APPLIED ORGANOMETALLIC CHEMISTRY, VOL. 5, 393-397 (1991)

Tributyltin analysis in seawater by GC FPD after direct aqueous-phase ethylation using sodium tetraethylborate

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Using sodium tetraethylborate as an alkylation reagent, one-step ethylation and extraction of organotin compounds in seawater was performed directly in the aqueous phase to obtain optimal reaction conditions. No further purification or concentration of the extract was required before GC FPD analysis. The detection limit for monobutyl-dibutyl- and tributyl-tin was approximately 0.4 ng dm^{-3}, which is adequate for any currently known toxicity effect. The linearity range (0–200 ng dm^{-3}) was solely dependent on the detector response curve. Compared with the Grignard reaction after extraction in an organic solvent, this new ethylation procedure reduces the number of analytical steps, thus saving time and improving reliability.

Keywords: Tributyltin, ethylation, seawater, analysis, gas chromatography, flame photometric detection, organotin speciation

INTRODUCTION

Tributyltin (TBT), used as an antifouling agent in paints for boats and submerged marine structures, has very high toxicity for some non-target species. At concentrations as low as 20 ng dm^{-3} TBT can inhibit Pacific oyster reproduction,[1] and populations of *Nucella lapilus* decrease dramatically[2] as soon as levels reach 1 ng dm^{-3}. Sublethal effects and disturbance of oyster calcification have been noted[3] at concentrations above 2 ng dm^{-3}. No other chemical used directly in the marine environment has shown such adverse effects.

New regulations in some countries require the control of TBT levels of at least 1 ng dm^{-3} in coastal waters. Results of surveys conducted along French coasts in recent years[4,5] indicate that much remains to be done if this objective is to be reached.

Current techniques for analysis of TBT in seawater are time-consuming and not entirely reliable. These include atomic absorption spectrometry of monobutyl-, dibutyl- and tributyl-tin hydrides after cryogenic trapping[6–9] and gas chromatography with a flame photometer as specific detector (GC FPD), which has been increasingly used in recent years because of greater sensitivity and reliability.[10–12]. Both these techniques require an initial procedure to convert butyltin ions into their corresponding volatile derivatives. Butyltin alkylation using a Grignard reagent involves several steps (extraction, drying, alkylation, concentration), thereby increasing the risk of error and uncertainty. Hydridization by sodium tetrahydroborate in aqueous phase is easier to perform, but the instability and volatility of butyltin hydrides can lead to a loss of compounds and consequent underestimation of contamination.

We have developed a novel organotin alkylation procedure applied directly in aqueous phase using sodium tetraethylborate, a reagent previously used for speciation or organoleads,[13] organomercury,[14] or tributyl-, dibutyl and monobutyl-tin in sediments.[15] The optimal conditions for measurement of butyltin species in seawater at the sub-nanogram level are also considered. With this procedure, derivatization and extraction of ethyl derivatives are done simultaneously, requiring only one flask for the entire operation. The method is as easy as that for hydridization and provides the same advantages as for alkyl derivatives from the Grignard reaction.

MATERIALS AND METHODS

Reagents

Dimethyltin dichloride (DMT), tripropyltin chloride (TPT), monobutyltin trichloride (MBT), dibutyltin dichloride (DBT) and tributyltin acetate (TBT) were purchased from Johnson

0268-2605/91/050393-05$05.00

Received 25 February 1991
Revised 30 May 1991

Matthey, Alfa Products GmbH, Germany. These standards were used without further purification. Methanolic stock solutions, acidified by 10% acetic acid and containing 1 μg dm^{-3} as Sn, were prepared monthly and stored in dark glass bottles at 4 °C. Working solutions were prepared daily from these stock solutions. Tetraethyltin and tetrabutyltin (Merck) were used to check the intrinsic sensitivity of the flame photometric detector.

Sodium tetraethylborate was obtained from Strem Chemicals GmbH, Germany. Solutions were prepared by dissolving 1 g in 50 cm^3 of deionized water. Aliquots of this solution were stored at −20 °C in borosilicate bottles for less than two weeks. To reduce blank values, these solutions were cleaned before use by two successive isooctane extractions.

