Derivatization methods for the determination of organotin compounds in environmental samples

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In the analysis of organotin compounds, derivatization is required in order to achieve more volatile compounds prior to the use of techniques based on GC separation. Derivatization can be considered as one of the main critical steps in organotin analysis, since low yields of derivatization and losses of analytes can easily occur at this stage, and lead to an underestimation of their content in environmental samples. Furthermore, experimental conditions which are not perfectly under control may induce degradations, and alter the original speciation in the sample. Hydride generation, and alkylation by Grignard reagents or by $NaBEt_4$, are the derivatization methods usually applied for organotins. The advantages and disadvantages of these methods are reported.

Keywords: Derivatization methods; Organotin compounds; Environmental samples; Grignard derivatization; Hydride generation

1. Introduction

Environmental concern about organotin compounds has increased considerably in recent years, particularly owing to the extensive use of these compounds as active components in antifouling paints (mainly tributyltin (TBT)) and in pesticide formulations (mainly triphenyltin (TPhT)). Their direct introduction into the marine environment, together with their high toxicity towards 'non-target' organisms such as oysters and mussels, has caused environmental and economic damage such as that observed in the past in the Bay of Arcachon in France, in the Crouch Estuary (UK), and in the Sado Estuary (Portugal) [1]. Di- and monosubstituted compounds are the main degradation products of TBT and TPhT in the environment.

Nowadays, the release of TBT from antifouling paints is recognized worldwide as being one of the main contamination problems for the marine environment, and the use of TBT-based antifouling paints is almost everywhere restricted by law [2,3]. In order to control the effectiveness of these legal provisions and to evaluate the distribution and fate of organotins in the marine environment, many analytical methods have been developed in the last twenty years. Of these, most of the separation methods are based on the use of hyphenated techniques using liquid or gas chromatography. Gas chromatography-based methods have been used most, owing to their high resolving power and easy coupling to sensitive and selective detectors: atomic absorption spectrometry (AAS), atomic emission spectrometry (AES), the flame photometric detector (FPD), and mass spectrometry (MS). Many papers reviewing analytical techniques for organotin determination are in the literature [4–8]. Owing to the low volatility of the organotins in the environment, a derivatization reaction is required before a gas chromatography-based technique is to be applied. Furthermore, derivatization also permits a reduction in the occurrence of possible interferences during the subsequent analytical steps, and particularly at the detection stage.

The derivatization reactions applied most commonly for organotin analysis are hydride generation with $NaBH_4$, ethylation with $NaBEt_4$, and alkylation with Grignard reagents [9]. It is worth

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stressing that derivatization as well as extraction must be considered one of the most critical steps in organotin analysis. Low yields in derivatization, as well as degradation phenomena (especially for phenyltins) can heavily affect the quality of the results. A validation, or at least a careful study, of the procedure being used in the laboratory for the particular matrix, along with its particular interferences, is necessary [10]. This validation is, however, almost always hindered by a lack of commercially available derivatized standards.

Recently, the synthesis of derivatized standards for ethylation and Grignard derivatization has been carried out at the Free University of Amsterdam [11] within the framework of EC-funded projects (Standards, Measurements and Testing Programme), and allowed the establishment of optimization and validation studies.

2. Hydride generation

Volatile organotin hydrides are formed by the reaction of organotin compounds with an aqueous solution of sodium borohydride, according to the following reaction:

$$R_nSn^{(4-n)+} + NaBH_4 + H^+ \rightarrow R_nSnH_{(4-n)} + H_2$$

where R is the organic substituent and *n* ranges from 1 to 3.

Hydridization can be performed on-line or off-line and is generally used when the final determination is performed by AAS [5,10]. The hydride generation conditions, concentration of the reductant solution, the pH, and the types of acid used must be selected according to the element considered and the nature of the matrix [4,5,7,10]. In the case of aqueous matrices, the hydride generation method is easy to apply, and allows high preconcentration factors, and the separation of the analytes of interest from potential matrix interferences as well as high hydridization yields [4,12].

