UNIVERSITY OF SOUTHAMPTON

FACULTY OF NATURAL AND ENVIRONMENTAL SCIENCE

School of Chemistry

Improving the measurements of butyltin compounds in environmental samples

by

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Thesis for the degree of Doctor of Philosophy

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ABSTRACT

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Tributyltin compounds (TBT) are highly toxic pollutants, mainly introduced to the environment as a marine antifouling agent. The main aim of this work was to evaluate the present methods for the measurement of TBT and its breakdown products dibutyltin and monobutyltin in environmental sediments and waters and to improve upon their measurement.

For the analysis of sediments, a triple hexane/acetic acid extraction was employed of tropolone complexes of the organotin compounds. Grignard reagent derivatization and measurements by gas chromatography with pulsed flame photometric detection were then employed. The TBT detection limit of 5 µg Sn/ kg in sediment was below the UK environmental quality target (EQS) value for TBT in sediment (1-2 mg Sn/kg). A pilot investigation was carried out on a small dockyard in Southampton to evaluate if the total amount of tin could be used to predict the presence of TBT. Due to different sources of tin contamination in the studied area no clear overall correlation of TBT with total tin was found.

As part of the investigation into the determination of butyltin in sediment, the extraction of TBT from paint particles deposited in sediment during boat refurbishment and the removal of sulphur interferences were investigated. For the extraction of TBT in paint, a pre-treatment procedure was developed based on pre-treatment with dichloromethane (DCM). This treatment improved the extraction of TBT from the paint, but TBT losses can occur during DCM removal by evaporation. Sulphur interferences were successfully removed from the sediment extract by improving the clean-up procedure. This procedure was based on treatment of the hexane extract with activated copper and then passing the hexane extract through a C_{18} solid phase extraction column.

For the determination of butyltins in water a doubly functionalized mesoporous silica was synthesized and used to extract butyltin compounds from water based on a solid phase dispersion technique. Butyltin chlorides were collected from the water on the surface of the HOC_{18}-nanoscavenger, hexylated using a Grignard reagent and quantified by GC-PFPD. Another approach was based on ethylation of tributylin chloride using $NaEt_4B$ followed by extraction of the ethylated species on the HOC_{18}-nanoscavenger. TBT detection limits of 1.5 and 3 ng Sn/ L were achieved using the Grignard and $NaEt_4B$ approaches respectively and were regarded satisfactory, as they were below or near to the UK EQS for water (2 ng/ L). The Grignard approach was more efficient than the $NaEt_4B$ approach, but the latter was more precise.

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DECLARATION OF AUTHORSHIP

I, Awad Al-Rashdi, declare that the thesis entitled: Improving the measurements of butyltin compounds in environmental samples and the work presented in the thesis are both my own, and have been generated by me as the result of my own original research and is expressed in my own words. I confirm that:

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- I have clearly mentioned the presence of quoted or paraphrased material and referenced all sources;
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ABBREVIATIONS

AAS	Atomic absorption spectroscopy
AcONa	Sodium acetate
AED	Atomic emission detector
BET	Brunauer–Emmett–Teller method
BJH	Barrett–Joyner^{o}–Halenda method
C_{18}	Octadecyl
C_{12}TMABr	Dodecyltrimethylammonium bromide
$C_{18}SiCl_3$	Octadecyltrichlorosilane
DBT	Dibutyltin
$DBTCl_2$	Di(*n*-butyl)tin dichloride
DCM	Dichloromethane
DEA-DCC	Diethylammonium diethyldithiocarbamate
DHDBT	Di(*n*-butyl)di(*n*-hexyl)tin
DMD	Dimethyldioxirane
DPhT	Diphenyltin
ECD	Electron capture detector
EDTA	Ethylenediaminetetraacetic acid
EQS	Environmental quality standard
ES	External standards
ETAAS	Electrothermal atomic absorption spectroscopy
FID	Flame ionisation detector
FPD	Flame photometric detector
FTIR	Fourier transform infrared spectroscopy
GC	Gas chromatography

