of individual congeners, and in particular the toxic CBs. Although there has been considerable improvement in the determination of the CBs for monitoring purposes, some development is still required to improve the method of identification and measurement of the mono-*ortho* and non-*ortho* CBs. There are two main problems associated with the determination of these toxic CBs in biota at concentrations of $< 0.1\ \mu g\ kg^{-1}$. Firstly, the sample size must be increased from 1–5 g to 10–50 g, the actual mass depending on the concentration present and the final method of determination. This requirement in itself can bring problems when

TABLE 1

Chlorobiphenyls determined in environmental matrices [a]

CBs selected IUPAC No.	Substitution pattern	Potential GC interferences without further separation		P450 induction potential of CBs
		SE 54 type column	HT 5 type column	
Tri- and di-ortho CBs				
52 [b]	2,5,2′,5	None	None	None/weak
44	2,5,2′,3′	None	None	None/weak
101 [b]	2,4,5,2′,4′	(90), 84	60	P450 IIB
128	2,3,4,2′,3′,4′	(167), 162	159, 174	P450 IA/IIB
138 [b]	2,3,4,2′,4′,5′	163, 160	None	P450 IA/IIB
149	2,4,5,2′,3′,6′	123, (118)	77	None
153 [b]	2,4,5,2′,4′,5′	(132, 105)	None	P450 IIB
158	2,3,4,6,3′,4′	(186)	175, 178	No data
170	2,3,4,5,2′,3′,4′	190	None	P450 IA/IIB
180 [b]	2,3,4,5,2′,4′,5′	None	193	P450 IIB
194	2,3,4,5,2′,3′,4′,5′	None	None	No data
209	2,3,4,5,6,2′,3′,4′,5′,6′	None	None	No data
Mono-ortho CBs				
28 [b]	2,4,4′	(31)	None	None
70	3,4,2′,5′	None	None	None/weak
105	2,3,4,3′,4′	132, (153)	141	P450 IA/IIB
114	2,3,4,5,4′	None	None	P450 IA/IIB
118 [b]	2,4,5,3′,4′	(149)	None	P450 IA/IIB
156	2,3,4,5,3′,4′	171, 202	172	P450 IA/IIB
Non-ortho CBs				
77	3′4′3′,4′	110	149	P450 IA
126	3,4,5,3′,4′	129, 178, 166	167, 185, 202	P450 IA
169	3,4,5,3′,4′,5′	(170, 190)	None	P450 IA

[a] CBs in parenthesis are normally separated on a well optimised system with a capillary column length of $\geqslant 50$ m and a film thickness of ca. 0.22 μm. [b] These CBs are used for routine monitoring purposes.

livers of only ca. 2 g total weight from individual fish must be analysed in order to relate the contaminant concentration to specific biological activity. Secondly, the removal all of the co-extracted lipids from the sample is essential since these compounds degrade the chromatographic columns, reduce the separation of similar CBs and interfere with the final detection whether by electron capture (ECD) or mass spectrometry (MS) [6].

This paper reports on the various methods that have been examined in this laboratory for the clean-up and separation of CBs including non-*ortho* and mono-*ortho* CBs at the trace (μg kg^{-1}) and the ultra trace (pg kg^{-1}) level in fish and sea mammal tissue. The methods, results and the discussion on the various options are presented in the order of the development, beginning with the final determination and building the method to include the clean-up and initial sample handling and extraction.

MATERIALS AND METHODS

Chemicals

All solvents used were of the highest purity and were obtained as glass distilled grade from Rathburn Chemicals (Walkerburn). The individual pure, solid CBs were obtained from the Community Bureau of Reference (CB 28, 52,101, 105, 118, 128, 138, 149, 153, 156, 163, 170 and 180) either as certified or well-characterised materials, from Promochem (CB 44, 70, 77, 114, 126, 158, 169, 194) and the CLB mixture of 51 CBs in four solutions from the National Research Council of Canada (NRCC) (Halifax). The internal standards (2,4-dichlorobenzyl alkyl ethers) were prepared and characterised in this laboratory [18].

Sulphuric acid impregnated silica gel (SAISG)

Silica gel (Merck 60, 70–230 mesh) (500 g) was conditioned in a muffle furnace at 300°C for 4 h and cooled in a desiccator to room temperature.

TABLE 2

Instrument conditions for the determination of CBs in fish and sea mammal tissue extract

	Gas chromatograph		Mass spectrometer
Instrument	PYE 304	Varian 3500	HP5890 GC and HP 5989A MS Engine
Column type	CP Sil 8 CB	HT5	CP Sil 8 CB
Length	50 m	25 m	50 m
Internal diameter	0.22 mm	0.22 mm	0.22 mm
Film thickness	0.25 μm	0.22 μm	0.25 μm
Injection	Splitless, 1 μl autosampler	PTV on-column 0.5 μl autosampler [a]	Cold on-column
Carrier gas	Hydrogen		Helium/hydrogen
Linear velocity	40 cm s^{-1}		25 cm s^{-1}/40 cm s^{-1}
Make-up gas	Nitrogen 34 ml min^{-1}		CI Moderator gas: hydrogen 1.8 Torr
Detector	Electron capture ^{63}Ni		Quadrupole 70 eV, 300 μA
Detector temperature	320°C		Source 150°C
Column programme			
Isothermal 1	80°C for 1 min		80°C for 1 min
Linear rate 1	15 K min^{-1} to 150°C		3 K min^{-1} to 280°C
Isothermal 2	150°C for 10 min		280°C for 10 min
Linear rate 2	3 K min^{-1} to 280°C		
Isothermal 3	280°C for 10 min		
Sample final volume	100 μl	500 μl	50 μl

[a] Other GC conditions for Varian 3500 were the same as the PYE 304.

The activated silica gel was transferred to a ground glass stoppered container and the concentrated sulphuric acid was added carefully in stages, e.g., 50 g at a time and mixed by shaking or rotating the stoppered container. The next portion of the acid was added only when the container was cool enough to handle. When all of the acid was added the contents were mixed for 2–3 h [a]. The final proportion of sulphuric acid to silica gel used was 1:1 (w/w).

All other materials used in the sample preparation have been reported elsewhere [6,19,20].

Final separation and detection

Gas chromatography. The Philips 320 gas chromatograph and autosampler were fitted with a 50 m × 0.22 mm i.d. CPSil 8 CB fused-silica column and a ^{63}Ni electron capture detector. The samples were injected (1 μl) into a splitless injector at 275°C and chromatographed at 80°C for 1 min and at 3 K min^{-1} to 280°C using hydrogen as carrier gas at a liner velocity of 40 cm s^{-1}. The chromatograph and data collection were controlled by a microcomputer operation Minichrom (VG Data Systems).

The Varian 3500 GC system and autosampler were fitted with 25 m × 0.22 mm i.d. HT5 CB fused-silica column and a ^{63}Ni ECD. The conditions are given in Table 2. The chromatograph and data collection were controlled by a microcomputer operation Minichrom (VG Data Systems).

Mass spectrometry. The HP5989A MS Engine was interfaced with an HP5890 GC system fitted with a 50 m × 0.22 mm i.d. CPSil 8 column which was connected directly into the ion source of the MS. The negative ion chemical ionisation was generated using hydrogen as the moderator gas at 1.8 Torr. The spectra were obtained at 70 eV over a scan range of 100 to 450 a.m.u.

[a] SAFETY: Extreme care should be exercised when handling the silica–sulphuric acid powder. The silica powder which may become airborne with handling large quantities and is, in effect, microfine concentrated acid. The material should always be handled in a fully operational fume cupboard and a filter mask should be worn.

Separation of CB groups

Liquid chromatography. Method 1. The CBs were separated using a Cosmosil 5-PYE LC column [2-(1-pyrenyl)ethyldimethylsilylated silica gel], particle size 5 μm, Nacalai Tesque (Promochem). The LC system consisted of a Gilson 321 autosampler fitted with a 50-μl loop, a Gilson 392 pump and 401 diluter to dispense the sample into the LC loop. The fractions from the 5-PYE column were monitored with a Philips PU 4020 UV detector set at 254 nm and collected with a Gilson 202 fraction collector. The injection and fraction cycle was controlled by the Gilson autosampler and the data collection was controlled by an Apple IIe microcomputer running Chromatochart software.

The cleaned-up samples from silica gel were concentrated to ca. 80 μl in a Chromcol tapered vial and eluted with hexane at a flow-rate of 0.5 ml min^{-1}. Two internal standards, 2,4-dichlorobenzyl hexyl and dodecahexyl ether [18] were added (1 ml of 1 μg g^{-1}) to each fraction, the sample was reconstituted in isooctane and the CB content determined by capillary GC.

Method 2. The same LC system was used as in Method 1, but the size of the injection loop was increased to 200 μl. The sample was evaporated to 50 μl in a Chromcol tapered vial. The sample was washed from the sides of the vial and very gently evaporated to 50 μl again. The injection needle was adjusted so that the tip rested in the base of the cone of the vial and the whole sample was withdrawn into the needle and flushed into the 200-μl loop. The sample was eluted with *n*-hexane at 0.5 ml min^{-1} and the fractions collected according to the preset calibration with a CB mixture.

Separation of similar trace organochlorine contaminants

Silica gel chromatography. The lipid free extract was chromatographed onto silica gel (3 g) deactivated with water to 3% (w/w) in a 6 mm i.d. glass column and eluted with hexane (6 ml) to separate the CBs from the organochlorine pesticides and chlorinated camphenes [20]. An additional 2–3 ml hexane were required, compared

with the routine CB method [20], to completely remove all of the non-*ortho* CBs, which are retained slightly on the silica gel compared with the other non-planar CBs.

Lipid removal. Three methods of lipid removal (clean-up) were investigated, in turn, using an extract of fish and blubber tissue. Only two methods were used; the third, gel permeation chromatography, was discarded and is not described here.

Saponification. The lipid in the soxhlet extract was hydrolysed by saponification with 20% (w/w) potassium hydroxide in ethanol (300 ml) at 60°C for 1 h. The reaction mixture was cooled and diluted with distilled water (20 ml) and extracted with hexane (2 × 50 ml). The hexane extract was evaporated to ca. 1 ml in a clean, dry stream of air and cleaned-up by passing the sample sequentially through an alumina and silica gel column [20] to remove any trace of remaining lipid and co-extracted material and to separate the CB fraction from other unhydrolysed pesticides.

SAISG. Sulphuric acid impregnated silica gel (25 g) was added slowly to the soxhlet extract in dichloroethane (DCE) (25 ml) and mixed thoroughly until the acid had reacted completely with the lipid (2–5 min). The resultant mixture was slurried into a glass column (20 mm i.d.) containing a further 25 g of SAISG. The contents of the beaker were transferred to the column including the washings. When the sample had completely soaked into the column the CBs were eluted with a further 200 ml of DCE, evaporated to a small volume with the rotary evaporator, and, finally a clean air stream and then reconstituted into hexane.

Extraction. Samples of fish or blubber tissue (up to 20 g) were taken and macerated with anhydrous sodium sulphate. The resultant powder was extracted in a soxhlet for 4 h with dichloromethane (DCM) (100 ml). A 1 ml portion of CB 209 (50 ng g^{-1}) was added to the weighed powder and re-weighed prior to extraction to determine the recovery of the CBs. The bulk of the DCM was reduced in a rotary evaporator and then completely removed by adding hexane and evaporation in a clean, dry stream of air. A portion of the soxhlet extract was taken to determine the amount of extractable lipid residue by removing the solvent in a weighed dish.

RESULTS

Detection and gas chromatographic separation

The capillary GC was optimised and calibrated for CB analysis according to the methods described earlier [21,22]. The columns selected for the analysis and the instrumental conditions are given in Table 2. The repeatability of the final measurement with the same calibration files has a maximum coefficient of variation of 1–3% for all CBs, and was often much less. The reproducibility of the calibration was between 5–7%. The GC calibration comprised of a five point curve with a deviation from linearity between adjacent points of $< 5\%$. A calibration test solution was injected with each batch of 10 samples and the GC was recalibrated when the test failed to meet the $\pm 5\%$ compliance level. The separation and identification of each of the CBs determined in these studies has been reported before [6]. The identification of the CBs was confirmed using a second GC column and negative ion chemical ionisation (NICI) MS with selected extracts from each batch of samples. An example of ECD chromatograms of the three CB fractions from the PYE LC column for a cod liver extract and a harbour porpoise blubber extract is given in Fig. 1.

LC fractionation of CBs

The CBs were separated according to their *ortho* substitution pattern using a pyrenyl LC column and hexane as an eluant at 0.5 ml min^{-1}. The initial studies were conducted using a column of 150 mm length to separate the non-*ortho* and mono-*ortho* CBs [6], but subsequently a 250 mm column was used to obtain a more discrete separation between some of the congeners. The capacity factors (k') for each of the CBs determined are given for both columns in Table 3. Of the CBs determined, some were still split between the fractions. In particular CB 118 and CB 180 were split between the first and second fractions. However, it was not critical to isolate each of these congeners in a single fraction since they

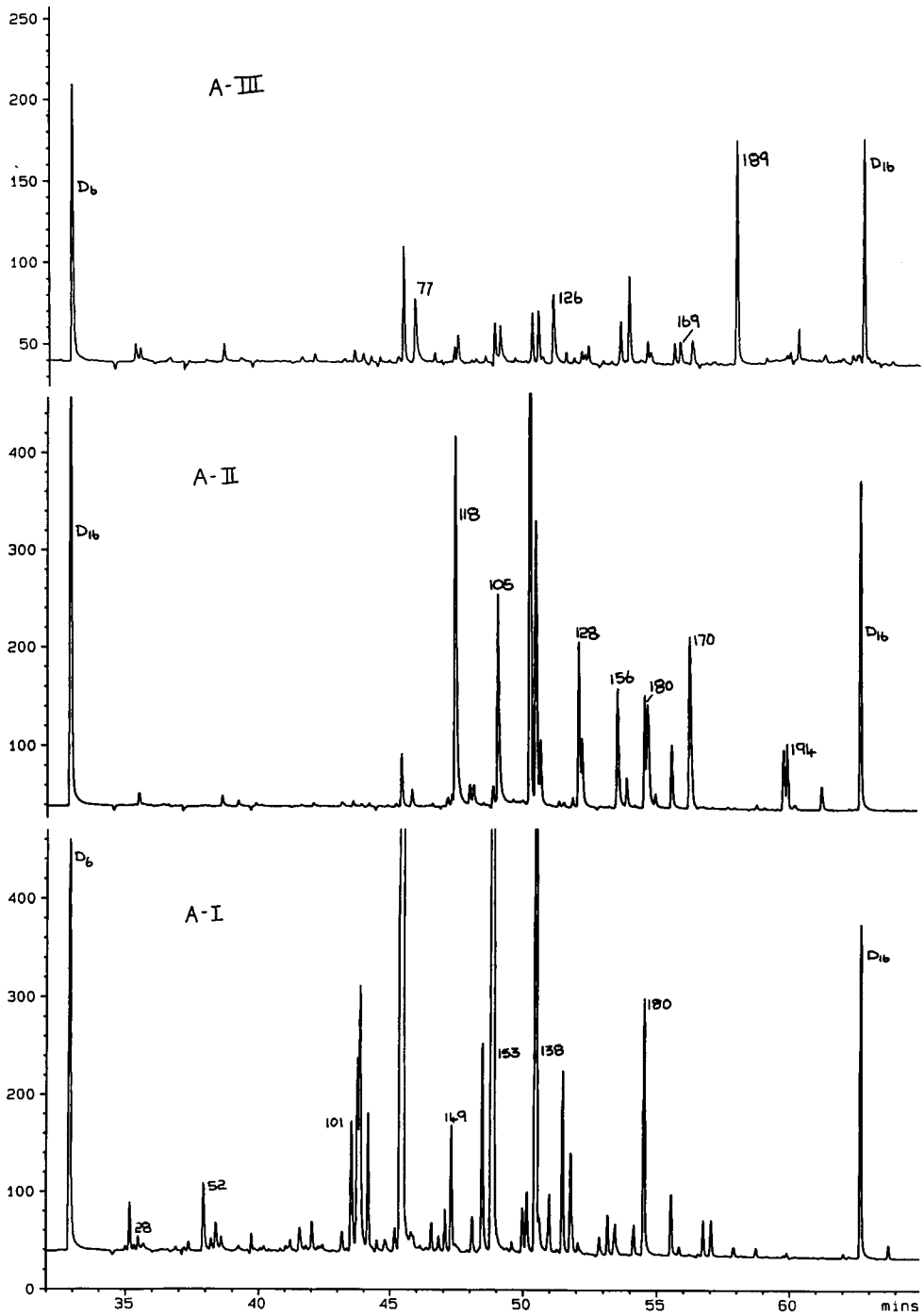

Fig. 1. Chromatograms of CBs from the three fractions of the pyrenyl LC column from extracts of cod liver (A-I, A-II, and A-III) and harbour porpoise blubber (B-I, B-II and B-III) (see Fig. 3). Column: CPSil 8, 50 m × 0.22 mm i.d.