Tris solution was prepared by dissolving 40 g tris(hydroxymethyl)aminomethane (Merck) in 100 cm^3 deionized water.

Bromophenol Red solution was obtained by dissolving 100 mg in 9.75 cm^3 of NaOH 0.02 mol dm^{-3} before dilution to 250 cm in water.

Methanol and acetic acid for analysis, and isooctane for spectroscopy, were obtained from Merck GmbH.

All glassware was cleaned with detergent, rinsed in deionized water, cleaned with a 10% nitric acid solution and then rinsed again, before decontamination overnight in an oven (450 °C).

Samples

Clean seawater was taken from the Atlantic Ocean, 50 miles off the coast, and later used as ‘reference seawater’ for calibration studies, stored in clean 60 dm^3 polyethylene containers. Other seawater samples (after filtration, if necessary) were stored in dark glass bottles with 0.4% acetic acid added.

Ethylation/extraction

When sodium tetraethylborate is used as alkylation reagent, ethylation and extraction of organotin compounds can be performed in seawater in a one-step procedure. A 250 cm^3 borosilicate flask with a narrow neck (Fig. 1) was used for this operation. Seawater and 50 μl of Bromophenol Red solution were introduced and pH was then adjusted to 6 using an appropriate quantity of Tris solution and acetic acid (light pink color). Deionized water was added to obtain a level 2 cm high in the neck. Iso-octane (1 cm^3) and tetraethylborate (100 μl) were then added successively. Vigorous swirling was obtained by magnetic stirring (1500 rpm for 30 min). The swirling was then stopped and the organic phase transferred into a vial for injection into the gas chromatograph. The efficiency of the procedure was checked using a tripropyltin internal standard.

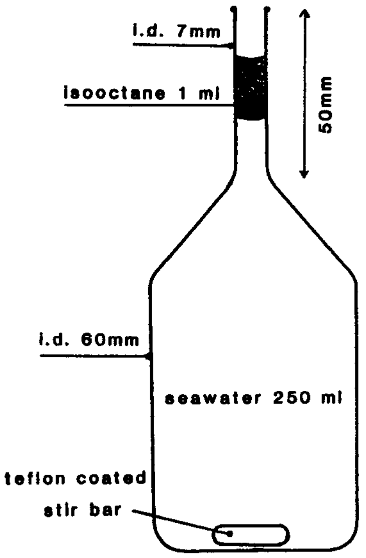

Figure 1 Glass reactor for organotin ethylation/extraction.

Apparatus

A DI 200 gas chromatograph from Delsy–Nermag was used for this study. The extract 2 μl) was injected at 200 °C using the splitless mode. A CP Sil 5 CB capillary column (inner diameter 320 μm, length 25 m, film 0.4 μm) was used for alkyltin separation. The column temperature was programmed at 70 °C for 1 min and then heated to 230 °C at 12 °C min^{-1}. The carrier gas was hydrogen (12 cm^3 min^{-1}). A combination of hydrogen (45 cm^3 min^{-1}) and air (30 cm^3 min^{-1}) was used for the flame. A commercial flame photometric detector was modified by addition of a quartz burner to increase sensitivity. The performance of this system was regularly tested by injection of a mixture of tetraethyl- and tetrabutyl-tin. The detection limit was approximately 0.2 pg as Sn for each tin compound.

RESULTS AND DISCUSSION

Chromatography

A typical chromatogram is shown in Fig. 2. The memory effect on the quartz burner gave some peak tailing which had no effect on calibration when peak height was used instead of peak area. Typical detector calibration curves (Fig. 3) show that the response factor was twice as high for the first compound. In general, the response factor was dependent on hydrogen velocity through the column. This might also account for the decreasing response during the temperature programming cycle which reduces carrier gas velocity. A recent review[16] indicates that sensitivity in different studies ranged between 0.04 and 80 pg for GC FPD analysis of organotins. It is apparent that a subpicogram-sensitive detector is required for TBT analyses at the nanogram level in water.