GMOS	Glycidoxypropyltrimethoxysilane
HDPE	High-density polyethylene
HexSH	Hexanethiol
HG	Hydride generation
HOAc	Acetic acid
HOC_{18}-nanoscavenger	Dual functionality nanoscavenger
HPLC	High performance liquid chromatography
ICP-AES	Inductively coupled plasma atomic emission spectrometry
ICP-MS	Inductively coupled plasma mass spectroscopy
ICP-ES	Iinductively coupled plasma–emission spectrometry
IMO	International marine organisation
IS	Internal standard
IUPAC	International union of pure and applied chemistry
LC l	Liquid chromatography
LLE	Liquid-liquid extraction
LOD	Limit of detection
MAE	Microwave-assisted extraction
MBT	Mono(*n*-butyl)tin
$MBTCl_3$	Mono(*n* -butyl)tin trichloride
MEPC	Marine Environmental Protection Committee
MHTBT	Tri(*n* -butyl)mono(*n* -hexyl)tin
MIP-AES	Microwave induced plasma atomic emission spectrometry
MMA	Methyl methacrylate
MS	Mass spectroscopy
MPhT	Monophenyltin
NaDDC	Sodium diethyldithiocarbamate
NSDE	Nanoscavenger solid phase extraction

***n*-Bu_3EtSn**	Tri(*n*-butyl)ethyltin
***n*-Hex_2S**	Di-n-hexylsulphide
***n*-Hex_2S_2**	Di-n-hexyldisulphide
OSPAR	Oslo Paris convention
OTC	Organotin compounds
PDMS	Polydimethylsiloxane
PFPD	Pulsed flame photometric detector
PLE	Pressurized liquid extraction
PTFE	Polytetrafluoroethylene
PTV	Programmed temperature volatilisation
PVC	Polyvinyl chloride
PVDF	Polvinylidene fluoride
Ref	Reference
RSD	Relative standard deviation
SD	Standard deviation
SEM	Scanning electron microscopy
SFE	Supercritical fluid extraction
SFC	Supercritical fluid chromatography
SPDE	Solid phase dispersion extraction
SPE	Solid phase extraction
SPME	Solid phase microextraction
TBT	Tri(*n*-butyl)tin
TBTCl	Tri(*n*-butyl)tin chloride
TBTO	Tri(*n*-butyl)tin oxide
TBT–SPC	Tributyltin self-polishing copolymer
TBTM	Tributyltin methacrylate
TEOS	Tetraethoxysilane
TGA	Thermogravimetric analysis

THMBT	Trihexylmono(*n*-butyl)tin
TMOS	Tetramethoxysilane
TPhT	Triphenyltin
TPTCl	Tri(*n*-pentyl)tinchloride
WFD	Water framework directive
XRF	X-ray fluorescence

1 The environmental chemistry of tributyltin

1.1 Background to the Issue

The term 'marine biological fouling' refers to the accumulation of microorganisms, plants and animals on underwater surfaces, such as ships' hulls, oil rig supports, buoys, fish nets and fishing tackle[1]. On submersed structures, some of the problems from fouling are: (1) an increase in roughness, which in turn leads to an increase of weight and drag as a vessel moves through the water; (2) the increased drag on the ship causes higher fuel consumption, with substantial consequences in terms of economics and emissions[2]; (3) an increase in dry-docking operations: hull cleaning, paint removal and repainting, which in turn leads to high financial cost and large amounts of toxic wastes generated during the process[3, 4]. The marine industry spends an estimated one billion dollars annually to overcome problems caused by biofouling[5]. Effective antifoulants are therefore needed to prevent this undesirable accumulation.