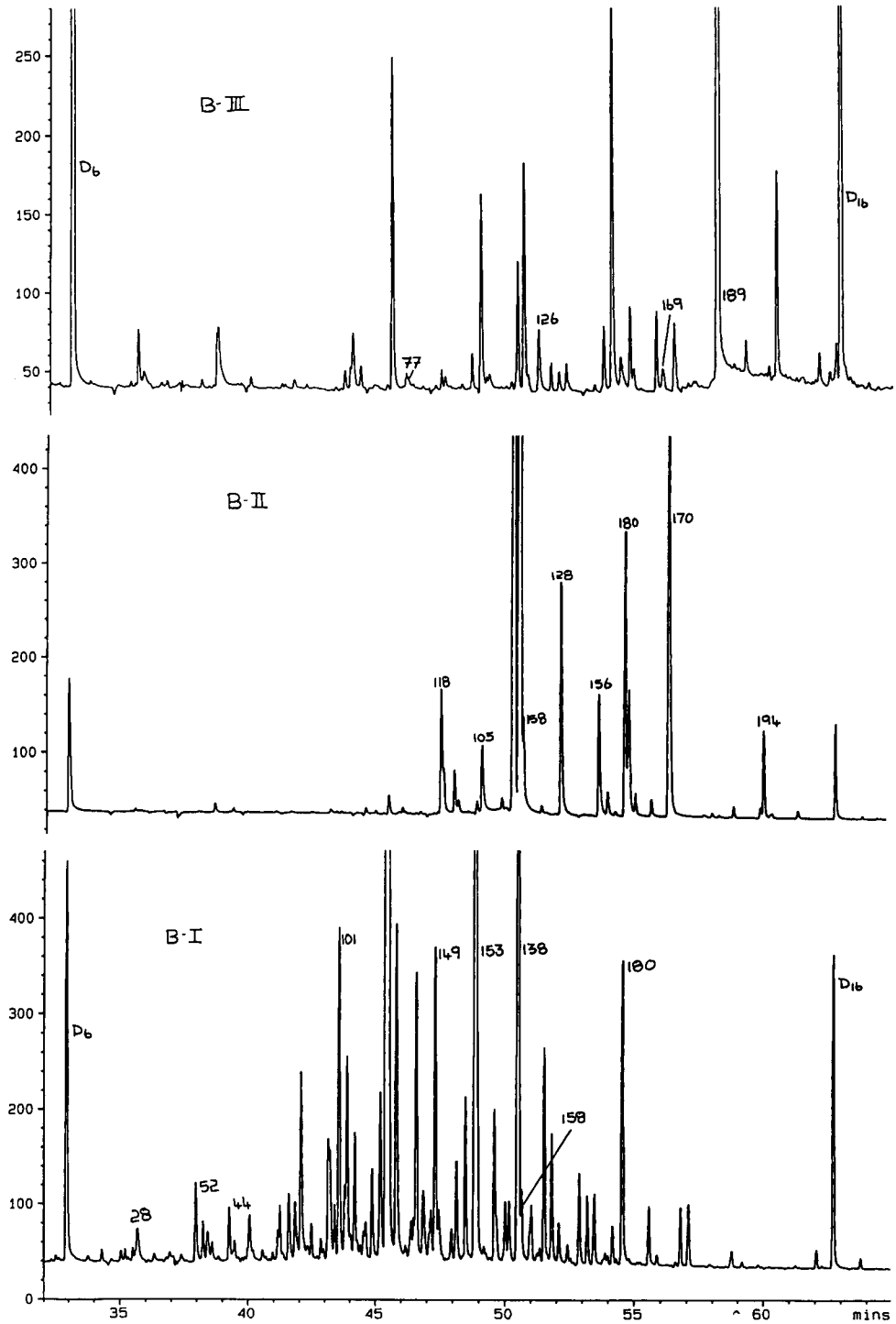

Fig. 1 (continued).

TABLE 3

Long term repeatability of k' values for CBs on the pyrenyl LC columns

CBS IUPAC No.	150 mm PYE colum		250 mm PYE column		Fraction
	k'	C.V. (%), $n = 10$	k'	C.V. (%), $n = 10$	
28	0.42	2.05	0.53	3.3	I
52	0.42	2.34	0.53	2.6	I
101	0.45	2.18	0.59	3.3	I
149	0.48	3.42	0.53	3.2	I
153	0.49	2.47	0.69	3.7	I
180	0.63	2.99	0.88	3.2	I/II
138	0.63	2.99	0.84	2.3	I
118	0.70	2.7	0.96	1.9	I/II
163	0.72	3.44	0.96	4.5	II
128	0.73	2.8	0.96	1.9	II
170	0.81	3.16	1.10	3.1	II
105	0.80	1.24	1.17	3.0	II
156	1.00	1.38	1.41	3.2	II
77	1.20	3.28	1.74	3.5	III
126	1.74	1.68	2.39	2.6	III
169	2.22	5.39	3.24	3.1	III

can be determined directly on both the CPSil 8 and the HT5 phases without any interference from other congeners. The CBs which do require further isolation on the LC column are the di-*ortho* chloro congeners CB 138, CB 128, CB 170, the mono-*ortho* congeners CB 105, and CB 156 and the non-*ortho* chloro congeners CB 77, 126 and 169 (Table 1). The long term reproducibility of the k′ values for the CBs with the 150 mm column measured between 27 November 1990 and 31 January 1991 ($n = 10$) ranged between 1.24% and 5.39% and with the longer, 250 mm column, over the period 28 November 1991 to 4 December 1991 the k' values ranged between 1.9% to 4.5%. The k' values were measured over a period when the column was in regular use separating cleaned-up extracts of fish and sea mammal tissue.

The recovery of the CBs from LC was initially measured by filling a fixed volume loop (50 μl) with a mixture of the CBs in isooctane, spiked

TABLE 4

Repeatability and recovery of CBs from the PYE LC fractionation at three concentration levels

CB	Fraction	Level 1			Level 2			Level 3		
		Mean (μg kg^{-1})	C.V. (%) $n = 3$	Rec. (%)	Mean (μg kg^{-1})	C.V. (%) $n = 3$	Rec. (%)	Mean (μg kg^{-1})	C.V. (%) $n = 3$	Rec. (%)
28	I	27.5	5.4	91.1	80.5	4.2	88.7	358	13.8	85.2
52	I	42.6	6.3	90.4	131	0.9	95.4	595	10.6	97.0
44	I	28.2	0.7	90.4	84.6	2.9	93.5	411	8.2	104
70	I	28.0	4.1	94.5	83.8	3.1	94.5	391	10.4	101
101	I	32.3	2.4	92.6	105	2.2	99.0	448	13.7	99.1
149	I	28.0	3.6	93.7	94.6	4.4	100	387	10.3	102
153	I	28.8	4.0	95.4	97.2	4.2	99.6	399	11.3	101
138	I/II	29.1	1.1	99.3	91.9	5.4	107	319	5.7	89.6
158	I/II	30.0	4.3	95.1	93.2	2.6	100	391	4.9	95.8
180	I/II	28.2	3.0	95.6	86.0	5.9	103	306	8.1	86.4
118	II	26.4	4.0	93.5	87.7	3.9	97.7	341	6.2	87.5
114	II	27.2	1.0	95.6	83.8	3.8	96.2	354	4.4	90.3
105	II	33.3	2.5	96.4	90.8	4.4	97.1	347	3.1	87.1
128	II	23.5	2.9	92.1	76.0	1.1	102	296	3.1	95.4
156	II	26.0	0.2	93.2	80.8	2.3	96.2	333	1.7	91.7
170	II	31.3	0.7	95.9	88.2	4.9	106	412	4.6	104
194	II	34.3	2.4	96.4	103	2.6	103	413	6.4	95.1
209	I	28.9	7.4	88.5	92.9	1.7	102	359	8.9	96.1
77	III	10.0	3.1	93.6	29.3	3.5	91.0	68.6	9.8	91.8
126	III	15.2	1.2	91.3	29.0	0.78				
163	II	15.1	2.6	89.5	38.7	2.3	96.2	89.9	10.6	106
169	III	11.9	1.9	88.2	44.7	1.2	95.3	63.7	8.3	98.3

TABLE 5

Recovery of non-*ortho* CBs from spiked cod liver oil after saponification to remove the lipid, and clean-up on silica gel

CB IUPAC No.	Mean ($\mu g\ kg^{-1}$)	C.V. (%)	Recovery (%)
77	17.6	23	63
126	35.5	11	75
69	11.6	11	53

with a known amount of CB 209 (Method 1). However, it soon became apparent that the complete transfer of the sample extract to LC was more preferable since the concentration of the non-*ortho* CBs were so low in may of the samples that a loss of 30–50% of the sample at this stage would result in a significant reduction in the sensitivity of the method. The sample loop injector/dispenser was, therefore, adjusted to transfer the maximum amount of the vial's content to the LC sample loop (Method 2). In the initial stages the efficiency of the transfer was monitored by weighing the sample vials before and after injection. The net transfer obtained was 98.3% ± 2.4% ($n = 9$).

The repeatability and recovery of the CBs from the pyrenyl LC column was measured at three different concentration levelsto determine the efficiency of the sample transfer to the LC system and the recovery from the column. The results are given in Table 4. The concentration range of the CBs was from 10 $\mu g\ kg^{-1}$ at level I to 595 $\mu g\ kg^{-1}$ at level III with recoveries between 88% and 106%.

Removal of lipids

Saponification. A series ($n = 5$) of the Laboratory Reference Material (LRM 110), cod liver oil (2 g) were spiked with the non-*ortho* CBs 77, 126 and 169 and saponified at 60°C for 1 h, extracted from the reaction mixture, separated into fractions on the liquid chromatograph and the non-

TABLE 6

Saponification of NRCC CLB mixture at 60°C in KOH–ethanol for 90 min

CB IUPAC No.	CUSUM $\Sigma(C_t - C_{t+1})$	Decomposition	CBs IUPAC No.	CUSUM $\Sigma(C_t - C_{t+1})$	Decomposition
18/5	19	? [a]	138	−20	None
54	15	None [b]	129	4	None
31	-		82/159/182	11	None
52	11	None	183	12	None
49	12	None	128	9	None[c]
44	11	None	185	32	
40/103	5	None	156/121/202	12	None
121	4	None	173/200	14	None
60	11	None	180	14	None
101	13	None	191	16	? [a]
86	14	None	170	7	None
87	−9	None	201	24	[a]
77/154	18	? [a]	203/196	45	[c]
151	2	None	189	13	None
118	7	None	208/195	54	[d]
143	9	None	207	70	[d]
114	8	None	194	28	[a]
153	15	None	205	57	[d]
105	7	None	206	72	[d]
141	11	None	209	100	[e]
137	13	None			

[a] > 20% probable decomposition. [b] None = < 20%, i.e., within the error of measurement. [c] > 30% decomposition has certainly occurred. [d] > 50% decompostition is very extensive. [e] > 80% complete decomposition.

ortho CBs determined by GC-ECD. The results are given in Table 5. The recovery of the three non-*ortho* CBs was low compared with the values obtained for the routine monitoring congeners which were plotted against the laboratory mean values on the Shewhart control charts. For explanation see Discussion.

The experiment was repeated with the addition of a known amount of CB 209 as an additional *non-planar* recovery congener, but after saponification at 60°C for 1 h the CB 209 had declined to < 50% of the original value. In view of the potential degradation of CB 209 the saponification was repeated with an aliquot of the NRCC CLB mixture of 51 CBs in isooctane. A series of reactions were performed at 60°C for 10, 20, 40, 60 and 90 min with the ethanolic potassium hydroxide and the reaction mixture cooled, extracted and the CBs measured by GC-ECD. The results of the analysis are given in Table 6. The initial concentration of each CB was taken as 100% and the percentage value of the subsequent measurement was subtracted to give a measure of any decline in the concentration of each CB. This calculation was repeated sequentially through the time series and finally these values were totalled to give an overall indication of the loss of any of the congeners. This is given in Table 6 as the cumulative summation, CUSUM $\Sigma(C_t - C_{t+1})$. Those CBs which appear to have decreased in concentration with the time of the saponification are also in Fig. 2 to show the rate and the extent of the decomposition.

SAISG columns. The original SAISG column, 6 mm i.d., was suitable for removing ca. 200 mg lipid per sample, but with a larger sample mass of 10–20 g it was necessary to increase the amount of reactant column packing. An 80 mm i.d. column fitted with a sinter-glass frit was made to hold 150 g of SAISG. A sample of cod liver oil (10 g) was spiked with a standard mixture of CBs and diluted in hexane (20 ml). The mixture was applied to the column and eluted with DCE. Each 50 ml fraction was collected and the eluate concentrated to 1 ml in isooctane, cleaned-up on a silica gel column (3 g) and the CB content

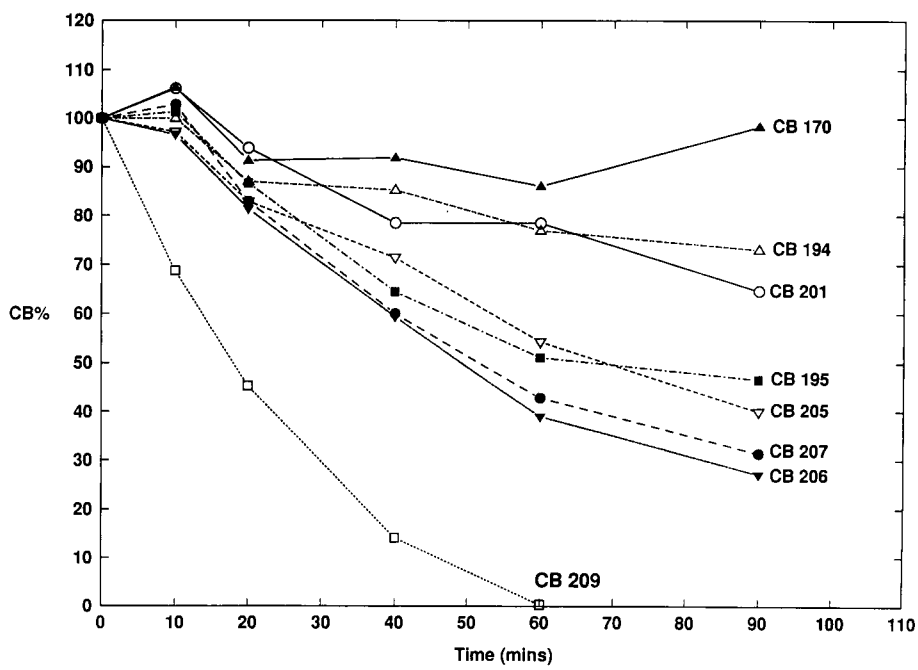

Fig. 2. Changes in the concentration of CBs of a standard NRCC-CLB mixture (51 CBs) after reaction with ethanolic potassium hydroxide.

determined by GC-ECD. The 50 ml fractions were collected up to 600 ml. Although the mass of column packing was sufficient to remove all of the lipid, the packing attenuated the elution of the CBs and a measurable quantity of the CBs was removed after 600 ml eluting with DCE. Only ca. 80% of the CBs were eluted within the 600 ml. The packing was clearly effective in sample clean-up, but it also appeared to trap some of the CBs. Approximately half of the SAISG was consumed, i.e., only half wasdarkened by the formation of carbon from the dehydrated lipid and so the column charge was reduced to 50 g and the experiment repeated.

Although the bulk of the CBs were eluted in a smaller volume, ca. 80% in 300 ml, measurable quantities of CBs were still eluted after 600 ml of DCE, indicating a tailing effect of the column. With the smaller mass of packing (50 g) the microfine carbon reaction products also began to elute from the column. The geometry of the column was therefore changed to reduce the breakthrough of the carbon and a 20 mm i.d. column was tested. This column was initially filled with 50 g of the SAISG and the elution volume and recovery of a standard mixture of CBs determined. The CBs were eluted with DCE and collected in 25 ml fractions up to 150 ml. None of the CBs eluted in the first 25 ml, which was effectively the bed volume of the column. However, all of the congeners were removed from the silica gel in the following 50 ml of solvent (Table 7).

The experiment was repeated with 2, 5 and 10 g of fish oil added to the CB mixture. With the smaller column, the reaction of the sulphuric acid with the lipid at the head of the column was too localised and the heat generated from the dehydration process caused the hexane to boil. A further modification to the method was made.

The 20 mm i.d. glass column was filled with 50% of the total charge of SAISG (25 g). The remaining 50% was added to the cooled sample

TABLE 7

Elution profile of CBs from SAISG (50 g) column (20 mm i.d.)

CB IUPAC No.	Percentage recovery of CBs from the SAISG column				Total % recovery
	Elution volume of 1,2-dichloroethane (DCE)				
	25 ml	50 ml	75 ml	100 ml	
28	–	72.1	24.6	–	96.4
52	–	74.1	24.0	–	98.1
44	–	65.5	30.6	–	96.0
70	–	68.1	32.6	–	100.6
101	–	76.6	21.7	–	98.3
149	–	76.9	23.0	–	99.8
118	–	66.9	29.0	–	95.9
114	–	73.8	25.9	–	99.7
153	–	77.8	20.7	–	98.5
138	–	75.9	28.2	–	104.1
158	–	80.6	25.5	–	106
128	–	68.7	34.2	–	103
156	–	68.5	30.5	–	98.9
180	–	80.3	21.3	–	101.6
194	–	81.7	25.5	–	107.2
209	–	88.9	11.6	–	100.6
77	–	45.4	41.9	–	90.3
163	–	77.2	23.9	–	101.0
126	–	57.0	40.0	–	97.0
169	–	61.9	38.1	–	100.0

TABLE 8

Recovery of CBs with 10 g of lipid from the SAISG (50 g) column (20 mm i.d.)

CB IUPAC No.	Accumulative recovery of CBs (%)			
	Volume of dichloroethane eluted through SAISG column			
	50 ml	100 ml	150 ml	200 ml
28	72	95	96	97
52	76	98	99	99
44	75	95	96	97
70	75	98	99	99
101	0	96	99	99
149	0	93	96	97
118	0	94	97	98
114	0	92	96	97
153	0	94	97	98
105	0	94	96	97
138	0	94	97	98
158	0	92	96	98
128	0	93	96	98
156	0	93	96	98
180	0	93	96	98
170	0	93	96	98
189	0	87	91	93
194	0	92	95	99
209	64??	97	98	99

extract, in stages in a beaker, and mixed thoroughly. Once the dehydration reaction had subsided, the resultant mixture was cooled and added to the top of the prepared column and eluted with DCE.

The recovery of the CBs through the 20 mm i.d. column with SAISG (50 g) was measured by cleaning-up a spiked fish oil (10 g) and measuring the CB content in each 50 ml fraction, up to 500 ml (Table 8). All of the measurable quantities of CBs were eluted within 200 ml. The recovery of the CBs through this method, followed by 5-PYE LC separation, was measured by spiking a cod liver oil containing low levels of indigenous CBs at three levels of concentration. Three replicate, independent determinations were made at each of the three levels and the recovery calculated from the regression analysis of the spiked values and the measured values. These data are given in Table 9.

DISCUSSION

Detection and gas chromatographic separation

Recent work by Duinker et al. [23], De Boer et al. [24–27] and Larsen et al. [28,29] on the determination of retention indices of individual CBs on different stationary phases has meant that the choice of column phase is no longer restricted to the SE 54 type on which Mullins et al. [30] undertook the original studies of the retention indices of all 209 congeners. Schultz et al. [31] have also investigated the composition of the main key industrial Aroclor and Clophen chlorobiphenyl mixtures using multi-dimensional gas chromatography (MDGC) to identify all CBs which were present above the 0.05% level. These studies listed those CBs which might be expected in environmental samples but of equal importance, those CBs which are absent or present at $< 0.05\%$. Those CBs which are absent in the

TABLE 9

Recovery of CBs from fish oil (10 g) spiked at three concentration levels [a]

CB IUPAC No.	Level ($\mu g\ kg^{-1}$)	C.V. (%)	Level ($\mu g\ kg^{-1}$)	C.V. (%)	Level ($\mu g\ kg^{-1}$)	C.V. (%)	Recovery [a] (%)	C.V. (%)
28	225	7.7	402	9.8	660	2.2	71	12
52	577	1.0	831	8.3	1135	1.7	67	9
44	241	6.9	330	4.9	473	3.2	72	18
70	360	3.7	556	7.8	805	1.4	73	9
101	750	2.6	1008	4.9	1337	6.2	79	11
149	583	6.1	810	1.2	1082	4.8	83	7
118	7.3	9.1	1.85	4.8	1273	1.7	92	11
114	185	8.7	441	4.2	613	2.5	85	7
153	1284	2.3	1510	7.1	1848	2.6	84	13
138	1219	1.3	1583	3.6	1852	2.3	93	10
158	273	3.4	536	4.9	727	2.7	82	6
128	290	4.3	571	5.0	757	1.4	93	10
156	190	3.3	376	4.8	529	1.5	76	6
180	512	4.9	787	4.2	948	3.4	83	13
194	253	5.1	483	6.8	666	3.6	80	8
209	240	12.1	492	2.8	7.4	2.2	86	4
77	24	8.8	48	13	65	4	114	20
126	30	8	63	15	84	8	99	13
169	24	7	44	10	62	4	93	8

[a] Recovery determined by a linear regression of the data for each CB at each spiked concentration level.

industrial mixtures will also be absent in the environmental samples. As a result a number of CBs which, in principle, may co-elute on a particular GC stationary phase, in practise, do not due to the absence of one of the congeners. The combination of the studies by Schultz et al. [31], De Boer et al. [24–27] and Larsen et al. [28,29] has meant that with the exception of CBs 69, 75 96 and 182 the retention times of all CBs known to be present in a formulation or environmental samples are now available on five different stationery phases; CPSil 8, CPSil 5, CPSil 19, CPSil 88 and HT5. In these studies we have used CPSil 8 and HT5 columns which are adequate for the measurements being made provided a column length of $\geqslant 50$ m $\times$ 0.22 mm i.d. and a film thickness of $\geqslant 0.20$ μm is selected and the non-*ortho* and mono-*ortho* CBs are separated prior to the final detection.