Calibration

External calibration was performed for MBT, DBT and TBT in reference seawater using concentrations of 0–200 ng dm^{-3} (Fig. 4). Regression coefficients (respectively 0.9996, 0.9989 and 0.9993) demonstrated good calibration linearity in the range. For higher concentrations of alkyltins in water, the extract had to be diluted according to detector linearity. In the normal range usually found in natural water (0–1000 ng dm^{-3}), there was no limitation for the derivatization step. As response factors obtained for calibration of TBT, DBT and MBT in seawater ranged exactly between those previously obtained for pure tetraethyl- and tetrabutyl-tins, it might be considered that the derivatization step was complete.

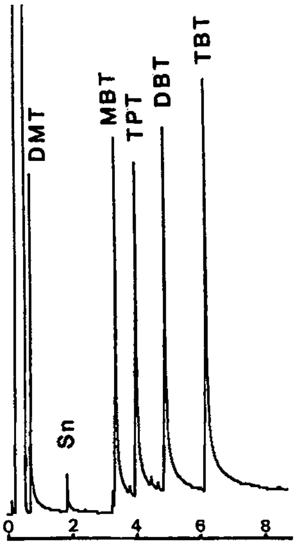

Figure 2 Typical chromatogram of organotin standards. Concentrations (ng dm^{-3} as Sn) were DMT 40, DBT 40, TBT 60.

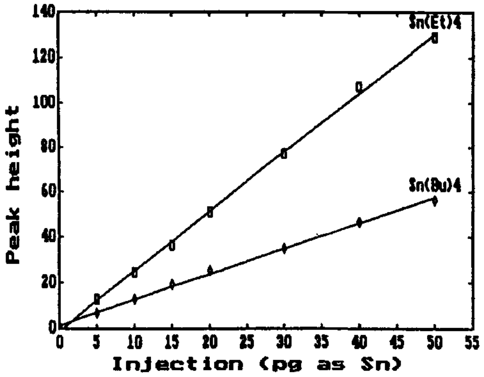

Figure 3 Calibration curves of the flame photometer detector with tetraethyl- and tetrabutyl-tin standards.

Detection limit

In optimal gas chromatography conditions, as described above, and with a detector sensitivity of 0.2 pg of injected tin, the TBT detection limit in seawater was approximately 0.4 ng dm^{-1} (a signal three times as high as baseline noise).

Reproducibility

A reproducibility test was performed using a mixture of MBT, DBT, TBT, TPT and DMT. Seven replications at concentrations of 60 ng dm^{-3} as Sn in reference seawater gave the results indicated in Table 1. The relative standard

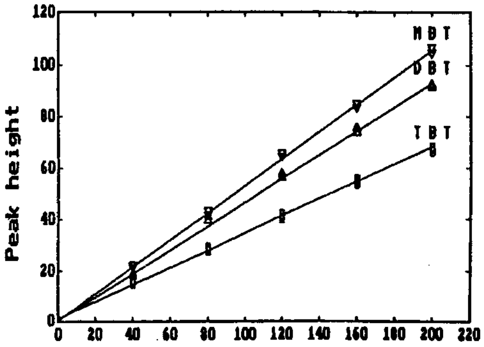

Figure 4 Calibration curves for MBT, DBT and TBT in reference seawater.

Table 1 Peak height reproducibility test for MBT, DBT, TBT, TPT and DMT analyses in reference seawater. Concentrations are 60 ng dm^{-3} (as Sn).

Sample	MBT	DBT	TBT	TPT	DMT
1	53	50	35	43	70
2	53	47	35	44	56
3	54	52	40	46	61
4	56	52	39	44	57
5	55	49	37	43	51
6	55	49	38	46	59
7	55	50	36	44	60
Mean	54.4	49.8	37.1	44.3	59.1
SD	1.13	1.77	1.95	1.25	5.81
RSD	2.09	3.55	5.25	2.83	9.83

deviation (RSD) was 5.25% for TBT and lower for the other butyltins. Reproducibility was lower for DMT (RSD = 9.83%), probably because of poor resolution with the solvent peak in gas chromatography.