The oxygen and the other volatile compounds present in the sample are stripped from solution, then an excess of a $NaBH_4$ solution (generally 4%) and an acidic solution (generally able to lead to a pH around 2) are injected directly into the reaction flask. The generated hydrides are then swept into a cold-trap for methods involving a separation based on cryogenic trapping and U-tube GC chromatography [4,7].

The nature and concentration of the acid used may strongly affect the hydridization yield. Acidic conditions contribute to neutralizing the NaOH (1%) added to stabilize the $NaBH_4$ solution (when applied), and to generating hydrogen by decomposition of the $NaBH_4$. In the case of organotin compounds, acetic acid seems to provide better results, in terms of yields of organotin hydrides, than nitric or hydrochloric acid. The derivatization of butyltin compounds needs stronger reducing conditions than that of methyltin compounds, probably for steric reasons [4,7].

Severe interferences can be observed when hydridization is applied to more complicated matrices such as sediments, biota, or even complex aqueous matrices (e.g. wastewater). In particular, high metal concentrations, which easily occur in harbor sediments and industrial wastewater, can lead to an inhibition of the hydride formation [13–15]. Studies on the multi-element interferences effect has confirmed that butyltin compounds are prone to interferences owing to the presence of metals during hydride generation [10,12,16]. According to the papers cited above, the interferences seem to be related to the severe reduction conditions associated with the $NaBH_4$ reaction, that lead to the formation of metal borides. These inorganic species can then probably react with the organotin hydrides by attacking the Sn-H bonds.

The influence of organic substances on the hydridization yields has also been investigated [12,16]. In this case, synthetic solutions of organic solvents, PCB, pesticides, and humic substances were tested. No significant effect on the signal suppression was observed, except in the case of the addition of humic substances. The presence of these compounds gave problems in terms of reproducibility, even if the sensitivity remained good, owing to the negative influence of foam production during the stripping step. The same problem of foam formation and loss of reproducibility was observed in the analysis of biota samples with high amounts of fat (e.g. for fish) [10].

In the case of solid samples (sediments, biota), the determination of phenyltin species by the hydride generation technique is generally hindered, owing to the low yields and poor reproducibility of hydridization. Furthermore, in this case the production of poorly volatile compounds makes the detection more complicated [4,5,7,10,17].

Procedures for reducing interferences in hydride generation for speciation analysis, such as the addition of masking agents such as EDTA, KI, L-cysteine, or ascorbic acid, or the use of separation techniques such as co-precipitation or the application of chelating resins, have been reviewed [10].

Hydride generation techniques were applied successfully in the certification of butyltin compounds in coastal sediment (CRM 462), but they failed in the case of a more complex matrix (harbor sediment with a very high organic carbon content) with a lower butyltin concentration (RM 424) [8]. In the certification of butyl- and phenyltin compounds in mussel tissue, hydride generation techniques could not be applied, even for the determination of butyltin compounds alone, because of the problems cited above.

Typical hydride generation reactions used in recent certification campaigns for butyltin determinations [8] are described in Table 1.

3. Alkylation by Grignard reagents

The transformation of organotin salts (R_xSnX_{4-x}) into less volatile compounds is obtained by reaction with a Grignard reagent (R′MgX) in a suitable solvent. Alkylation by the Grignard reaction is the most widely used derivatization technique for organotin determination, and is generally performed by methylation, ethylation, propylation, butylation, pentylation, or hexylation [4,7–9]. This technique permits the formation of very stable derivatives such as the mixed tetra-alkyltins ($R_xSnR'_{4-x}$) which are more suitable for GC separation.

The Grignard reaction is generally performed on organic extracts (taking care that the organic solvent of the extracts is compatible with the Grignard reagent) by the addition of a suitable amount of Grignard reagent. The excess of Grignard reagent is generally destroyed by adding acidic (sulfuric or hydrochloric) or saline (ammonium chloride) solutions. In order to minimize violent reactions, it is preferable to add a few drops of water and shake gently before the addition of the quenching solution. Finally, the so-formed tetra-alkyltins are back-extracted into an organic solvent and purified prior to GC determination.