LC fractionation of CBs

Normally, it is necessary to make a series of group separations prior to the final resolution of the CBs and organochlorines by HRGC. The cleaned-up extract, at this stage, will contain other organohalogens, such as organochlorine pesticides (OCPs), polychlorinated naphthalenes (PCNs), polychlorodibenzo-*p*-dioxins (PCDDs), polychlorodibenzofurans (PCDFs), polychlorinated camphenes (PCCs) (Toxaphene) as well as the CBs [32]. The OCPs and the more abundant CBs are normally the predominating two groups of OCs, and these can easily be separated on silica gel [19,20,33] and determined separately. Most of the PCCs can also be separated from the CBs and the OCPs on the silica gel by increasing the polarity of the solvent from a *n*-alkane to between 5–15% methyl-*tert*-butyl ether in hexane [20]. This separation is normally adequate when the predominant monitoring CBs are to be determined.

However, with the exception of CB 118, and to a lesser extent CB 105, all mono-*ortho* CBs and the non-*ortho* CBs are present at substantially lower concentrations compared with the remaining CBs. It is, therefore, necessary to separate the non-*ortho* and mono-*ortho* CBs into different groups since: (i) the range of concentrations of the CBs is normally too large for all congeners to be measured without additional dilution or concentration; and (ii) some of the key CBs are not resolved from each other on a single GC column, regardless of the column phase. The methods available for the isolation of the CBs into separate fractions, prior to GC analysis, utilise the spatial planarity of these compounds.

These methods of separating the CBs according to their planar structure have been extensively reviewed by Creaser et al. [15] and Wells [16]. The main techniques which have been investigated have used active adsorption columns of silica gel [34], alumina or Florisil [35–37], active carbon [38–42], glass fibre impregnated with active carbon [43,44], porous graphitic carbon HPLC [45–49] and the pyrenyl silica LC columns [6,50,51].

Active adsorption columns have been shown to separate non-*ortho* CBs from other congeners [52], but great care is required in optimising and maintaining the highly active adsorbent. The column packing degraded quickly on use and this technique is not particularly suited to automation. Active charcoal will separate the non-*ortho* and the mono-*ortho* CBs from the remaining congeners, both as a micro column and supported on glass fibre. Most of these carbon systems reported are specific to each laboratory with no standardised or recommended column type. These columns were developed primarily from their use in isolating the planar PCDDs and PCDFs. The main problem with their use has been to obtain a sufficiently clean blank at the ultra trace level and to obtain the necessary separation of CBs which may interfere in the subsequent GC analysis, e.g., CB 77 and CB 110 [53].

The porous graphitic carbon (PGC) developed by Knox et al. [45] has been used by Creaser and Al-Haddad [46] to separate CBs, PCDDs and PCDFs. The *ortho* and non-*ortho* CBs are separated using a single solvent with an LC system that can be fully automated. However, the mono-*ortho* CBs are not separated from the bulk of the congeners using this column.

Zebühr et al. [49] used two coupled LC columns to improve the isolation of the CBs in fish tissue according to their *ortho*-chloro substitution pat-

tern. They coupled an aminopropyl RP column (250 × 10 mm i.d.) with a Hypercarb column (100 × 4.7 mm i.d.). The samples were eluted with *n*-hexane through the aminopropyl column to isolate the aliphatics and mono aromatics, e.g., hexachlorobenzene. The second fraction containing the diaromatics PCDD/Fs, PCNs and CBs, was switched to the second Hypercarb column. The polyaromatics were removed from the first column by backflushing. The diaromatic fraction was first eluted from the PGC with hexane (2–4 *ortho*-chloro CBs) and then with hexane–DCM (1:1) to remove the mono-*ortho* chloro CBs. ThePGC column was washed with DCM–methanol (1:1) and then backflushed with toluene at 40°C to isolate the PCDD/FS and the non-*ortho*-chloro CBs together.

The silica bonded phase, 2-(1-pyrenyl)ethyldimethylsilyated (*PYE*) silica [50,51] which can separate the *ortho*- and non-*ortho*-chloro CBs on the basis of the degree of planarity and chlorination. This column material separates structurally similar molecules with different π-electron densities resulting from the spatial configuration of the aryl rings, and has sufficient resolution to isolate the non-, mono- and other *ortho*-chloro CBs. Initially, this type of column was, like the activated carbon and the PGC, used to separate the toxic CBs 77, 126 and 169. However it is also possible to separate other key CBs which can remove a number of ambiguities that sometimes occur in the final determination of the toxic CBs where MDCG is not available [6,50,54]. Using the retention pattern of the 168 CBs available on this PYE column [6,50,54] it has been possible to three different fractions which will isolate predominantly the tri- and di-*ortho*-chloro CBs (fraction I) from the mono-*ortho*- (fraction II) and the non-*ortho*-chloro CBs (fraction III).

These fractions separate pairs of CBs which have similar retention times on an SE 54-CPSil 8 type column and overcome some of the problems encountered using the activated carbon columns without MDGC [53]. For example CB 129 and CB 178 which normally co-elutes with CB 126 are separated into fractions I and III, and CB 110 (fraction II) are fully separated from CB 77 (fraction III). The two CBs 138 and 163 are also separated into fractions I and II respectively [6], although a full 100% split was difficult to maintain since any small shift in retention times of 3–5 s can result in ca. 5% of the more abundant CB 138 in fraction II.

The retention times using the pyrenyl column are very repeatable within a batch calibration, but are susceptible to change if the sample contains any lipid residues. Both Haglund et al. [50] and Wells and Echarri [6] found it essential to remove all lipophilic co-extracted materials prior to separating the CBs on the PYE column when the column was degraded by poorly cleaned-up samples. The column efficiency was restored by flushing the column with 10–20 bed volumes of ethyl acetate (100%) between sample batches, and then reconditioning with a similar volume of *n*-hexane. The pyrenyl column was remarkably robust and, provided the lipid content of the sample was not over excessive (e.g., totally uncleaned sample), it was possible to fully restore the column by cleaning in this way.

A constant stable retention time for each compound as measured by the k' value is essential. The stability of the k' values were measured by injecting a series of CB mixtures to determine the retention values at regular intervals during the working life of the column. This was done initially to calibrate the column and to determine the elution volumes of the three fractions. Although the column was relatively stable the long term retention index repeatability was between 1.9% and 4.5%, which at the worst case, is too inaccurate to set the fraction volumes without a regular calibration check (Table 3). Although the short term k' repeatability was $< 0.7\%$ the long term stability was affected by the presence of trace amounts of lipid from the sample due to incomplete clean-up. This was particularly noticeable when GPC or adsorption chromatography was used as a sample preparative technique. It was the most stable when SAISG columns were use to prepare the lipid samples.

The recovery of CBs from the pyrenyl column was almost 100% (Table 4). Any losses incurred at this stage of the analysis were due to incomplete transfer of the sample from the vial or incorrect fractionation or collection of the sam-

ple. This section of the sample preparation proved to be highly reliable and repeatable. The geometry of the sample injector/dispenser was such that it was possible to transfer 95–99% of the vial's content. The transfer was, in fact more precise than any attempt to compensate for small losses at this stage by the addition of a "recovery" standard such as CB 209 or CB 53.

Removal of lipids

Extracts containing ca. 250 mg of lipid from biological tissue or 50 mg from sediment is usually sufficient to determine the more abundant CBs in all but the cleanest samples. However, the larger sample size for the planar CB analysis can result in extracts containing up to 10–20 g of lipid all of which must be efficiently removed prior to further sample treatment [3,6,41]. Traces of lipid (e.g., 1 mg) may only be a fraction of the total extract (ca. 1 : 10 000) but this becomes very significant when the sample is concentrated to 1 ml. This amount of lipid, should it remain in the extract, will degrade the pyrenyl column when separating the planar CBs.

Gel permeation chromatography (GPC)

GPC columns, 300–400 × 25 mm i.d., packed with Biobeads SX3 200–400 mesh can remove up to 500 mg of extractable lipid in a single injection. The two main advantages of this technique are that the method can be fully automated and that other chemically sensitive contaminants can be separated from the fat without being destroyed [40]. Tuinstra et al. [47] have used this technique for the removal of animal fat in the determination of planar CBs. Haglund et al. [51], Haglund [55] and Jansson et al. [32] had a very similar system to separate the CBs in extracts of reindeer tissue, fish liver and seal blubber. Separation is made primarily between lipid material > 500 Å which is the first to elute from the column followed by the CBs.

GPC was used to attempt a separation of the lipids and CBs in an extract of fish liver. Although this method is generally useful for removing lipid from samples there are two main disadvantages when determining the *toxic* CBs. The large mass of lipid, ca. 10–20 g, is difficult to remove with a single injection onto a GPC column. The maximum amount of lipid that can be removed from a standard column is ca. 500 mg. To increase the size of the system to cope with 10–20 g lipid would require a prohibitively large column and an excessive volume of solvent to elute the CBs. The second disadvantage of the GPC system is that it is difficult to completely remove *all* of the lipid. Since the triglycerides elute prior to the smaller contaminants the "tail" of the lipid peak intrudes into the second fraction. The amount of lipid in the "tail" is significant because there is a relatively large mass of triglyceride that has to be removed relative to the concentration of the contaminants. Grob and Kälin [56] found that much of the tailing was coming from the injection port and the connecting tubing. Although they reduced the lipid level by appropriate switching, it was not completely eliminated. Even a 0.01% carry over on 1 g of lipid will leave an unacceptably high level of co-extractant in the extract. Until this inherent problem can be solved the low-molecular-weight fraction usually requires further clean-up to remove the trace lipids, e.g., SiO_2 prior to analysis.

Our early attempts to use GPC without further clean-up caused severe degradation of the pyrenyl LC column when separating the CBs. The separation was completely degraded and traces of lipid remained in the final fractions also degrading the GC capillary columns. For these reasons the GPC was discarded as a suitable technique for the analysis of CBs when such large quantities of co-extracted tissue material must be removed.

Saponification

Lipids can be saponified by heating the extract in a small volume of solvent with 20% ethanolic potassium hydroxide at ca. 70°C for 30 min. Van de Valk and Dao [57] found that CB 180 was partially degraded during the saponification of sewage sludge when the reaction temperature was above 70°C for more than 30 min. However, when a standard CB mixture in hexane was treated in the same way there was no measurable degradation.

We initially used CB 209 as a recovery standard, but were only able to recover ca. 50% from

the saponification mixture, although most of the other CBs (Table 1) appeared to be unaffected by the reaction. The poor recovery of the non-*ortho* CBs (Table 5) was initially thought to be due to losses at the saponification stages, but it was subsequently discovered that the non-*ortho* CBs were retained slightly on the silica gel columns [34] even though they were deactivated to 3% (w/w) with water. This observation differs from the work reported by Storr-Hansen et al. [34] which may indicate the variability of such adsorbents in reproducing separation of some CBs. A full recovery was obtained (> 95%) when the elution volume was increased by 2–3 ml to remove all of the non-*ortho* CBs.

All those CBs tested in the Canadian NRCC CLB mixture which were fully substituted on one of the biphenyl rings showed some measurable degree of hydrolysis. These were CBs 209, 208, 207, 206, 205, 203, 195 and to a lesser extent CB 185. CB 194 and CB 196 with five chloro groups on both rings were also partially hydrolysed. The degradation of CB 170 was < 10% over 90 min, but CB 209 was almost completely eliminated after 30 min (Fig. 2). All other CBs, including CB 180 in the CLB mixture were not affected by the reaction at 60°C.

The remaining CBs (CBs 116, 142, 160, 166, 173, 181, 186, 190, 192, 198, 199 and 204) which are also fully chlorinated on one ring were not tested in this mixture, but it is quite possible that these congeners would also be degraded by the alkaline hydrolytic mixture. However, in terms of measurement of environmentally important CBs, this degradation may be a hypothetical problem since seven of these fully substituted CBs (CBs 116, 142, 166, 181, 186, 192 and 204) do not occur in the commercial mixtures [31] and are therefore unlikely to be present in biological tissue.

These particular hydrolytic reactions were only studied with pure solutions. Van der Valk and Dao [57] found that there was a measurable decomposition of CB 180 with a sewage sludge extract, but not with the pure solution. In view of this data it is likely that the hydrolysis observed in the CLB standard mixture could be more pronounced in the extracts of environmental samples due to additional catalytic effects.

Sulphuric acid impregnated silica gel

The main alternative, destructive clean-up method to saponification is oxidative dehydration with concentrated sulphuric acid adsorbed onto silica gel. The degradation primarily removes lipids and wax ester, but many other co-extractants are also removed at the same time [6,32]. The main advantage of this technique is that it is fast, efficient and can remove large quantities of lipid (20 g or more).

It was clear from the early development of the SAISG clean-up column that the method was very efficient. A 20 g lipid solution (20 g cod liver oil in 50 ml DCE) was dehydrated with SAISG and the final eluate contained no measurable traces of remaining lipid. The main difficulty with this method was to obtain the best geometry of the column to minimise the effects of the heat of dehydration with such a bulk of lipid. Ideally, the chromatographic column should be relatively narrow, but this restriction caused excessive overheating when the sample was added. The modification made was to mix 50% of the column charge with the sample in a beaker and denature the lipid prior to packing. This technique eliminated the problems of overheatingand mixed the acid and lipid more evenly. Although this modification was successful with 2 g of lipid, there was a problem when 5 or 10 g of lipid were dehydrated with the formation of a emulsion. Initially the sample extract was dissolved in hexane and mixed in the beaker with the SAISG. However, by changing the supporting solvent to DCE, it was both possible to eliminate the formation of the emulsions and to suspended the silica more easily in the solvent for slurry packing, due to the higher density of the DCE.

All of the selected CBs were removed from the SAISG column in 75 ml of solvent after applying a standard CB solution (Table 7) and there was no trace of any congener in the subsequent fractions. However, when the fish oil (10 g) was spiked the volume of DCE required to remove the bulk of CBs increased to ca. 100 ml, and there was a substantial tail of CBs in the following fractions. After 200 ml of DCE had been eluted > 93% of all CBs which could be removed had been collected (Table 8), but a small propor-

tion of the CBs (5–10%) were retained on the column. Initially we considered whether the more planar CBs would have been preferentially trapped, since a large proportion of the head of the column was microfine carbon. However, there was no apparent effect of spatial structure of the CBs on their recovery from this column. In fact, the non-*ortho* CBs were amongst the highest recoveries. Those CBs which had the lowest recoveries were the less chlorinated congeners; CB 28, CB 52, CB 44 and CB 70.

When the column packing was discarded it appeared to be highly viscous mixture of silica, sulphuric acid, carbon and water. It is likely that a small percentage of CBs were trapped in this mixture which was difficult to remove in situ, even with a large (~ 600-800 ml) volume of solvent. However, further developments to improve

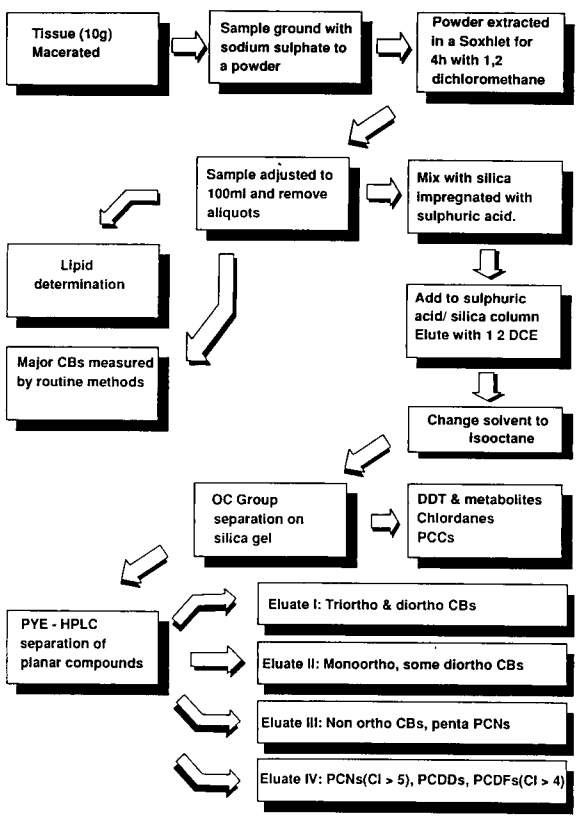

Fig. 3. Schematic diagram of the method for the preparation and separation of CBs prior to the determination by GC-ECD or GC–MS.

the recovery of the lower chlorinated CBs was not really necessary since each of these particular congeners could be determined without any ambiguity on a smaller sample size by well established methods [6,20,21].

The recoveries of the non-*ortho* and mono-*ortho* CBs were both repeatable and quantitative. A number of workers have used ^{13}C labelled congeners, particularly for the determination of the non-*ortho* CBs [47,58,59]. This approach has the added advantage of eliminating much of the error associated with the recovery at each stage in the analysis. However, the isotope dilution technique also has a number of disadvantages for method development that should not be overlooked. Firstly, the intrinsic efficiency of a method should be known. Methods with low recoveries are also often highly variable. Recoveries should be maximised and variability reduced to a minimum and this requires developing the method initially without the use of ^{13}C congeners. Once the method is fully developed it may then more useful to use the isotope dilution technique. However, isotope dilution limits the final detection to using a MS and increases the cost of the analysis, since the ^{13}C labelled compounds are expensive and not always available for all compounds of interest.