The history of antifouling methods dates back to early times, but the topic remains an important issue for research today. Some of the harmful effects of biofouling have been recognized and fought against from very ancient times. First attempts were started as early as the third century BC. Pitch and copper sheathing were used by the ancient Phoenicians and Carthaginians, while early Greeks used wax, tar and asphaltum on their ships' hulls[6]. In the fifth century BC. coatings of arsenic and sulphur mixed with oil were used. By the mid 1800s, various kinds of paints had been developed, based on the dispersion of active ingredients in a polymeric vehicle. Copper oxide, arsenic and mercury oxide were used intensively at that time[7]. By the early 1960s, organotin compounds had been introduced as an active ingredient in the form of so-called 'free-association' paints[1]. Ten years later, a different paint system based on the use of organotin self-polishing copolymers was introduced[8]. Among all the different solutions proposed throughout history, organotin self-polishing copolymer paints have been the most effective antifoulants. Organotins quickly became very cost-effective coatings and by the mid-1980s were used on over 80% of the commercial fleet[9]. 'Tributyltin' (TBT) and its derivatives 'dibutyltin' (DBT) and

'monobutyltin' (MBT) are not compounds in their own right, but only constituent parts of molecules in the class of organotin substances. They are colloquial descriptors that refer to different forms of butyltin compounds, such as butyltin halides and butyltin oxide in which three, two or one butyl chains are attached covalently to a tin atom (in the Sn (IV) oxidation state). These compounds are released continuously into the water, are persistent and impact on organisms. The toxic effects of TBT compounds have been investigated since the early 1980s after researchers in France discovered that TBT released from antifouling paints caused abnormal and reduced growth in the pacific oyster[6]. In addition, various biological effects of TBT such as shell deformation in oysters and growth retardation in many other species have been well documented. However, the most harmful effect of TBT is the commonly observed effect of imposex, the development of male characteristics in female species, in the dog-whelk.

These facts forced the development of national legislations all over the world. The use of antifouling paints containing TBT has been banned on vessels under 25m in France since 1982, in England since 1987 and in the United States since 1989. More recently, the Marine Environmental Protection Committee (MEPC) of the International Maritime Organization (IMO) banned the use of TBT-based paint products on ships from 1 January 2003. By the beginning of 2008, the presence of such paints on ships had to be completely removed or covered[10].

Despite widespread restrictions and the strict bans on its use, significant concentrations of this compound and its degradation products, dibutyltin (DBT) and monobutyltin (MBT), are still found in water, sediments and sewage sludge[11]. Thus, it is very important to develop simple, sensitive, selective, rapid and economical methods for the quantification and identification of tributyltin compounds in environmental samples.

1.2 The process of marine biofouling and its prevention

Biofouling is one of the most important problems currently facing marine technology. In the marine environment, submersed structures such as platforms, jetties and ship hulls are under attack from the marine microorganisms and plants (Figure 1). These organisms are primarily the attached forms occurring naturally in the shallower coastal waters. Nearly 4000 species have been identified on fouled structures[12]. With time, this settlement can result in the formation of a thick, uneven and hard crust. The aggressive biofouling process creates adverse effects on all immersed structures, limiting their utilization and accelerating their corrosion. Worldwide, the marine industry spends an estimated one billion dollars annually to overcome problems caused by biofouling[13]. Although the development of antifouling technologies for shipping has gone on for more than two centuries, metal-containing antifouling paints continue to be the method of choice.

Fig. 1 Example of massive shell fouling[12]

1.2.1 Marine biofouling process

The fouling process has been considered to consist of four general stages (Figure 2): (1) initial biofouling begins when the surface of a vessel is submerged in water. Organic molecules, such as polysaccharides and proteins are rapidly accumulated on every artificial surface, and give a slimy surface film, a so-called conditioning film[14, 15]. Physical forces, such as Brownian motion, electrostatic interaction and van Waals forces control this process[15]; (2) - bacteria and unicellular organisms then settle on this modified surface, forming a microbial biofilm[16]; (3) the presence of adhesive exudates and the roughness of irregular microbial colonies enable more particles and organisms to be trapped. The transition from a microbial film to a more complex form that typically includes multicellular organisms, grazers and decomposers is regarded as the third stage of fouling[15, 17]; (4) the final stage involves the settlement and the growth of larger marine organisms and seaweeds. This macrofouling has rapid growth rates and high adaptability to different environments[15, 18, 19]. This macrofouling is divided, in engineering terms, into soft fouling (algae, soft corals, sponges and hydroids) and hard fouling (barnacles, mussels, tubeworms and other organisms)[19].