It is worth stressing that Grignard reagents are hazardous chemicals. They react violently with acids, water, alcohols, ketones, etc., and should be handled with extreme care by well-trained personnel using appropriate safety precautions (gloves, glasses, etc.). Excess Grignard reagent must be destroyed before clean-up and analysis. Extreme care must be taken by the operators in this step. The careful dropwise addition of water, keeping the vial in a cold water bath, is necessary to reduce the risks of too violent a reaction. The addition of hexane (or isooctane) before this step reduces the risks of evaporation losses [6]. In contrast to hydride generation, Grignard derivatization allows the determination of different species of organotins (methyltins, butyltins, phenyltins) in different environmental matrices (water, sediment, biota) with high derivatization yields and reproducibility. However, it is characterized by additional analytical steps, owing to the necessity of destroying the Grignard reagent (by quenching), and the back-extraction of the mixed tetra-alkyltins. The increase in analytical steps increases the risk of contamination, decomposition, and losses.

Experimental conditions under which the Grignard reaction is carried out must be investigated accurately and maintained under control. Some examples of Grignard reactions are given in Table 1. The influence of many experimental parameters on the derivatization yields has been studied extensively [9,17–20].

A recent study of derivatization parameters was carried out on samples of mussel, previously highly homogenized in order to reduce to a minimum the

Table 1
Examples of experimental conditions for derivatization reactions used in butyltin determinations in sediment, adapted from [8]

Reaction	Experimental conditions
Hydride generation (with $NaBH_4$)	10% $NaBH_4$ in 1% NaOH in milli-Q water (after acetic acid extraction)
	4% $NaBH_4$ in seawater (after acetic acid extraction)
Grignard derivatization	Ethylation with EtMgCl (2 mol l^{-1}) in tetrahydrofuran
	Pentylation with PeMgBr (1 mol l^{-1}) in diethyl ether
	Pentylation with PeMgBr (2 mol l^{-1}) in diethyl ether
Ethylation	Ethylation with 2% $NaBEt_4$

variability between samples [9]. A common characteristic was observed in all the determinations: a very poor reproducibility for MPhT, probably owing to problems during the extraction step rather than the derivatization step.

Among all the parameters tested, only the concentration of the Grignard reagent had a significant influence on the efficiency of derivatization. Lower yields were observed by using diluted (1:4, in ether) instead of concentrated Grignard reagent. The reaction time (0, 15, 30 and 60 min), temperature (25 and 50°C), and shaking of the sample during the reaction, showed no significant influence, suggesting that the reaction is instantaneous even at room temperature. The same results were found by Harino et al. [18] who concluded, on the basis of a study of the effect of the reaction time on the propylation reaction of organotin compounds, that 10 min is more than enough for reaching the maximum yield.

In the same study cited above [9] the influence of the different Grignard reagents (hexyl, pentyl, propyl, ethyl and methyl) on the derivatization yields was also tested. It was observed that only for the less volatile compounds, TPhT and DPhT, was the same recovery obtained for the different alkylations tested (Fig. 1). For the other organotins, decreasing recoveries were obtained by reducing the length of the alkyl group, this decrease being