Blank measurements

Careful measurements of blank values are essential when determining these planar CBs at the ultra trace level. Blanks were determined using a fish liver oilobtained from the northern North Sea. This oil contained levels of monitoring CBs (see Table 1) below 100 pg kg^{-1}, and was effectively used as a field blank. The limits of determination were based on the matix "noise" from these samples, and the minimum concentration measurable by spiking these oils.

Conclusion

The overall preferred method for the analysis of CBs in fish and sea mammal tissue is given in the schematic diagram in Fig. 3. It is preferable to determine the more abundant CBs, i.e., CB 28, 52, 101, 118, 153 and 180, by taking a fraction of the total extract and making the determination directly by clean-up with alumina and silica prior

to GC-ECD. The non-*ortho* and mono-*ortho* CBs which occur at the ultra-trace level are more appropriately separated on the pyrenyl LC column in two fractions prior to measurement by GC-ECD or MS. In this way all of the congeners which are to be determined are presented to the instrumentation at a concentration which is within the normal calibration range of the detectors.

The limit of determination for the CBs using this method with a sample intake mass of 10 g, wet weight, is of the order 10–50 pg kg^{-1} with an overall variance of $\leqslant 15\%$, using GC-ECD.

One of the authors (IE) wishes to acknowledge the financial support from the Commission of the European Communities bursary given during the tenure of this work. The authors wish to than B. Griepink, P. Quevauviller and E. Maier of the Community Bureau of Reference for their interest and support of this project and for the staff of the chemistry section of the Marine Laboratory. In particular A. Kelly, J. Robson, D. Campbell and L. Campbell for their help in the laboratory and with the instrumentation.

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Analytica Chimica Acta, 286 (1994) 451–456
Elsevier Science B.V., Amsterdam

2,4,6-Trichlorophenyl alkyl ethers as retention index markers in capillary gas chromatography with electron-capture and mass spectrometric detection

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(Received 17th February 1993; revised manuscript received 19th July 1993)

Abstract

A homologous series of eight 2,4,6-trichlorophenyl alkyl ethers (TCPEs) was synthesized to provide a retention index (RI) reference system for routine analysis by high-resolution gas chromatography (HRGC) with electron-capture (ECD) or mass spectrometric (MS) detection. The chemical structure of the RI markers was chosen in order to mimic to some extent the molecular interaction between the GC system and important families of environmental contaminants such as halogenated, low-volatile compounds. Owing to the three chlorine atoms in the TCPEs, a good signal is obtained with ECD. With MS detection the different TCPEs give a common strong signal due to the 2,4,6-trichlorophenol fragment. This allows the monitoring of all the TCPEs with a unique single ion monitoring (SIM) trace. A reference scale of retention indices was set (RI_{TCPE}) with absolute rounded values for the TCPEs (e.g., RI-T2 = 1400, RI-T4 = 1600). The retention indices of the TCPEs referred to *n*-alkanes were also calculated (stationary phase: 5% diphenyl–95% dimethylpolysiloxane). In addition to their use as RI markers, the TCPEs can be used as single or multiple internal standards and as test substances to ascertain possible discrimination phenomena in the GC part (injection block and column) of a GC–MS system.

Keywords: Gas chromatography; Gas chromatography–mass spectrometry; Retention index markers; Trichlorophenyl alkyl ethers

Thirty-five years after its introduction by Kováts [1], the retention index (RI) system is still "perhaps the most significant contribution to the theory and practice of gas chromatography (GC)" [2]. The RI system can contribute to validating the identity of signals in GC and also in liquid chromatography (LC) [3–5]. This is particularly true for GC, where capillary colums and industrial production have led to a dramatic increase in the resolution potential and to better reproducibility. Nevertheless, the RI system is rarely used routinely. One reason is the lack of a universally accepted RI marker series, detectable by electron-capture detection (ECD). As low concentrations of the classical RI markers, the *n*-alkanes, are not detectable by ECD, some ECD-detectable RI markers have been proposed: *n*-alkyl trichloroacetates (ATA) [6,7], monobromo-*n*-alkanes [8], chlorobenzenes and chlorobiphenyls [9], dichlorobenzyl alkyl ethers [10], alkyl bis(trifluoromethyl)phosphine sulphides (M series) [11], nitroalkanes [12] and alkyl nitrates [13].

The choice of possibly a unique and generally accepted RI reference series for the high-resolution (HR) GC–ECD would be desirable. In view of this choice and in order to broaden the range of candidate RI markers, in this work a homologous series of eight 2,4,6-trichlorophenyl alkyl

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SSDI 0003-2670(93)E0423-5

ethers (TCPEs or T2, T4, T6, T8, T10, T12, T14, T16, with alkyl = C_2–C_{16}) was synthesized. The TCPEs can be detected not only by flame ionization detection (FID), atomic emission spectrometry (AES) and mass spectrometry (MS), but also by ECD. A "problem-oriented" approach, aimed at the separation of semi-volatile halocarbons and to their detection by ECD and MS, was preferred to a "universal marker" approach. Owing to some structural parameters in common with important families of halogenated environmental contaminants (biphenyls, terphenyls, biphenyl ethers, dibenzodioxins, dibenzofurans and some classes of pesticides), the TCPEs are likely to interact with apolar or semi-polar stationary phases to some extent in a similar way to the analytes. Better reproducibility is therefore to be expected.

EXPERIMENTAL

Synthesis

The Williamson synthesis of the alkyl aryl ethers was applied in eight separate reactions: 2,4,6-trichlorophenol (4.2 g) and potassium iodide (10 mg) as catalyst were dissolved in dry ethanol (32 ml), stirred and heated under reflux. After addition of the alkyl bromide (2–6 g), the solution was stirred and held at boiling temperature for 6 h. The ethanol was distilled with a Vigreux column under a water-pump vacuum. The reaction products were dissolved in a 5% NaOH solution (15 ml), followed by extraction in a separating funnel with diethyl ether (3 × 15 ml). The organic phase was washed with distilled water until a neutral reaction of the water was attained. The solvent was evaporated in a rotary vacuum system. The total yield of the ether synthesis was 40–60%. The product was weighed and stored at −18°C. The purity of the synthesized compounds, checked by HRGC with FID, ECD and MS detection, was $\geqslant$ 95%. A further purification step involving distillation or sublimation will be applied before using the TCPEs in routine analytical work. The molecular weights of the eight TCPEs ranged between 225.35 (T2) and 421.35 g mol^{-1} (T16).

Instrumentation

An HP 5840A gas chromatograph (Hewlett-Packard, Palo Alto, CA) was used with FID and ECD. The column was DB-5 (5% diphenyl–95% dimethylpolysiloxane) (J & W Scientific, Folsom, CA) (25 m × 0.25 mm i.d.; 0.1 μm; phase ratio β = 625). The carrier gas was hydrogen at 15 psi. Splitless injection was used. The temperatures were injector 250°C, FID 280°C and ECD 300°C and the column was programmed from 90°C (3 min) at 2, 3 and 5°C min^{-1} to 270°C (held for 5 min).

A GC HP 5890 gas chromatograph (Hewlett-Packard), was used with a VG TS 250 double-focusing mass spectrometer with EBE configuration (VG Analytical, Manchester), with electron impact (EI) ionization at 30 and 70 eV. The column was Ultra 2 (5% diphenyl–95% dimethylpolysiloxane) (Hewlett-Packard) (50 m × 0.20 mm i.d.; 0.33 μm; phase ratio β = 151). The carrier gas was helium at 33 psi. Splitless injection was used. The temperatures were injector 280°C and detector source 180°C and the column was programmed from 90°C (3 min) at 2.5°C min^{-1} to 310°C (held for 5 min).

Chemicals

2,4,6-Trichlorophenol (purity 98%) and eight *n*-alkyl bromides (C_2, C_4, C_6, C_8, C_{10}, C_{12}, C_{14} and C_{16}) (purity 97%) were obtained from Fluka (Buchs, Switzerland). Polychlorobiphenyls (PCBs) (IUPAC [14] Nos. 28, 52, 101, 118, 138, 153 and 180) (purity 99%) were purchased from Promochem (Wesel, Germany).

RESULTS AND DISCUSSION

The individual TCPEs and a mixture of all of them were diluted in isooctane at different concentrations (1 μg, 10 ng, 1 ng, 100 pg and 10 pg μl^{-1}). Storage of the solutions for 6 months on the laboratory bench in clear glass bottles did not alter the ECD signal. Injection temperatures of 250 and 280°C were used without significant thermal degradation of the compounds. Signals much over the quantification limit were obtained on injecting the following amounts of a single TCPE: FID 10 ng, MS 1 ng (SIM 50 pg) and ECD 10 pg.

The presence of three chlorine atoms in the TCPE molecule ensures a good ECD response also in the lower picogram range. Peaks with very little tailing were obtained using a 5% diphenyl–95% dimethylpolysiloxane stationary phase (DB-5, Ultra 2).

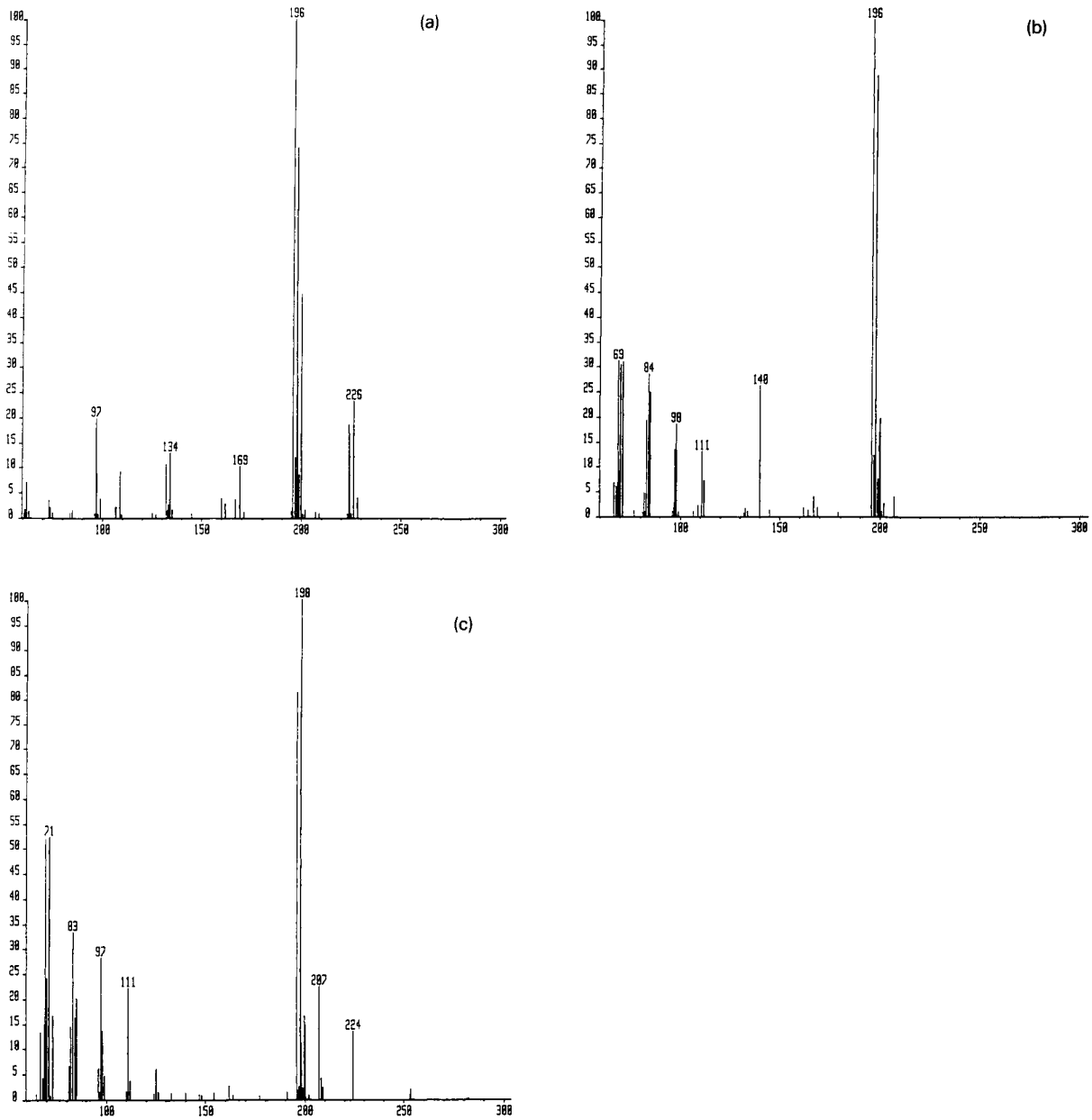

Fig. 1. Fragmentation of (a) 2,4,6-trichlorophenyl ethyl ether (T2, M.W. 225.35), (b) 2,4,6-trichlorophenyl decyl ether (T10, M.W. 337.35) and (c) 2,4,6-trichlorophenyl hexadecyl ether (T16, M.W. 421.35). EI ionization at 70 eV. Trichlorophenol fragment at m/z 196, 198 and 200.

With MS detection (EI, 70 eV) the signal of the molecular ion is weak or at the limit of detection for all the synthesized TCPEs; its intensity decreases with increasing alkyl chain length. The predominant signal is the 2,4,6-trichlorophenol ion fragment (*m/z* 196, 198, 200) (Fig. 1a, b and c). The fragmentation pattern obtained at 30 eV is very similar to that obtained at 70 eV, indicating that the 2,4,6-trichlorophenol fragment is particularly stable. 2,4,6-Trichlorophenol itself with EI ionization gives a strong molecular ion pattern (*m/z* 196, 198, 200). The strong signals of the 2,4,6-trichlorophenol fragment are useful for monitoring the homologous TCPEs.

The retention indices of the TCPEs and of some selected PCBs were calculated with Van den Dool and Kratz's equation for temperature-programmed elution [15]. Using the *n*-alkanes as a reference system, a linear temperature programme of 2.5°C min^{-1} and an Ultra 2 column, the TCPEs give RI_{alkane} values between 1405.6 (T2) and 2895.4 (T16) (Table 1). The TCPEs T4–T16 show increments of the RI_{alkane} values greater than 200 RI units for each CH_2CH_2 group (ΔI between 213 and 216). The RI_{alkane} increment between T2 and T4 is smaller (202 RI_{alkane} units), indicating a non-linear progression of the RI values of the lowest members of the series. This is also the case with other RI markers series [7] and with the *n*-alkane series itself [16].

The purpose of this work being to substitute the *n*-alkanes as a reference system, rounded values of retention indices (RI_{TCPE}) were assigned to the single TCPEs (e.g., RI-T2 = 1400, RI-T16 = 2800). When the logarithm of the adjusted retention time (log t_s) of the TCPEs is plotted against their retention index (RI_{TCPE}, isothermal elution at 130, 150, 170, 190 and 210°C), a linear relationship is observed. Plotting the gross retention time of the TCPEs obtained with a temperature programme of 2°C min^{-1} against the RI_{TCPE} value, the eight TCPEs lay very close to a linear regression through eight points (Fig. 2). A more precise calibration of the RI values would require optimization of the GC conditions oriented for this purpose. However, this is not the case in routine environmental analysis, where the operating parameters are rather regulated so as to obtain time and separation efficiency, e.g., using double ramp heating of the column instead of a linear temperature programme. Table 1 reports the retention indices of the TCPEs with reference to the *n*-alkanes ($RI_{alkanes}$) and those of thirteen trichloroacetates

TABLE 1

Retention indices of 2,4,6-trichlorophenyl alkyl ethers (TCPEs), alkyl trichloroacetates (ATAs) and polychlorobiphenyls (PCBs) on a 5% phenyl–95% dimethylpolysiloxane column with temperature programming at 2.5°C min^{-1}

Substance	$RI_{alkanes}$	ΔI [a] $RI_{alkanes}$	RI_{TCPE}
T2	1405.6	–	1400
T4	1608.0	(202.4)	1600
T6	1822.2	214.2	1800
T8	2035.8	213.6	2000
T10	2251.9	216.1	2200
T12	2466.8	214.9	2400
T14	2682.5	215.7	2600
T16	2895.4	212.9	2800
Mean RI_{alkane} increment for CH_2CH_2 in the TCPE series		214.6	
ATA7		–	1474.8
ATA8		(114.6)	1589.4
ATA9		96.8	1686.2
ATA10		102.3	1788.5
ATA11		97.7	1886.2
ATA12		96.2	1982.4
ATA13		98.2	2080.6
ATA14		95.9	2176.5
ATA15		97.8	2274.3
ATA16		96.4	2370.7
ATA17		98.1	2468.8
ATA18		96.7	2565.5
ATA19		98.2	2663.7
ATA20		97.3	2761.0
Mean RI_{TCPE} increment for CH_2 in the ATA series		97.6	
PCB 28			1853.4
PCB 52			1920.4
PCB 101			2079.7
PCB 118			2192.9
PCB 153			2241.0
PCB 138			2292.2
PCB 180			2428.9

[a] $\Delta I = RI_{nC} - RI_{nC-2(1)}$.

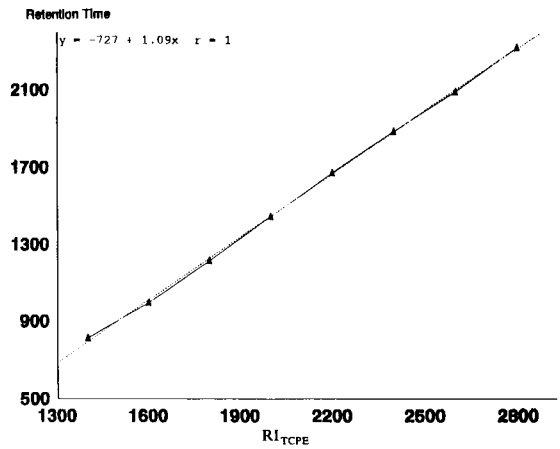

Fig. 2. Plot of the retention index (RI_{TCPE}) of eight TCPEs (T2–T16) against their retention time. The dotted line is the linear regression obtained using the eight points.

(ATAs) and of seven PCB congeners with reference to the TCPE (RI_{TCPE}).

The average RI_{TCPE} CH_2 increment of the homologous series of ATAs (97.6 RI units) is lower than the RI_{TCPE} CH_2 increment of the TCPEs (normalized to 100.0 RI units). This effect is probably due to the different structural features of the two series: a carboxylate group in the ATAs and an aromatic ring in the TCPEs. When the ATAs and the TCPEs are injected together, the members of the TCPE series show an increasing delay with respect to the ATA compounds, which increases with increasing length of the alkyl chain. Owing to their structural analogy to some families of analytes (e.g., PCBs, polyhalogenated dibenzodioxins and dibenzofurans or PHDD/F), the TCPEs are better suited than the ATAs as RI markers for these analytes.