Time effect on the alkylation/extraction step

Ethylation of organotin ions occurs in the water phase and could proceed rapidly. However, TBT and DBT chlorides are already soluble in the organic phase before derivatization, so that the duration and mixing efficiency of this step must be adequate to obtain the complete reaction required for better reproducibility. Therefore, signal response as a function of reaction time was checked using a mixture of MBT, DBT and TBT in seawater. The results (Fig. 5) indicate that a steady state is reached within 30 min in the mixing conditions described above. Reaction times respectively of 8 and 6 min have been reported for organoleads[13] and methymercury.[14] However, the procedures used were homogeneous-phase reactions. For organotin ethylation, previous results reported by Ashby and Craig[15] cannot be compared as they proceeded in an ethanolic phase.

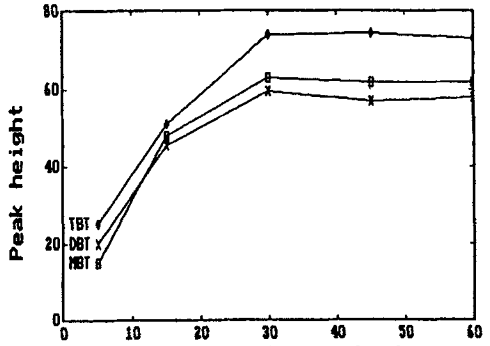

Figure 5 Time effect on signal response for MBT (40 ng dm^{-3}), DBT (40 ng dm^{-3}), TBT (80 ng dm^{-3}) and DMT (40 ng dm^{-3}) in reference seawater.

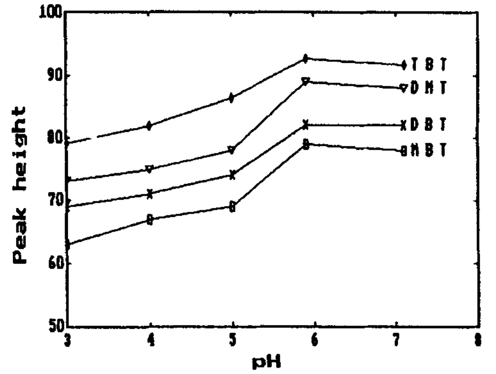

Figure 6 pH effect on signal response for MBT (40 ng dm^{-3}), DBT (40 ng dm^{-3}) and TBT (80 ng dm^{-3}) in reference seawater.

pH effect on alkylation yield

For preservation purposes, acid is often added to a sample to obtain a pH < 3. However, as addition of alkaline sodium tetraethylborate increases sample pH, it was necessary to determine the true effect of pH on the ethylation reaction. The results (Fig. 6) indicate that pH effects in the 3–7.2 range could decrease the response factor by about 32%, with the best response occurring at pH 6–7. These findings are in agreement with previous results obtianed with organoleads.[13] The best results for mercury compounds were obtained in the 4–7 range,[14] with acetate buffer (pH 4.9) being finally chosen for the ethylation step because of its convenience.

CONCLUSIONS

The Grignard reaction has generally been used for organotin alkylation prior to gas chromotography. However, this procedure must be carried out in a clean, dry organic solvent and requires final clean-up and concentration. These operations are time-consuming and costly, and reliability and accuracy may be decreased due to the number of steps involved. Hydride generation of volatile organotin derivatives has been performed directly in aqueous phase using sodium tetrahydroborate. However, organotin hydride derivatives are not really stable, so that contamination can be underestimated. The high volatility of hydrides could also be a drawback if a concentration step is required before GC FPD analysis.

Direct ethylation of organotins in aqueous phase using sodium tetraethylborate is a novel method, and ethylation and simultaneous extraction of derivatives has to our knowledge never been reported for organometallic contaminants in seawater. The new procedure described here would thus seem useful to any laboratory carrying out studies or monitoring in the organotin field. For ecological purposes, it enables measurements to be performed in seawater at the sub-nanogram level. The one-step ethylation/extraction operation is easy to perform, saves time, is less costly and provides improved results.

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