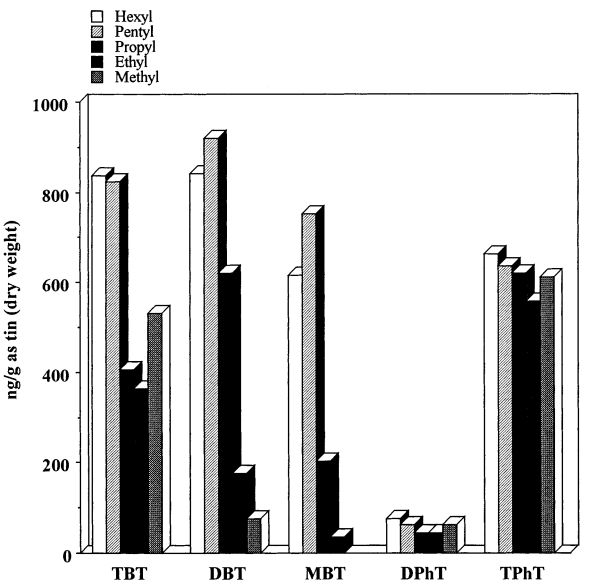

Fig. 1. Comparison between different Grignard reagents in the determination of organotin compounds in mussel tissue (final volume = 0.3 ml).

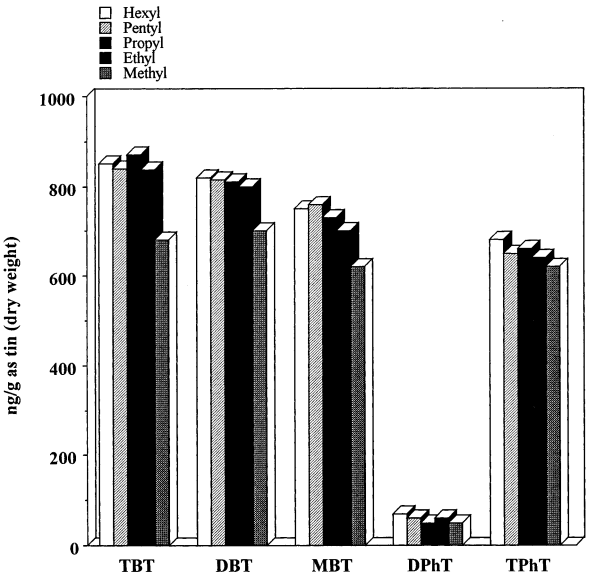

Fig. 2. Comparison between different Grignard reagents in the determination of organotin compounds in mussel tissue (final volume = 1 ml).

proportional to the volatility of the compound (the methylated derivative of MBT was not even detectable). Considering this fact, it was thought that the decrease in recovery could be caused by losses owing to volatility in the final preconcentration steps under a nitrogen flow, rather than to differing efficiencies in the derivatization depending on the alkyl group. The hypothesis was confirmed by repeating the same comparison and stopping the preconcentration step at 1 ml instead of 0.3 ml. The results showed that only the methylation of butyltin compounds led to slightly lower results, whereas in all the other cases the yields were comparable (Fig. 2). An alternative to avoid losses by volatility, allowing the same preconcentration factors, would be to exploit a large-volume injector.

Qualitative, even if not quantitative, evidence of an interconversion between TPhT and MPhT during Grignard derivatization has been observed [21]. Davies and Smith [22] described the alkylation reaction for tin as a transmethylation reaction, which is not dependent on the associated anion. This statement was confirmed by a methylation study performed by Stäb et al. [19] who found, contrary to Müller [17], that it is not necessary to generate halogenated organotins prior to the Grignard reaction for obtaining alkylation. Other major results of the methylation study were that quench-

ing with ammonium chloride provided very good results in terms of organotin recovery, and as organotins with larger alkyl groups (e.g. pentylated derivatives) are less sensitive to degradation, pentylation may be more favorable than methylation. The same authors found, however, that Grignard reagents containing small organic groups are more reactive, which guarantees high derivatization yields.

The application of the Grignard derivatization technique to sediments with high concentrations of sulfur is often hindered. In this case, the elemental sulfur is co-extracted with the organotin and is alkylated during the Grignard derivatization, leading to the formation of dialkyl mono-, di- and trisulfides [23]. These sulfur species interfere with the final determination of the organotin by GC-MS or GC-FPD. Suitable methods for the elimination of the sulfur interference in the organotin determination seem to be the oxidation with dimethyldioxirane (DMD) of all sulfur compounds to the corresponding sulfones, followed by an alumina clean-up step [24], and the use of a $AgNO_3$-coated silica column in the clean-up step, allowing almost quantitative removal of sulfur compounds after 2 h [25]. In this last case, however, phenyltins were completely lost after treatment.