In the analysis of the PCBs the use of the two congeners, PCB 52 and PCB 180, as internal reference compounds has been proposed [17] for the identification of other congeners through relative retention data (RR_{52+180}) based on the equation

$$RR_{52+180} = 2t_{PCB}/t_{PCB52} + t_{PCB180} \quad (1)$$

On plotting the retention indices of the PCBs (RI_{TCPE}) against their relative retentions

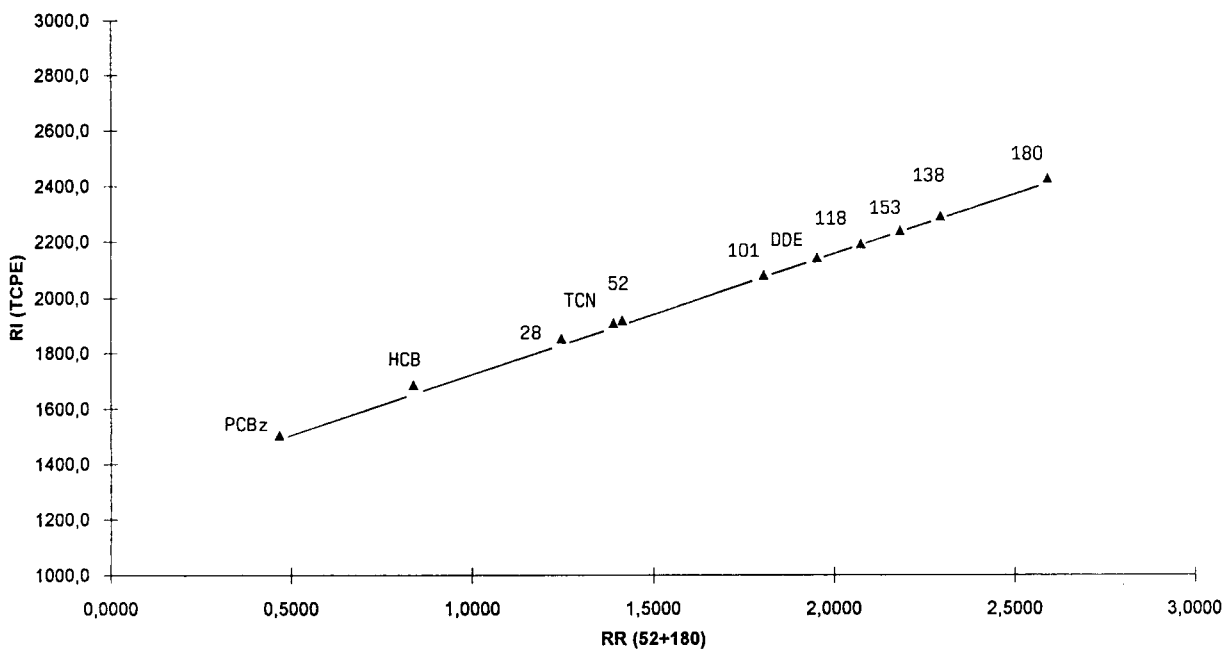

Fig. 3. Plot of RI_{TCPE} against the relative retention ($RR_{PCB52+PCB180}$) for seven PCBs and of some other chlorinated compounds. PCBz = pentachlorobenzene; HCB = hexachlorobenzene; DDE = 4,4′-dichlorodiphenyldichloroethene; TCN = tetrachloronaphthalene. HRGC–ECD with a DB-5 column (25 m × 0.25 mm i.d.; 0.1 μm) programmed at 2°C min^{-1}.

(RR_{52+180}) (Fig. 3), a linear correlation is obtained, confirming the advantage of a homologous series structurally similar to the analytes.

A single TCPE or few of them can also be used as internal standards to be added to environmental samples before extraction and clean-up. The elution range covered by the T2–T16 series on low-polarity stationary phases allows the bracketing of several families of environmental contaminants.

The synthesized series of TCPEs is also suitable for monitoring the performance of the GC part of a GC–MS system. Mass-dependent discrimination of the response of the MS detector can be ruled out by detecting the common trichlorophenol signal; this enables one to ascertain possible discrimination phenomena in the remaining part of the system (injection block and column).

Conclusions

The TCPEs are a useful RI reference system in the GC analysis of halogenated low-volatile, non-polar compounds. They could probably be used as RI markers also for other non-halogenated ECD-detectable analytes (e.g., nitro-aromatic compounds, alkyl nitrates, quinones). A good ECD signal and a strong trichlorophenol fragment signal (m/z 196, 198, 200 amu) ensure good detectability using ECD and MS-SIM.

The authors are grateful to Mr. Thomas Wiedmann for his assistance with the mass spectrometric analyses. M.M. is grateful to the Community Bureau of Reference (BCR) of the Commission of the European Communities (CEC) for a bursary (B/BCR*-913002).

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Analytica Chimica Acta, 286 (1994) 457–468
Elsevier Science B.V., Amsterdam

Optimisation of the liquid chromatographic separation of pirimicarb and its metabolites V–VII: application to a soil sample used as a candidate reference material

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(Received 17th February 1993; revised manuscript received 12th May 1993)

Abstract

The simultaneous separation of the *N*-dimethylcarbamate pirimicarb and its metabolites V–VII was carried out by liquid chromatography (LC) using diode array detection (DAD). Two columns, a 250 × 4.6 mm i.d. column packed with 5-μm particles with a cyanopropyl modified silica stationary phase and a 150 × 4.6 mm i.d. column packed with 3-μm particles with a base deactivated C_8 modified silica stationary phase, were compared. The addition of a buffer in the eluent, for both columns, was needed to improve the peak shape of metabolite V and the resolution of the metabolites V–VII and consequently the pH dependence of the retention time was tested. Additionally chromatograms of two soil samples of different origin, spiked at a concentration level of 1 $\mu g\ g^{-1}$ for pirimicarb, analysed by LC-DAD, gas chromatography (GC) with nitrogen phosphorus detection and GC–mass spectrometry are shown. Detection limits and reproducibility data of pirimicarb in a candidate reference sample using LC-DAD and a cyanopropyl column are reported.

Keywords: Liquid chromatography; Pirimicarb; Soils

Simultaneous analytical methods used for the isolation of polar pesticides and their degradation products are of general interest in addition to the screening methods for pesticide residue analysis in environmental matrices. This is evident from the number of publications dealing with the analysis of degradation products of various pesticides like carbaryl [1,2], carbofuran [3–5], aldicarb [6–8] and atrazine [9,10] in environmental matrices. Different mechanisms can affect the formation of these degradation products in the environment such as physicochemical degradation, e.g., photolysis and hydrolysis, or biological degradation by microorganisms [11]. In the case of Pirimicarb it has been reported that its degradation takes place by hydrolysis of the carbamate group, to form the metabolites V (2-dimethylamino-5,6-dimethyl-4-hydroxypyrimidine), VI (2-methylamino-5,6-dimethyl-4-hydroxypyrimidine) and VII (2-amino-5,6-dimethyl-4-hydroxypyrimidine). Metabolite V is the major degradation product (84%), when pirimicarb is applied to the soil surface, [12]. The structures of the mother compound pirimicarb and the three metabolites V, VI and VII are shown in Fig. 1. Pirimicarb is an *N*-dimethylcarbamate, so it can not be analysed with the

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SSDI 0003-2670(93)E0465-J

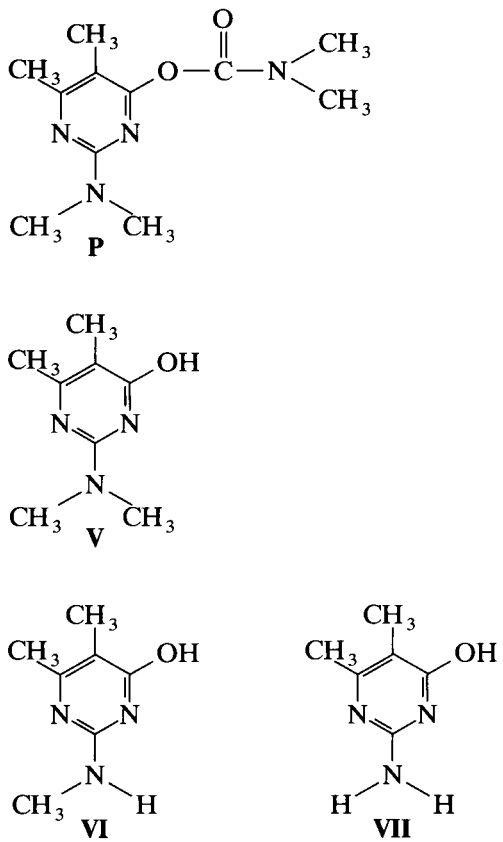

Fig. 1. Chemical structures of pirimicarb (P) and its metabolites V, VI and VII.

post-column fluorescence method developed for the analysis of *N*-methylcarbamates [13–19]. The metabolites V–VII do not contain a *N*-methylcarbamate group and consequently cannot be analysed with this fluorescence method. Gas chromatographic (GC) [20–22] or liquid chromatographic (LC) [23–25] methods of analysis can be used for the determination of Pirimicarb, but not for the analysis of all the degradation products. The extraction, clean up and GC and LC methods are not sufficiently optimised for the simultaneous determination of pirimicarb and its metabolites V–VII.

An LC separation method for pirimicarb and its metabolites V–VII and a reproducible, selective and sensitive analytical method for quantification of pirimicarb in soil samples is required.

This paper gives a comparison between various LC columns for the separation of pirimicarb and its metabolites, the use of DAD(LC) and MS(GC) for the structural confirmation at trace levels, and the development of a method for the quantification of pirimicarb in a candidate reference material for the Community Bureau of Reference (BCR) of the Commission of the European Communities. In this paper these three aspects are discussed and a complete analytical method for the quantification of pirimicarb in two soil samples is presented.

EXPERIMENTAL

Chemicals

Water (for chromatography) and acetonitrile (gradient grade) used for LC separation and dissolution of the standard solutions, were obtained from Merck (Darmstadt). The pesticide grade solvents methanol, acetone and *n*-hexane, used for the extraction and sample clean-up of the soil samples, were also obtained from Merck. Pesticide grade ethyl acetate for the dissolution of the samples analysed by GC was obtained from Sharlau (Barcelona). Ammonium acetate (pro analysis) was obtained from Merck. Acetic acid (pro analysis) was purchased from Fluka (Buchs). Butyric acid, sodium salt, 99% used as eluent buffer was obtained from Aldrich (Steinheim). Butyric acid, 99%, was also obtained from Aldrich. Pirimicarb [dimethyl 2-dimethylamino-5,6-dimethyl-4-pyrimidinyl ester] was purchased from Labor Dr. Ehrenstorfer (Augsburg). The metabolites V [2-dimethylamino-5,6-dimethyl-4-hydroxypyrimidine], VI [2-methylamino-5,6-dimethyl-4 hydroxypyrimidine] and VII [2-amino-5,6-dimethyl-4-hydroxypyrimidine] were gifts from Dr. P. Cabras (Cagliairi). Florisil (Korngrösze 0.150–0.250/60–100 or 100–200 mesh für die Saülen-Chromatographie) was obtained from Merck.

Apparatus

Two LC systems were used. The first LC system consisted of an LC 1090 Series 1 containing three pumps and a diode array UV–Visible detector from Hewlett Packard (Waldbronn). Sam-

ples were introduced via a HP 1050 auto sampler (Cotati, CA). The second LC system consisted of two Model 64 high pressure pumps with a dynamic mixing chamber from Knauer (Bad-Homburg), coupled with a Chrom-a-scope, and completed with Barspec software for data acquisition and data analysis and a rapid scanning UV–visible detector from Barspec (Rehovot). Samples were injected via a 25-μl loop with a Rheodyne valve (Cotati, CA). LC separations were carried out on a 250 × 4.6 mm i.d. column packed with Supelcosil cyanopropyl (PCN) 5-μm particles and a 150 × 4.6 mm i.d. column packed with Supelcosil deactivated C_8 (C8DB) 3-μm particles, both obtained from Supelco (Bellefonte, PA). A gradient analysis was performed at a flow-rate of 0.7 and 1.0 ml min^{-1} respectively for the C8DB and PCN columns. Eluents A contained: 90% 50 mM ammonium acetate buffer in water (for chromatography) with pH 6.0 and 10% acetonitrile (gradient grade). Eluents B contained 100% acetonitrile (gradient grade). The gradient programs used in the LC 1090 Series I liquid chromatograph from Hewlett Packard (Palo Alto, CA) for both columns are listed in Table 1. For the LC-thermospray (TSP)-mass spectrometer (MS) experiment a Model 5989A mass spectrometer equipped with a TSP interface coupled to an LC 1090 Series II solvent delivering system from Hewlett Packard was used.

GC separations were carried out on a 30 m × 0.25 mm i.d. bonded FSOT RSL 300 capillary column with a film thickness of 0.1 μm from Alltech (Eke) and a 30 m × 32 mm i.d. DB-5 column with a film thickness of 0.1 μm obtained from J&W Scientific (Folsom, CA). For the GC nitrogen phosphorus detector (NPD) experiments a 5300 Mega series gas chromatograph equipped with an EL 580 (flame controller) and SL 516 (injector and valve controller) from Carlo Erba (Milan) was used. GC–MS experiments were performed with a 5995 mass spectrometer from Hewlett Packard, interfaced with a 59970 C data system in the EI mode. In both GC methods 2 μl of the sample was injected with a splitless injector.

TABLE 1

Gradient programs used for the C8DB and the cyanopropyl columns with eluent A containing 50 mM ammonium acetate in 90% water and 10% acetonitrile with a pH value of 6.0 and eluent B containing 100% acetonitrile

Column	Time (min.)	Eluent A (%)	Eluent B (%)
C8DB	0.00	100	0
	9.00	50	50
	12.00	20	80
	17.00	100	0
PCN	0.00	100	0
	3.00	100	0
	13.00	40	60
	18.00	100	0

Procedures

Solutions. Three separate 100 mg l^{-1} stock solutions of pirimicarb and the metabolites V and VI in acetonitrile were prepared. The 100 mg l^{-1} stock solution of metabolite VII was prepared in acetonitrile–water (50:50). The working solutions were prepared by diluting the stock solution with acetonitrile. Before injection these standard solution were diluted two times with eluent A.

The ammonium acetate solution was prepared by dissolving 3.845 g ammonium acetate in 900 ml water. After adjusting the pH with acetic acid, 100 ml acetonitrile was added. The butyric buffer was prepared by dissolving 1.10 g butyric acid sodium salt in 500 ml water. After adjusting the pH with butyric acid, 50 ml acetonitrile was added

Sample pretreatment. The extraction and clean-up methods which were used for the analysis of pirimicarb in soil samples have been reported before [25] and described briefly in the following. The recoveries for pirimicarb, following extraction and clean-up were 110% and 111% respectively, using the procedure as described below.

(A) Extraction. Cellulose extraction thimbles (double thickness inner diameter, 22 × 80 mm) obtained from Whatman (Maidstone) were filled with 10 g soil, sieved with 125 μm sieves and freeze dried, from the Ebro delta (Catalunya, Spain). These thimbles were cleaned with methanol (for residue analysis) together with the Soxhlet system during 14 h prior to the Soxhlet

extraction of the soil samples. The soil was spiked with 1 ml standard solution with a concentration of 1 mg l^{-1}, dissolved in acetonitrile, and Soxhlet extracted within 1 h after addition.

The soils were Soxhlet extracted for 16 h with methanol (for residue analysis). The extracted solution was concentrated nearly to dryness with a rotary evaporator operating at 35°C and subsequently to dryness with a nitrogen flow.

(B) Sample clean-up. After evaporation to complete dryness the sample was dissolved in 500 μl *n*-hexane (for residue analysis). This fraction was eluted on a 15 cm long glass column containing approximately 2 g of Florisil (Merck) with 20 ml of a solvent mixture containing 60% *n*-hexane (for residue analysis) and 40% acetone (for residue analysis). The Florisil was activated at 350°C for 14 h and after cooling down to room temperature deactivated by adding 2% (w/v) water (for chromatography) and gently mixed. One day after deactivation the Florisil was ready for use. After collecting all of the extract, the eluent was evaporated to nearly dryness with a rotary evaporator and subsequently to dryness with a nitrogen flow. After clean up the samples were dissolved in ethyl acetate for GC analysis and in eluent A, with a pH value of 6.0 for LC analysis.

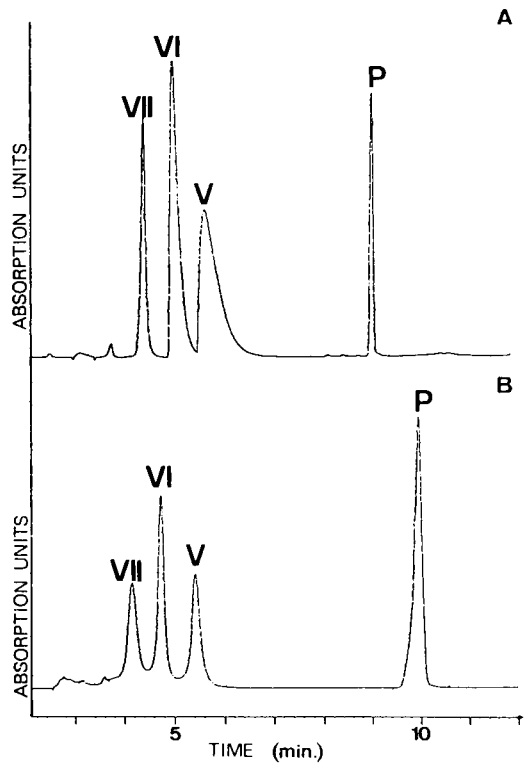

Fig. 2. LC chromatograms at 245 nm of pirimicarb (P), metabolite V, metabolite VI and metabolite VII with a C8DB 150×4.6 mm i.d. 3 μm Supelco column. Acetonitrile–water gradient elution without (A) and with (B) use of a 50 mM ammonium acetate buffer and pH 6,0. Flow-rate, 0.7 ml min^{-1}.

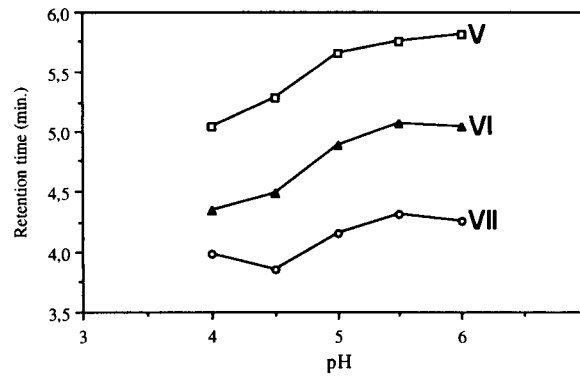

Fig. 3. Retention time of the metabolites V, VI and VII on a C8DB column as function of the buffer pH in a acetonitrile–50 mM ammonium acetate gradient system. UV detection at 245 nm. Flow-rate, 0.7 ml min^{-1}.

RESULTS AND DISCUSSION

The best choice for the simultaneous separation of pirimicarb and its metabolites V–VII was a reversed-phase (RP) C_8 column, [24] although no complete separation of the metabolites V–VII was obtained. This separation problem can be resolved by using a solvent gradient system. The quantification of pirimicarb in soil sample is difficult [25] using a conventional RP C_{18} column. This is mainly due to a severe tailing effect, caused by the presents of free silanol groups in the stationary phase and the high background signal at the beginning of the chromatogram, caused by a high content of early eluting compounds. In view of these problems, a deactivated C_8 ("no presence of free silanol groups") and a cyanopropyl column (recommended for the simultaneous analysis of carbamate pesticides)

where tested for the simultaneous separation of pirimicarb and its metabolites V–VII and for the quantification of pirimicarb in soil samples.

LC separation with a C_8 column

The C_8 column chosen in this study was a deactivated C8DB column. The results of the C8DB column (see Fig. 2) with a gradient elution system indicate complete separation of the metabolites V–VII, however the peak shape of the metabolite V was not satisfactory. The LC-DAD chromatogram at 245 nm of this separation, using an acetonitrile–water gradient from 5:95 100:0 in 16 min with a flow-rate of 1.0 ml min^{-1} is depicted in Fig. 2A. The flow-rate was changed to 0.7 ml min^{-1} in subsequent experiments because of the high back pressure of the column.

The primary, secondary and tertiary amino group of the metabolites V–VII respectively lead to the use of a buffer in the eluent. Ammonium acetate was chosen as buffer, because of its optimum buffer capacity in the range of pH 4–6 and because of its volatility. The volatility of the buffer is of importance when the LC system should be coupled to a TSP-MS system. The addition of an ammonium acetate buffer led to an improvement of the separation of the metabolites V–VII and

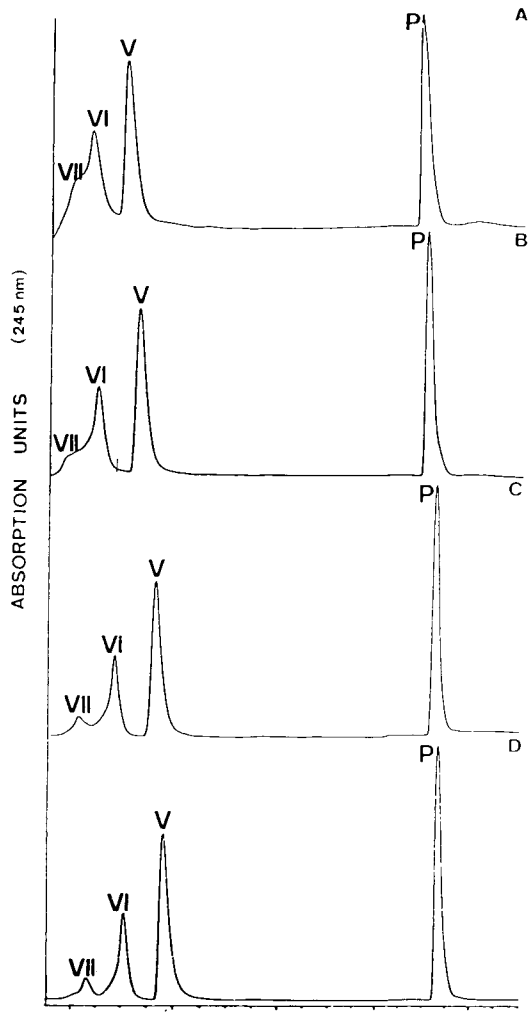

Fig. 4. LC-DAD at 245 nm of pirimicarb (P), metabolite V, metabolite VI and metabolite VII at pH (A) 4.0, (B) 4.5, (C) 5.0, (D) 5.5. Column: 150×4.6 mm i.d. packed with 3-μm C_8 particles. Flow-rate 0.7 ml min^{-1}. Acetonitrile–50 mM ammonium acetate gradient system (see Table 2).

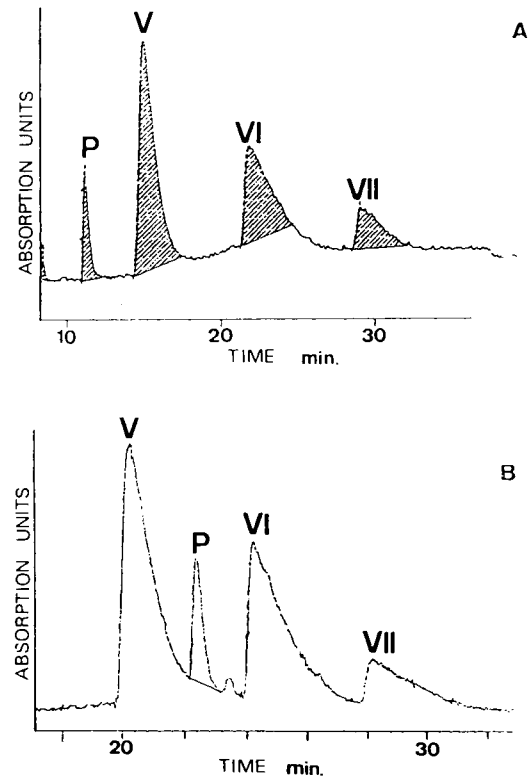

Fig. 5. LC-DAD of pirimicarb (P) and the metabolites V, VI and VII with two different gradient time tables listed in Table 3 and the 250×4.6 mm i.d. cyanopropyl-modified silica column packed with 5-μm particles. Flow-rate, 1.0 ml min^{-1}.

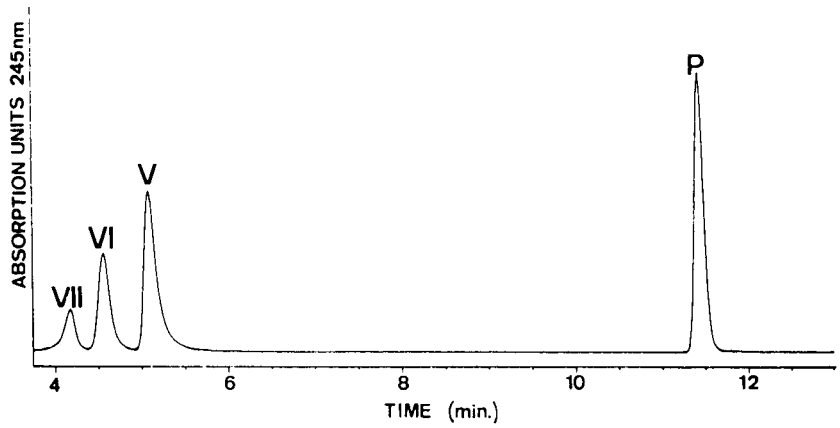

Fig. 6. LC-DAD of pirimicarb (P) and the metabolites V, VI and VII with the 250 × 4.6 mm i.d. PCN column packed with 5-μm cyanopropyl modified silica particles. The gradient time table is listed in Table 1, and eluent compositions are: eluent A, 100% acetonitrile and eluent B, 50 mM ammonium acetate in water–acetonitrile (90:10).

the peak shape of metabolite V. This improvement is illustrated in Fig. 2B. This separation was obtained with a solvent gradient system at a flow-rate of 0.7 ml min^{-1} and 245 nm.

Since buffer had been used, the dependence of the retention time on the pH was tested. Between pH 4.0 and 6.0 the retention times of the metabolites V–VII were virtually constant. The small variation in retention time is illustrated by Fig. 3, representing the dependence of the retention time as function of the buffer pH. The small differences in retention time gave a better separation of the metabolites V–VII (Fig. 4A–D). These separations obtained pH values of 4.0, 4.5, 5.0 and 5.5 respectively at 245 nm using a gradient elution with 100% acetonitrile and a 50 mM ammonium acetate solution in water–acetonitrile (90:10) as the two different eluents. The time table of this gradient elution is listed in Table 1. A flow-rate of 0.7 ml min^{-1} was used. The retention time of pirimicarb was pH dependent between pH 4.0 and 5.0. Above pH 5.0 the retention time was constant. The influence of the pH on the retention time is due to a mixed retention mechanism [26]. After using the C8DB column for three months the separation between the metabolites and pirimicarb, without using a buffer, as illustrated in Fig. 2A, had disappeared completely. The loss in the separation is caused by the loss of the stationary phase in endcapped columns [27]. However when the buffer was used the separation was maintained. However, in spite of these separation the changes in column performance still led to an overall unreliable analytical system. The increase in the amount of free silanol groups in the stationary phase and the change to the Hewlett Packard LC system, because of the different dead volume of the system, caused the slight differences between the chromatograms shown in Figs. 2B and 4A–D.

A butyric buffer was also tested at pH 4 because of its expected "ion pair like" separation behaviour with the tested compounds. A serious drawback of the butyric buffer was its high absorbance at 250 nm.

The signal measured at 245 nm, therefore, suffers from a strong background adsorption. No significant improvement was observed in the separation of the metabolites V–VII and consequently this buffer was not used in further experiments.

LC separation with a cyanopropyl column

In addition to the C8DB column, a PCN column was also tested. The influence of the eluent composition in an isocratic elution system on the retention time of pirimicarb was significant. Change from 20% to 70% acetonitrile in water caused a change in the capacity factor k' of pirimicarb from 2.4 to 6. Different acetonitrile–

TABLE 2

Gradient programs used for the separation of pirimicarb and its metabolites V, VI and VII in Figs. 5A (A) and 5B (B) with the PCN column using a two gradient elutions with eluent A containing a water–acetonitrile (90:10) and eluent B containing 100% acetonitrile.

	Time (min)	Eluent A (%)	Eluent B (%)
A	0.00	55	45
	8.00	55	45
	18.00	0	100
	33.00	0	100
	40.00	55	45
B	0.00	100	0
	8.00	100	0
	18.00	55	45
	33.00	55	45
	40.00	100	0

water gradient profiles gave good separation of pirimicarb and the metabolites V–VII. Although a good separation of pirimicarb and its metabolites V, VI and VII was achieved, the LC-DAD system at 270 nm using two different acetonitrile–water gradient systems illustrates the poor peak shapes and large tailing of the metabolites V–VII (Fig. 5A and B). The time tables of these two gradient systems are listed in Table 2. Compared to the LC-DAD chromatogram of these compounds with the C8DB column, without the use of an ammonium acetate buffer in the eluent as shown in Fig. 2A. The elution order of the three metabolites with the PCN column is reversed in comparison to the elution order of these compounds with the C8DB column. The strong tailing of the peaks can be explained by the presence of silanol groups. The eluents used for the separation with the C8DB column, were also used for the separation with the PCN column. In contrast to the C8DB column a flow-rate of 1.0 ml min^{-1} was applied. Addition of ammonium acetate to the mobile phase changed the elution profile. After addition of ammonium acetate, the separation of pirimicarb and the metabolites V–VII were similar both for the PCN column and with the C8DB column. The ammonium acetate also changed the elution order of the compounds; the metabolites V and VI eluted after metabolite VII.

The separation profile using LC-DAD at 245 nm with the PCN column is shown in Fig. 6 and gradient elution with ammonium acetate buffer at pH 6.0. Consequently a pH dependence test was performed. Changing the pH of the eluent did not have influence on the retention time of the metabolites V–VII.

The retention time of pirimicarb was altered from 10.30 min to 11.45 min when the eluent pH

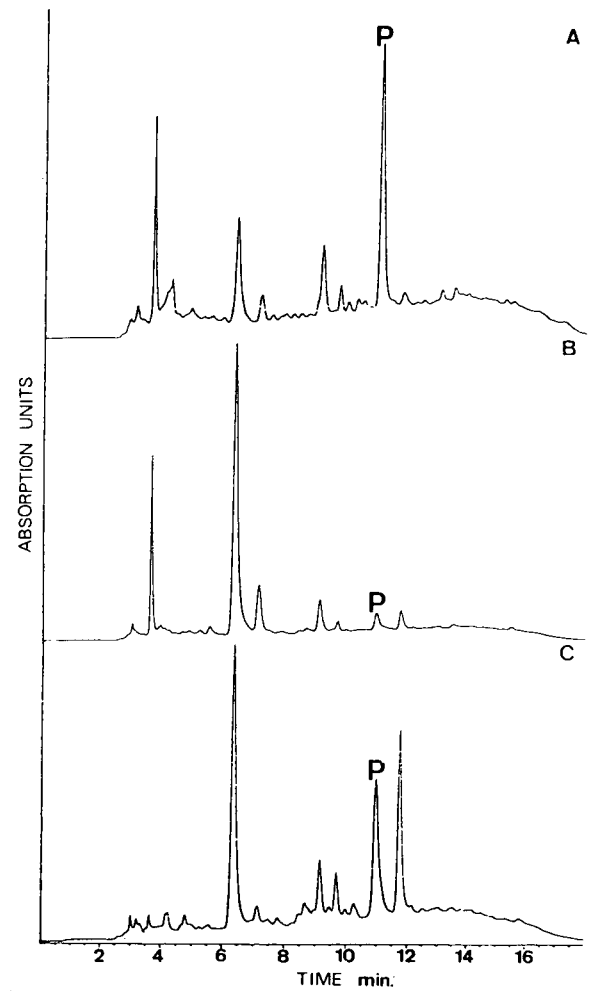

Fig. 7. LC-DAD of soil sample extract type I, using the 250×4.6 mm i.d. PCN column with 5-μm cyanopropyl modified silica particles, spiked at 1 mg l^{-1} level for pirimicarb (P) and at a 0.5 mg l^{-1} level for the metabolites V, VI and VII at three different absorption wavelengths: (A) 245, (B) 270 and (C) 311 nm.

was changed from 4.0 to 5.5. Above pH 5.5 the retention time of pirimicarb was constant.

Calibration curves of pirimicarb using the PCN column with and without the addition of ammonium acetate to the eluent were obtained at 245 nm and 311 nm. The calibration curves were linear between 50 μg l^{-1} and 10 mg l^{-1} at 245 nm and between 282 μg l^{-1} and 10 mg l^{-1} at 311 nm. The correlation coefficients of the calibration curves are fully satisfactory ($r^2 = 0.99$). For both calibration curves variances between 1 and 5% were obtained. At 245 nm the limit of detection (LOD) was 20 μg l^{-1} when 25 μl was injected. At 311 nm the absorption response was a factor 6 less. The PCN column is the preferred choice for the simultaneous analysis of pirimicarb and its metabolites V–VII.

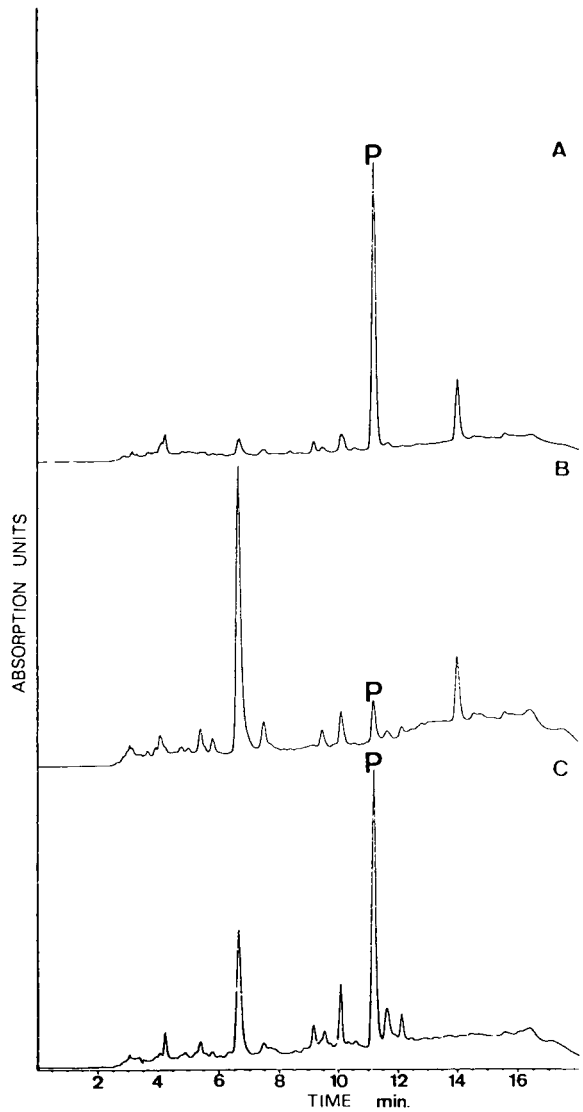

Fig. 8. LC-DAD of soil sample extract type II, using the 250×4.6 mm i.d. PCN column with 5-μm cyanopropyl modified silica particles, spiked at 1 mg l^{-1} level for pirimicarb (P) and at a 0.5 mg l^{-1} level for the metabolites V, VI and VII at three different absorption wavelengths: (A) 245 nm, (B) 270 nm and (C) 311 nm.

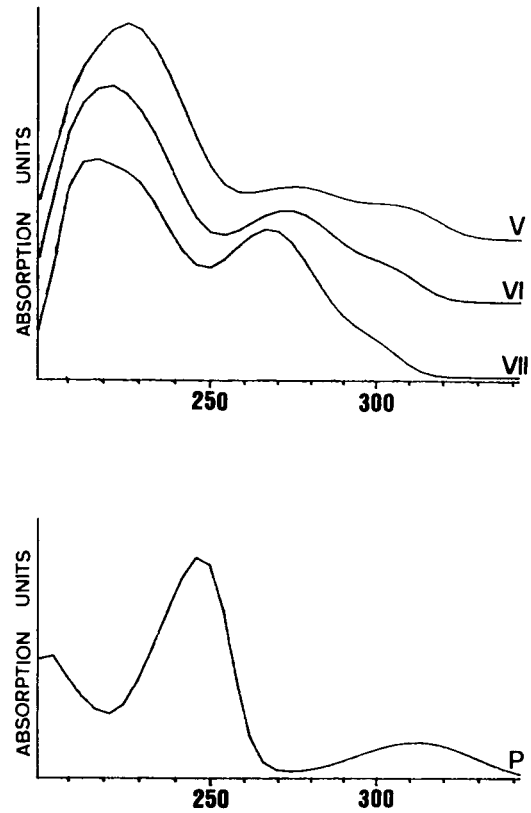

Fig. 9. UV spectra of pirimicarb (P) and its metabolites V, VI and VII.

Soil samples from the Ebro delta used as candidate reference materials of the BCR

The quantification of pirimicarb in soil samples suffered from a strong interfering effect, from early eluting compounds when using the deactivated base C_8 column. Consequently the PCN column was used for the quantification of pirimicarb in soil samples. A gradient system containing two eluents, respectively 100% ace-

tonitrile and 50 mM ammonium acetate with pH 6.0 in 90% water and 10% acetonitrile, was used for the separation of these compounds.