In addition to sulfur, sample extracts from sediments or mussels contain high amounts of co-extractants and after the addition of the Grignard reagent a precipitate is often found. Sonication of the solution is performed to improve sample-to-reagent contact by partial resolubilization of the precipitate [6].

Although Grignard derivatization is rapid and effective at room temperature, higher temperatures and reaction times are necessary to improve recovery from complex sample matrices. The reaction conditions used do not affect speciation, as is observed experimentally [6].

Finally, one must take into account the fact that non-derivatized compounds, contrary to the derivatized ones, are subject to easy decomposition, particularly when they are in solution at room (or higher) temperature. Thus, it is necessary to ensure the shortest time between extraction and derivatization to minimize the risk of decomposition [21].

Derivatization with different Grignard reagents has been applied successfully in a number of certification exercises, both on sediment (CRM 462, CRM 646) and biological samples (CRM 477) [8].

4. Ethylation by sodium tetraethylborate

The use of sodium tetraethylborate as a derivatizing reagent for organotin compounds seem to be convenient, especially in the case of aqueous samples for which the direct in situ derivatization is possible, with a consequent reduction in the analytical steps [7,26].

Martin and Donard [12] carried out a 2^3 factorial experimental design to determine the optimum experimental conditions for ethylation of organotins (dibutyltin, monomethyltin, and diethyltin) in simple aqueous solutions. The parameters considered were pH, $NaBEt_4$ concentration, and the time of reaction. The results showed that the yield of the ethylation reaction depends on the degree of substitution and the nature of the alkyl groups linked to the tin atom. The ethylation reaction is a nucleophilic reaction and the ability of organotins to be involved in nucleophilic reactions may depend on their degree of substitution. The concentration of $NaBEt_4$ did not produce a significant effect on dibutyltin, while it provided better results for monomethyltin and diethyltin in the higher range of values. The best pH for carrying out the reaction was found to be in the range 4–5. At lower pH (around 2) the formation of organotin hydrides, owing to the partial conversion of $NaBEt_4$ into $NaBH_4$, was observed. The time of reaction proved to be important more for the stripping efficiency of the ethylated organotin species than for the overall yield of the ethylation reaction. The presence of inorganic elements did not affect the ethylation reaction.

Similar results were obtained by studying the effect of pH, reaction time, and mixing in the GC-FPD determination of butyl- and phenyltin compounds after a one-step simultaneous aqueous ethylation (with $NaBEt_4$) and extraction (with isooctane) [27,28]. The best results were obtained at pH=4.8, under shaking for 30 min on a 420 rpm rotative shaking table. The pH was stabilized using an ethanoate buffer because the presence of ethanoate provided a higher ethylation yield [28].

A larger amount of $NaBEt_4$ reagent must be used for the direct ethylation of organotins in sediment and biological samples in order to achieve high yields. This is necessary to compensate for the consumption of reagents by side reactions with metals and other components in the matrices [26–32]. If organotins are extracted previously from the matrix

a smaller amount of $NaBEt_4$ reagent could be sufficient [32].