Two types of soil samples from separate locations in the Ebro delta (Catalunya, Spain) were analysed using the extraction and sample clean-up described in the procedure section. Soil type 1 contained 31% clay, 57% silt, 12% sand and 3.3% organic matter by mass. This soil type 1 was polluted with pirimicarb, and collected 3 days after spraying. Figure 7A–C shows the LC-DAD chromatograms with the PCN column at the wavelengths 245 nm (A), 270 nm (B) and 311 nm (C) of a sample from soil type 1 spiked at a soil concentration of 1 mg l^{-1} for pirimicarb. Soil type 2 contained 8% clay, 28% silt, 64% sand and 2.5% organic matter by mass. This soil type 2 was polluted with atrazine and collected three days after spraying. Figures 8A–C shows the LC-DAD chromatograms with the PCN column, at the wavelengths 245 nm (A), 270 nm (B) and 311 nm (C), of a sample from soil type 2 spiked with pirimicarb at the same soil concentrations as in soil sample 1. The difference of the matrix effect between the two types of soil sample can be seen by comparing Figs. 7 and 8.

In these figures it can be observed that the expected increase of the selectivity, when 311 nm was used, is not reached. This influence of the sample matrix is an important parameter for the validation of environmental analysis methods.

A lot of the matrix compounds are trapped at the beginning of the PCN column whereas in the C8DB column most matrix compounds elute at the beginning of the chromatogram. It should be pointed out that the cyanopropyl column has to be flushed, or backflushed, with 100% acetonitrile after use.

When 10 g of soil is extracted and sample preparation is carried out the LOD of the analytical system was 5 ng g^{-1} with 25 μl injections of soil sample, although the chemical noise can disturb this limit of quantitation. An LOD of 5 ng ml^{-1} for all the compounds, using 50-μl injections, was reported before [24].

A soil content of approximately 5 kg of soil type 1, was spiked a concentration level of 10 μg g^{-1} with pirimicarb. After homogenizing the soil was freeze dried. Using the complete analytical procedure, with the PCN column, five samples where randomly collected and analysed. The analysis resulted in a concentration of 9.8 ± 0.9

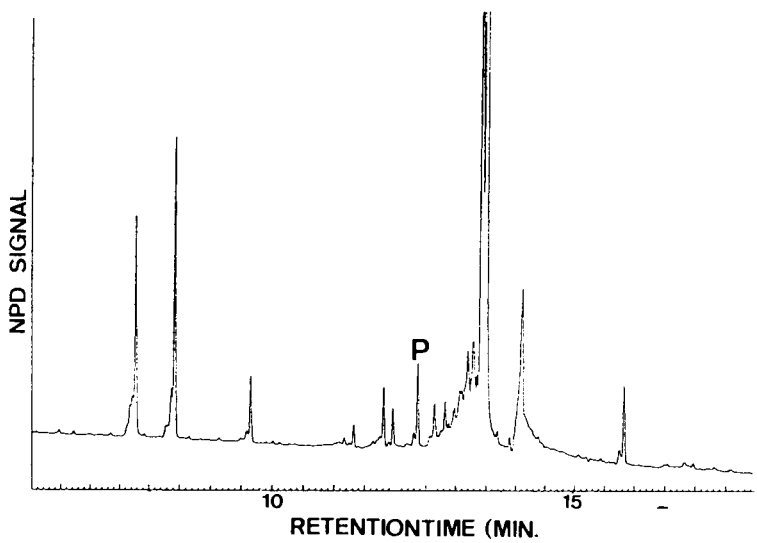

Fig. 10. GC-NPD of a soil sample, polluted with pirimicarb (P), from the Ebro delta with the DB5 capillary column.

$\mu g\ g^{-1}$. Using the student-t test for $n = 5$ the analysis resulted in a concentration of 9.1 ± 3.0 $\mu g\ g^{-1}$. From these results it can be stated that the candidate reference material can be analysed by using the described analytical method.

Gas chromatographic analysis

The differences between the UV absorption spectra of the metabolites V–VII is small (Fig. 9). These small differences are responsible for the fact that structural confirmation of the metabolites V–VII at trace levels in soil samples cannot be obtained from conventional DAD data. The use of GC with more sensitive and selective detectors, like NPD and MS for the structural confirmation of these metabolites is also not possible, because the metabolites V–VII are too polar to be separated with a GC system. Contrary to the metabolites, pirimicarb can be analysed by GC. The use of GC-NPD and GC-MS for the analysis of pirimicarb in the soil samples causes the increase of the sensitivity by a factor of 10 to 100 compared to LC-UV systems. Although an increase in the sensitivity is obtained, the expected increase of the selectivity was not obtained, as is illustrated in the Figs. 10 and 11. Figure 10 shows the GC-NPD chromatogram of a soil sample spiked with 1 mg g^{-1} pirimicarb. The

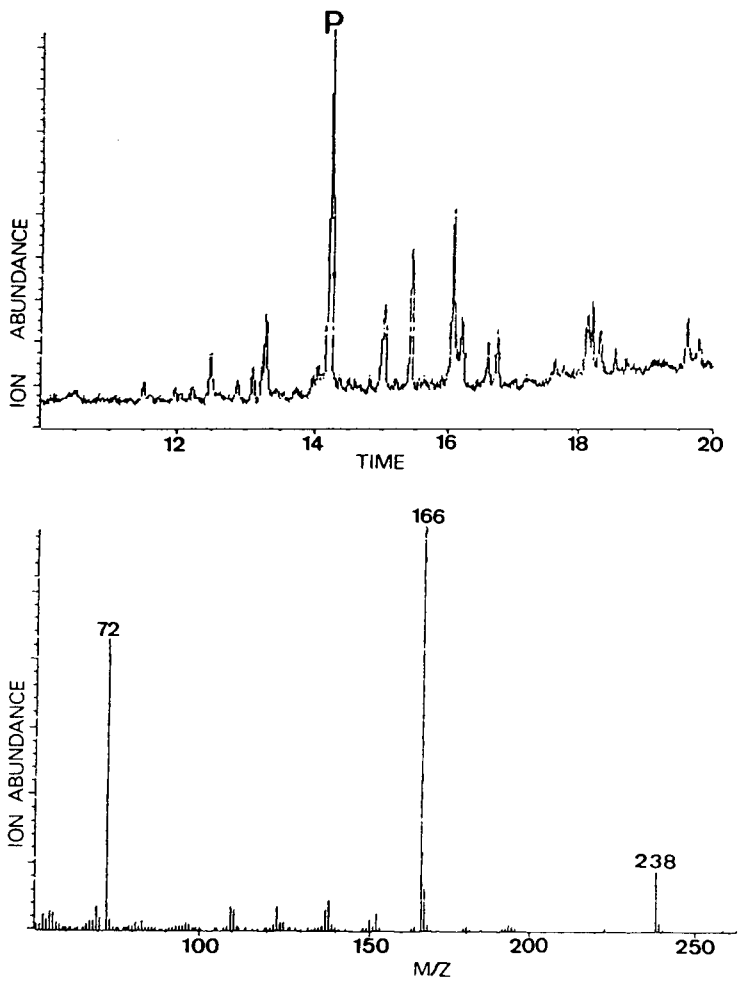

Fig. 11. SIM GC–MS of a soil sample spiked with Pirimicarb (P) at a level of 1 mg l^{-1} (A) and the EI mass spectrum of pirimicarb (B) with a DB5 capillary column. For GC temperature program see Table 3.

TABLE 3

Temperature program used for the analysis of pirimicarb with the DB 5 capillary column using NPD and MS

Rate (°C min^{-1})	Temperature 1 (°C)	Temperature 2 (°C)
15	60	100
8	100	220

optimized separation was obtained on a DB 5 column together with a temperature program (Table 3). The same column and temperature program were used for the analysis of the same types of soil samples with GC–MS. The chromatograms were obtained using selected ion monitoring (SIM) (Fig. 11). The mass monitored in the SIM mode was 166, which is formed by neutral loss of 72 [$(CH_3)_2NCO$] from the molecular ion with m/z 238. The LOD for GC-NPD and the GC–MS, in the SIM mode are 0.5 μg l^{-1} when 2 μl was injected, thus approximately two orders of magnitude more sensitive then the LC-UV-DAD system, as expected.

The two chromatograms presented in Figs. 10 and 11 still show a large amount of matrix compounds. The use of GC–MS/MS for the analysis of pirimicarb in soil samples increases the selectivity and consequently can overcome the problem of the matrix compounds. Also, changing from the electron impact (EI) to the chemical ionisation (CI) mode can increase the selectivity and additionally the decrease of the matrix effect.

Conclusion

The metabolites V–VII cannot be analysed by GC and consequently for the simultaneous analysis of the metabolites V–VII and pirimicarb the LC-DAD method with the PCN analytical column is the preferred choice. The optimum buffer pH for this separation, using a 50 mM ammonium acetate buffer, was 6.0.

The comparison of two LC systems for the analysis of pirimicarb in soil samples showed that, use of the PCN column resulted in a robust and reproducible analytical method. Detection limits of 5 ng g^{-1} for the analysis of pirimicarb in soil samples can be obtained using this analytical method. The analysis of pirimicarb in candidate reference materials distributed by the BCR can be performed with the described extraction and clean up protocols together with the LC and GC analytical systems.

For the determination of the mother compound, pirimicarb, the GC method meets the requirement for the low detection limits (1 μg l^{-1}) and the easy access to additional structural information for the confirmation purposes, but it must be emphasised that injection of relative dirty samples in a GC-NPD or GC–MS analytical system, can strongly influence the reliability of this analytical method.

Further work will contain the development of other clean up protocols for the simultaneous isolation of pirimicarb and its metabolites V–VII from soil samples and the application of more selective analytical methods like LC–TSP–MS for the simultaneous analysis and structural confirmation of pirimicarb and its metabolites V–VII at trace levels in soil samples.

M.H. has a fellowship from the Community Bureau of Reference of the Commission of the European Communities (BCR*-913001). This research project has been financed by the contract "Preparation and stability studies of an estuarine sediment containing pesticides" No. 5171/1/9/332/89/06-BCR-E(10) from the Community Bureau of Reference of the Commission of the Euorpean Communities. Hewlett Packard (Mr. R. Soniassy) is acknowledged for the loan of an LC-DAD type 1090 series apparatus. Dr. P. Cabras (University of Cagliari, Italy) is thanked for providing the metabolites V–VII of pirimicarb.

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Analytica Chimica Acta, 286 (1994) 469–476
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Susceptibility of bacterial strains to desiccation: a simple method to test their stability in microbiological reference materials

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(Received 17th February 1993; revised manuscript received 25th June 1993)

Abstract

The purpose of this work was to predict the behaviour of bacteria within spray-dried powders used for the production of microbiological reference materials. The survival of eighteen bacterial strains desiccated on anhydrous silica gel and stored at 22°C for at least 3 months was determined. According to their percentage survival after desiccation, storage and reconstitution after certain time periods a classification of these bacterial strains was possible. Gram-positive strains such as *Enterococcus faecium* showed a relatively high resistance to desiccation with more than 30% survival on each day of sampling. Certain Gram-negative strains such as *Aeromonas hydrophila* were very susceptible to this treatment; culturable cells could not be determined as soon as 1 week after desiccation. Other Gram-negative organisms (e.g., *Salmonella* serotypes, *Escherichia coli* and *Enterobacter cloacae*) were less susceptible to drying than this group but showed a lower resistance than the Gram-positive bacterial strains. The use of anhydrous silica gel is probably a suitable basis for the development of a screening system to characterize the susceptibility of bacterial strains to desiccation. Further, the suitability of methods to improve the stability of the concentration of microorganisms in dry materials could be investigated with the help of this desiccation system.

Keywords: Bacterial strains; Desiccation; Microbiological reference materials; Stability

Thousands of laboratories throughout the European Community (EC) are daily engaged in the microbiological analysis of water and food. Industrial laboratories monitor the quality of raw materials, the effectiveness of treatment processes and the quality of the end-product. Public laboratories monitor the quality of food, drinking water and recreational waters to determine whether existing national or international guidelines and standards are being satisfied. International standards for the microbiological quality of water and food are primarily those formulated by the EC and by WHO. It is not known how many microbiological analyses of water and food are performed annually within the EC, but the number must run into many millions. The large number of microbiological analyses indicate the importance of quality assurance of these tests.

Incorrect test results may have a great economic or public health impact. False-positive results can lead to the unnecessary condemnation of batches of food products or the unnecessary closure of bathing beaches. False-negative results, on the other hand, may lead to improper competitive positions or direct risks to public health due to the release of contaminated food products, the consumption of inadequately treated water or

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SSDI 0003-2670(93)E0398-Q

swimming in polluted water. The great potential impact of microbiological analyses of water and food, the analytical errors that are frequently detected and the immature state of quality assurance practices in microbiological laboratories warrant further investigations at all levels. The use of microbiological reference materials plays an essential role in this respect.

Reference materials are hardly known in microbiology. Therefore, efforts are being made in microbiological science to develop and agree on reference methods. However, the nature of microbiological tests is not such that they can be claimed to come close to the true value in all circumstances. Depending on the circumstances, such as the presence of competing microorganisms and type of sample, even the (agreed) reference methods can lead to systematic errors. Using such methods for certification makes the certified parameter method dependent.

In the past, several attempts have been made to produce microbiological reference materials. Most of these attempts relied on the preservative effect of low temperatures [1] and/or the addition of bacteriostatic compounds [2]. They have limitations with regard to the logistics of distribution and the period during which the samples can be used. Preservation by freeze-drying improves the stability of the product and thus reduces the constraints placed upon transport and timing of collaborative studies [3]. This method, however, also has its limitations, such as poor survival of some strains during freeze-drying and the fact that it is difficult to prepare a quantitative sample.

Investigations carried out by Van Schothorst and Van Leusden [4] and Beckers et al. [5] led to the choice of artificially contaminated spray-dried milk as the basic material for microbiological reference materials as prepared by the National Institute of Public Health and Environmental Protection [6]. Also in this process some strains will have a poor survival as with freeze-drying, but the advantage is that the preparation of quantitive materials is easy by diluting the contaminated spray-dried milk with sterile milk powder until the desired contamination level. Gelatin capsules are then filled with 0.2–0.3 g of the mixed powder and the resulting capsules are the final reference materials. The desired contamination level depends on the application of the final materials. A distinction is made between materials used for qualitative tests and those used for quantitative tests. The desired contamination level of the former type of material is 5 cfp per capsule and that of the latter is 500 cfp per capsule or 10^4 cfp per capsule (cfp = colony-forming particles).

During the spray-drying process, microorganisms are exposed to heat stress. Additionally, they are osmotically stressed during the drying process and the procedures of reconstitution [7]. The concentration of culturable bacteria in the spray-dried material decreases during storage depending on the storage conditions and due to sublethal damage of the bacterial cells caused by the stress factors heat and desiccation. In order to elucidate the significance of the effects of these two stress factors on the viability of the dried and reconstituted microorganisms, a study was set up to test these two factors separately. On the one hand, experiments were carried out to obtain more information concerning the heat susceptibility of certain bacterial strains. On the other, a drying procedure working without the use of extremely high or low temperatures was developed. This paper describes the application of anhydrous silica gel in a model desiccation system.

In 1962, Perkins [8] published a drying method for the preservation of fungal spores and mycelium. These microorganisms were suspended in skim milk and desiccated by mixing the suspension with anhydrous silica gel. This technique has been used successfully to preserve other moulds [9] and also bacteria [10] and viruses [11]. Depending on the strain and the storage temperature, the microorganisms survived under these conditions for up to several years. So far no quantitative data have been reported concerning the survival rate of bacteria exposed to this desiccation treatment.

The purpose of this work was to collect quantitative data (percentage survival) concerning the resistance of several bacterial strains to desiccation on silica gel and to determine whether this drying procedure would be suitable for screening

the susceptibility of microorganisms to desiccation.

EXPERIMENTAL

Bacteria strains and methods of cultivation

Four Gram-positive (strains 15–18) and fourteen Gram-negative (serotypes 1–9, strains 10–14) bacterial strains, as listed in Table 1, were selected. The distinction between Gram-positive and Gram-negative bacteria is very common in the field of bacteriology and is based on structural differences in the cell walls of the organisms belonging to these two groups. Owing to these structural differences the bacteria react in a different way to a staining procedure according to Gram (1884): the cell wall of Gram-positive bacteria retains the staining produced by iodine–crystal violet complex so that the cell appears blue under microscopic examination. Gram-negative cells lose this stain during a subsequent washing procedure with alcohol and can be stained with a red complex that contrasts with the former under microscopic examination. A number of the strains mentioned in Table 1 have already been used for the development of spray-dried reference materials (Nos. 1, 7, 10, 11, 12, 13 and 15). Nine different *Salmonella* serotypes (Nos. 1–9) were chosen to test whether the sensitivity against extremely dry conditions would vary among different serotypes of one genus. Two examples each of the serotype *Salmonella typhimurium* (Nos. 1 and 2), and the species *Enterococcus faecium* (Nos. 15 and 16) and *Lactobacillus plantarum* (Nos. 17 and 18) were selected to compare the resistance of different phagetypes resp. strains of one serotype resp. species to desiccation.

All organisms listed were grown in brain–heart infusion (BHI) (Oxoid, CM 225). Incubation temperatures and times are listed in Table 1.

Drying procedure

A 1-ml volume of each BHI culture was added to 9 ml of skim milk (dry weight 200 g l^{-1}, fat content 40 g l^{-1}) and mixed thoroughly. These suspensions were used for the following drying procedure. Silica gel particles (Kieselgel; Merck, Art. 7735) with a diameter of 5 mm were mixed with a certain amount of water to cause an extreme exothermic reaction which resulted in the breaking of the 5-mm particles into pieces of 1–2 mm. These smaller particles were dried at 175°C and were transferred in portions of 1 g into glass

TABLE 1

Bacterial strains and cultural conditions

Bacterial strain No.	Bacterial strain	Temperature (°C)	Time (h)
1	*Salmonella typhimurium*, phagetype 505	37	24
2	*Salmonella typhimurium*, phagetype 240	37	24
3	*Salmonella enteritidis*, lab. code 8215	37	24
4	*Salmonella hadar*, lab. code 7252	37	24
5	*Salmonella infantis*, lab. code 7198	37	24
6	*Salmonella livingstone*, lab. code 7295	37	24
7	*Salmonella panama*, lab. code alm 41	37	24
8	*Salmonella senftenberg*, lab. code 7202	37	24
9	*Salmonella thompson*, lab. code 7398	37	24
10	*Enterobacter cloacae*, lab. code WR 3	37	24
11	*Escherichia coli*, lab. code WR 1	37	24
12	*Pseudomonas aeruginosa*, lab. code alm 32	41.5	24
13	*Aeromonas hydrophila*, lab. code M 800	30	24
14	*Aeromonas sobria*, lab. code WA 40	30	24
15	*Enterococcus faecium*, lab. code WR 63	37	48
16	*Enterococcus faecium*, lab. code M 74	37	48
17	*Lactobacillus plantarum*, lab. code W 2	37	48
18	*Lactobacillus plantarum*, lab. code B 5	37	48

bottles with a volume of 12 ml. These bottles are normally used for the moisture-free storage of vaccines. The bottles were heat sterilized at 175°C for 5 h. For each bacterial species 0.1 ml of the skim milk suspension was added to 1 g of sterilized silica gel. Directly after adding the suspension it was mixed with the silica gel particles by using a whirl mixer (2000 rev. min^{-1}) for 30 s. The bottle was closed tightly with a rubber septum and an aluminium top. For each bacterial strain 36 glass bottles were filled using this method. The water activity (a_w) of the silica gel before and after adding the bacterial milk suspension was determined by using the Novasina, TH-2, RTD-33.

Storage

All bottles containing contaminated silica gel were stored in an incubator at 22°C. This temperature was chosen because one of the requirements for a microbiological reference material is the stability of the concentration of culturable bacteria at 20–25°C.

Reconstitution

The survival of desiccated organisms was determined 1 day after desiccation and subsequently at regular time intervals (7 or 14 days) over a period of at least 3 months. On each occasion of verification the contents of two glass bottles were analysed in parallel. A portion of 1 g of contaminated silica gel was mixed with 2 ml of precooled (10°C) evaporated milk (dry weight 200 g l^{-1}, fat content 40 g l^{-1}) and suspended on a whirl mixer (2000 rev. min^{-1}) for 60 s. A 2-ml volume of peptone (1 g l^{-1}) saline (8.5 g l^{-1} NaCl) solution was added, the whole mixture was suspended again and 1 ml of this suspension was serially diluted in peptone saline solution.

From all samples including the serial dilutions of the initial bacteria milk suspensions certain dilution steps were spread in duplicate on trypticase soy agar (TSA) (Oxoid, CM 131) and were incubated under optimum growth conditions (see Table 1).

Calculations

Viable counts per ml of original suspension (cfp/ml) and per ml dried material were determined. A colony-forming particle is a certain amount of microorganisms which grows by cell division and produces a large number of cells (= colony) that is visible to the naked eye [6]. With the help of this value the percentage survival of desiccated organisms after certain time periods could be calculated. Subsequently for all strains the initial decrease (ID), i.e., the decrease in the logarithm of colony-forming particles per ml within a defined time period after desiccation (see Table 2), was determined. It covers a period beginning with day $t = 0$, when the desiccation process was carried out, and ending on day $t = z$, representing the first day of the time period marked by a very slow decrease in cfp/ml. Depending on the bacterial strain the ID can be of different length. Regarding the time period starting with day $t = z$ and ending with the last occasion of verification for all strains except Nos. 12, 13 and 14, the decimal reduction time D was

TABLE 2

Survival of bacterial strains desiccated on anhydrous silica gel

Bacterial strain No.	Log cfp/ml on day $t = 0$ [a]	Initial decrease (ID) [b]	D value (days) [c]	a_w value of contaminated silica gel [e]
1	7.8	1.8(14)	1290	0.20
2	8.7	2.8(14)	475	0.20
3	9.4	3.4(21)	300	0.20
4	9.4	3.7(14)	340	0.20
5	8.3	2.5(14)	315	0.20
6	8.2	1.8(14)	248	0.20
7	8.3	1.4(14)	677	0.20
8	8.2	1.8(14)	419	0.20
9	8.5	2.7(28)	355	0.20
10	8.3	1.4(1)	23	0.20
11	8.2	2.0(1)	30	0.25
12	7.0	> 4(1)	n.d. [d]	0.25
13	8.1	> 4(1)	n.d.	0.25
14	7.4	> 4(1)	n.d.	0.25
15	7.9	0.2(7)	2114	0.25
16	7.8	0.1(7)	775	0.25
17	7.9	0.2(14)	396	0.25
18	7.7	0.2(21)	362	0.25

[a] Log cfp/ml of the original bacteria in milk suspension before drying. [b] Decrease in log cfp/ml within a defined period of storage (given in parentheses in days). [c] Decimal reduction time in days. [d] n.d. = not determined. [e] Water activity of the silica gel before addition of the bacteria in milk suspension: $a_w = 0.07$.

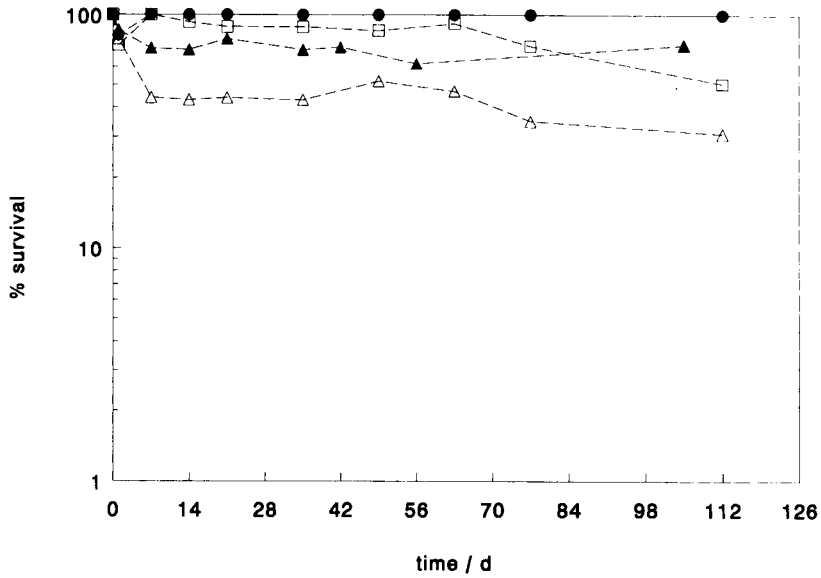

Fig. 1. Survival of Gram-positive bacterial strains desiccated on silica gel. ▲ = No. 15, *Enterococcus faecium*, lab. code WR 63; ● = No. 16, *Enterococcus faecium*, lab. code M 74; □ = No. 17, *Lactobacillus plantarum*, lab. code W 2; △ = No. 18, *Lactobacillus plantarum*, lab. code B 5.

calculated based on logarithmically transformed contamination levels. The D value is a direct measure of the resistance of an organism at a given stressful condition. It is the time required to inactivate 90% of a population [12]. In this case the D values are given in days. The calculation is based on the following equation:

$$D = t - t_z / \log \mathrm{cfp}_{tz} - \log \mathrm{cfp}_t$$

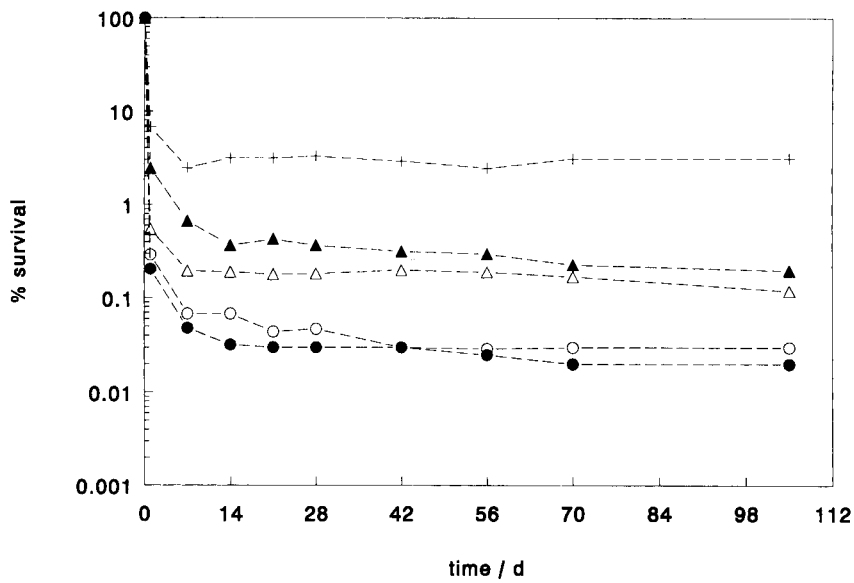

Fig. 2. Survival of *Salmonella* serotypes desiccated on silica gel. + = No. 1, *Salmonella typhimurium*, phagetype 505; △ = No. 2, *Salmonella typhimurium*, phagetype 240; ○ = No. 3, *Salmonella enteritidis*, phagetype 8215; ● = No. 4, *Salmonella hadar*, lab. code 7252; ▲ = No. 5, *Salmonella infantis*, lab. code 7198.

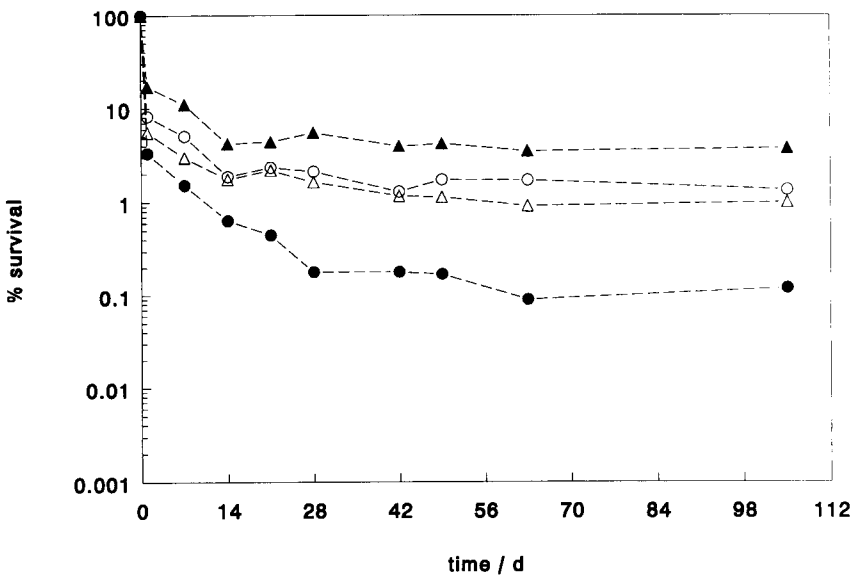

Fig. 3. Survival of *Salmonella* serotypes desiccated on silica gel. △ = No. 6, *Salmonella livingstone*, lab. code 7295; ▲ = No. 7, *Salmonella panama*, lab. code alm 41; ○ = No. 8, *Salmonella senftenberg*, lab. code 7202; ● = No. 9, *Salmonella thompson*, lab. code 7398.

RESULTS AND DISCUSSION

The investigation described in this paper was carried out to obtain quantitative data concerning the stability of several bacterial strains in the dried state. With regard to the osmotic stress caused by desiccation and according to the results of a number of stability tests on spray-dried mate-

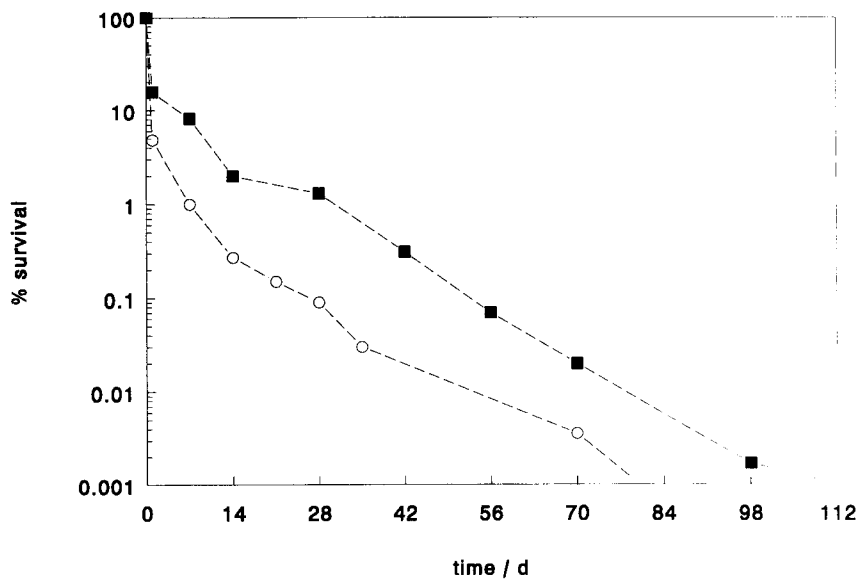

Fig. 4. Survival of Gram-negative bacterial strains desiccated on silica gel. ○ = No. 10, *Enterobacter cloacae*, lab. code WR 3; ■ = No. 11, *Escherichia coli*, lab. code WR 1.

rial [6], a long-term survival of 100% of the desiccated organisms stored at 22°C is obviously unrealistic. Therefore, the term "stability" was defined as follows: the reference material must remain stable over a defined period of time, that is, the number of organisms present must remain within defined limits [6].

The percentage survival of the bacterial strains tested (except Nos. 12, 13 and 14) as a function of time is shown in Figs. 1–4. Further data, such as the logarithms of colony forming particles per ml (log cfp/ml), D values and water activities (a_w), are presented in Table 2.

According to the survival rates resulting from this investigation, the bacterial strains tested could be classified into four typical groups. This classification was based on the parameters "initial decrease" and "D value" (see *Calculations*):

Group 1: initial decrease up to 1 log unit (Fig. 1). Further survival rates correspond to D values ranging between 362 (No. 18) and 2114 (No. 15) days. All Gram-positive bacterial strains tested, such as *Enterococcus faecium* (Nos. 15 and 16) and *Lactobacillus plantarum* (Nos. 17 and 18) belong to this group and survived a desiccation treatment on silica gel with a survival rate ranging mainly between 30 and 100% (see Fig. 1). Although the survival rates are in general high, there are differences between the strains of one species, *e.g.*, between *Lactobacillus plantarum* W 2 (90%) and *Lactobacillus plantarum* B 5 (45%).

Group 2: initial decrease higher than 1 log unit, ranging between 1.4 log units up to day 14 (No. 7) and 3.7 log units up to day 21 (No. 4) (Figs. 2 and 3). Further survival rates correspond to D values ranging between 248 (No. 6) and 1290 (No. 1) days. All *Salmonella* serotypes tested (Nos. 1–9) were classified into this group.

Group 3: initial decrease ranging around 1 log unit (Fig. 4). D values are 30 days (*E. coli*) and 23 days (*Enterobacter cloacae*). After 12 weeks of storage, reconstitution of these bacteria was no longer possible; no colonies could be found after distributing contaminated silica gel particles on nutrient agar plates and incubating these plates under optimum conditions. Strains belonging to this group are *Enterobacter cloacae* (No. 10) and *Escherichia coli* (No. 11).

Group 4: initial decrease higher than 4 log units. After 1 week of storage a reconstitution using the method described for group 3 did not reveal living organisms. The lack of data resulting from this extreme decrease in the contamination level made the calculation of D values impossible. Hence the following bacterial strains are obviously marked by a relatively high sensitivity against desiccation: *Pseudomonas aeruginosa* (No. 12), *Aeromonas hydrophila* (No. 13) and *Aeromonas sobria* (No. 14).

A distinct result of this investigation is the relatively high resistance of Gram-positive (for definition see Experimental) bacterial strains to desiccation. Within the group of Gram-negative species there obviously exist subgroups (here, e.g., groups 2 and 4) characterized by significantly different susceptibilities to desiccation. According to Brennan et al. [13], the desiccation of bacteria causes a viability loss that is related to damage to the cell wall and cytoplasmic membrane. Hence a structural difference of these cell compartments in Gram-positive and Gram-negative bacteria is obviously one of the factors responsible for the typical reactions of bacterial strains to the described stressful treatment. Also within the group of Gram-negative bacteria differences according to the structure of the cell wall and cytoplasmic membrane may play an important role.

To compare the behaviour of the bacterial strains desiccated in this model system with the data resulting from spray drying, the results of a stability test with *Salmonella panama* in spray-dried material were used. To carry out stability tests with artificially contaminated spray-dried milk, the final material, i.e., gelatin capsules filled with contaminated milk powder, were stored at temperatures of −20, 22, 30 and 37°C [6]. One trial was carried out using milk powder contaminated with *Salmonella panama*. The material used for the trial had been stored for 13 years at 5°C. Once per week 50 capsules were enumerated per storage temperature. At the start of a trial 200 capsules were used. The results of this trial corresponded to D values of 209, 207, 117 and 37 days at −20, 22, 30 and 37°C, respectively. The silica gel contaminated with the same bacterial strain

and stored at 22°C resulted, from day 14 after desiccation up to the end of the investigation period, in viable counts corresponding to a D value of 677 days. Hence this material reached a higher level of stability after a significantly shorter period of storage. The lower stability of the spray-dried material is most likely due to sublethal damage caused by a combination of desiccation and elevated temperatures during the drying process.

With regard to the spray-drying process, it should be mentioned that viable counts of *Pseudomonas aeruginosa* and *Aeromonas hydrophila* could not be found 1 week after carrying out the spray-drying procedure. Hence these bacterial strains showed the same susceptibility to spray drying and desiccation on silica gel.

After spray drying, Gram-positive bacterial strains such as *Enterococcus faecium* WR 63 showed larger initial decreases and needed longer periods for stabilization than after desiccation on silica gel. Thus, in spite of the generally high degree of stability, these bacteria showed less resistance against a combination of heat and desiccation. Compared with the Gram-negative strains, the Gram-positive organisms tested are marked by a short stabilization period and a high contamination level both in the spray-dried material and on the contaminated silica gel.

The drying procedure described here using silica gel seems to be a suitable basis for the development of a desiccation screening system; the procedure is simple and does not require expensive laboratory equipment. Microorganisms can be rapidly converted into the dried state. Methods to improve the stability of certain bacterial strains after desiccation can be investigated in detail. The screening of bacterial strains to test their suitability for spray drying is an important application of this model system: different strains of one species or different serotypes of one genus can be compared (see *Salmonella*, serotypes 1–9) to select an optimum organism. Further, data resulting from these studies can be used as a basis for the development of a desiccation method working without extreme temperatures. Such a method should be used for the production of reference materials containing bacterial strains marked by a high susceptibility against elevated temperatures and by a sufficient resistance to desiccation (i.e., according to screening with the silica gel model system they are classified into group 2 and 3).

In further studies a statistical method should be developed to determine exactly day $t = z$ (see *Calculations*), the day indicating a change in the regression of the contamination level. Hence regarding each bacterial strain tested with this model system and belonging to group 1, 2 or 3, it should be possible to determine the starting point of a period marked by a very slow decrease in the contamination level.

This study was carried out under a sectoral grant for research and technology offered by the EC, Community Bureau of Reference (BCR). Dr. E. Maier (BCR), Dr. A.H. Havelaar (RIVM), Dr. S. Notermans (RIVM), Professor Dr. J. Krämer and Dipl.-Ing. agr. R. Engels (University of Bonn) are thanked for their contributions.

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