De la Calle-Guntinàs [9] investigated the influence of pH, reaction time, and concentration of sodium tetraethylborate on the derivatization yields in the determination of butyl- and phenyltin compounds in mussel samples. The same recovery was obtained for all the compounds in the pH range 4.5–5.0. For TPhT, pH 4 was sufficient to achieve a maximum analytical signal; at higher and lower pH values a decrease in the recovery was observed. Two min (3 in the case of TPhT) were enough to provide the highest signals. Regarding the concentration of $NaBEt_4$ almost a steady recovery was obtained for concentrations equal to or higher than 0.3% (w/v) (0.2% for TBT). In the same work a comparison between Grignard derivatization (pentylation and ethylation) and ethylation with $NaBEt_4$ was carried out. The comparison was performed on highly homogenized mussel samples: the results are shown in Fig. 3. As can be seen, Grignard pentylation and ethylation provided very similar results, slightly higher than those provided by ethylation with $NaBEt_4$. Similar results were obtained by Chau et al. [32] who compared ethylation by $NaBEt_4$ and by Grignard reagent in the determination of butyl- and phenyltin compounds in mussel tissue (spiked and real samples) and in biological reference materials (NIES 11). In all the cases, ethylation by Grignard reagent provided the highest recoveries.

5. Conclusions

Hydride generation and ethylation with $NaBEt_4$ are particularly suitable for aqueous samples, both presenting the main advantage of being directly applicable to the samples. Simultaneous in situ derivatization/extraction is possible, reducing the number of analytical steps and thus the potential sources of errors. Ethylation, in contrast to hydride generation, presents high yields of derivatization not only for butyltin compounds but also for phenyltin ones. In the case of solid samples such as sediment and biological samples, hydride generation is often hindered by the presence of severe interferences whereas the main advantage of $NaBEt_4$ (its stability in water) cannot be exploited. Furthermore, $NaBEt_4$ in the presence of strong acids, which are often applied in the extraction of organotin compounds from solid samples, is not stable and decomposes. In this case the organotins have to be extracted into an organic solvent prior to the derivatization reaction.

Grignard derivatization offers the advantage of possible use for the determination of most of the organotin compounds present in the environment (methylated, butylated and phenylated species) in a large variety of matrices (water, sediments, biota). Among the different Grignard derivatizations, hexylation and pentylation provide derivatized compounds with relatively low volatility, which permits preconcentration steps in the sample pretreatment without the need for special precautions even though, owing to the low volatility, condensation problems in the interface have been described when the coupling of GC to AAS is used [5]. The use of propyl, ethyl, and, above all, methyl Grignard reagents offers the advantage of a higher reactivity, even though the high volatility of their products can lead to volatilization losses during the preconcentration steps, and careful precautions must be taken. Methylation has an additional disadvantage: it cannot be applied to the determination of methylated species. The same remarks can be made concerning ethylation with $NaBEt_4$.

Independently of the derivatization technique applied, the derivatization yields should – in principle – be verified in the chosen experimental conditions. However, the lack of commercially avail-

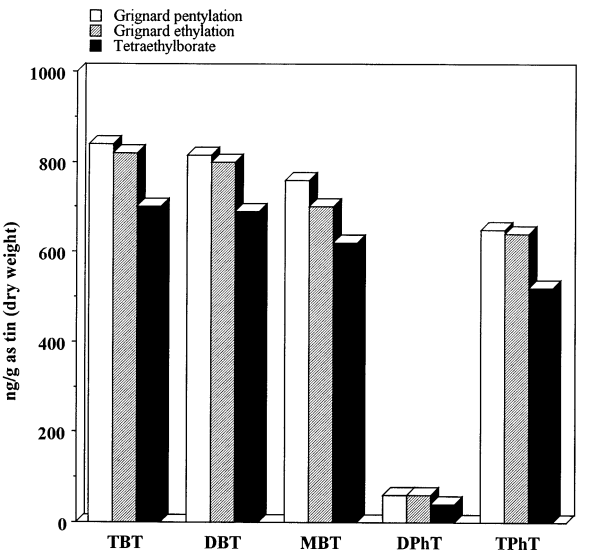

Fig. 3. Comparison between Grignard pentylation, Grignard ethylation, and ethylation with $NaBEt_4$ in the determination of organotin compounds in mussel tissue (final volume = 1 ml).

able derivatized organotin standards (pentylated, ethylated, etc. organotins) hampers the systematic quantitative evaluation of the derivatization yields. Recent advances have been made in this respect in the framework of certification campaigns [8].

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