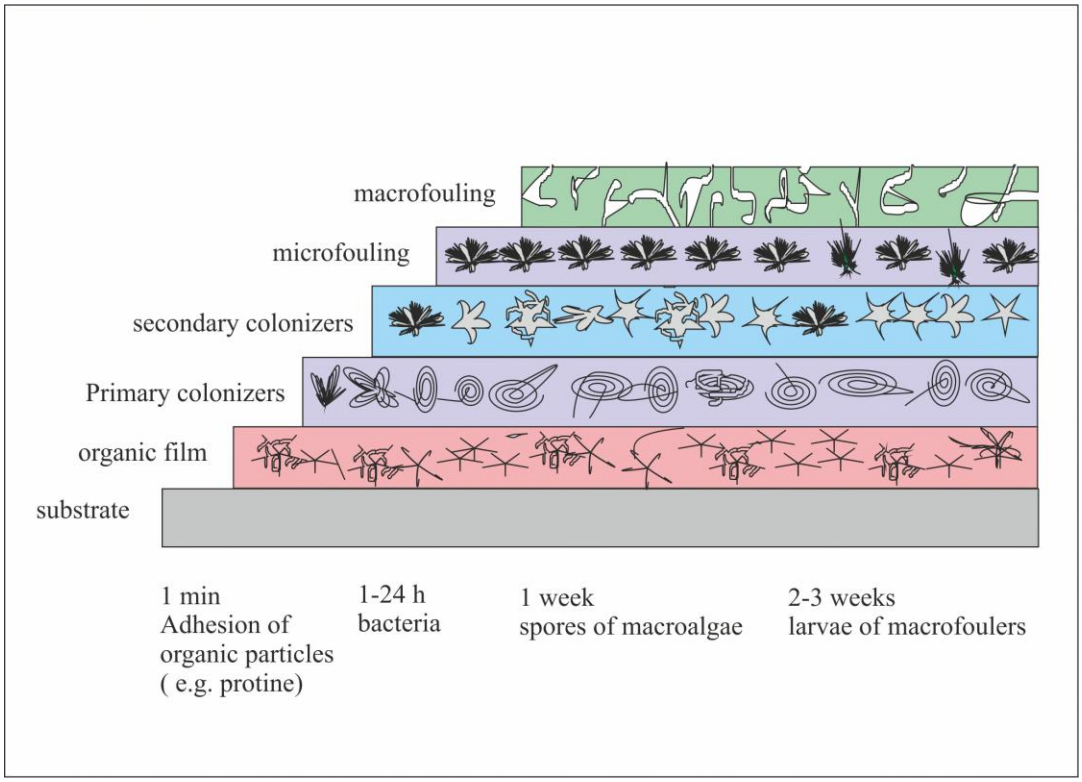


Fig. 2 Temporal structure of settlement (adapted from Yebra[12])

1.2.2 Antifouling paint systems

Antifouling is the process of preventing the growth of biological organisms. The use of antifoulants on ship hulls had been recognized very early and large number of methods to prevent biofouling have been tested over the years[7, 20]. Various chemical, electrical, radiation and thermal techniques have been used in these methods.[5] However, amongst all the different solutions proposed, chemically active antifouling paints have been the most successful in combating biofouling. The main idea of antifouling paints is that their active ingredients are dispersed in a matrix–the 'paint' from which they are 'leached' into the sea water[5], forming a thin layer of highly concentrated biocides around the submersed surface; the toxic concentration killing organisms attached to the surface. These paints are usually classified into three types, known as soluble and insoluble matrix paints and self-polishing paints according to the chemical characteristics of the binder, the film -forming components of the paint and their water solubility[21].

1.2.2.1 Soluble matrix paints

This type of paint system has been the basis of most traditional antifouling paints, which have been in use since the 19th century[12]. In soluble matrix paints, the active biocide is dispersed in a polymer binder that is physically dissolved in seawater[22]. classical film-forming binder in this system contains high proportions of rosin (a natural resin obtained from the exudation of pine and fir trees)[23]. On immersion in seawater, the rosin matrix dissolves releasing the biocides, usually Cu_2O particles, into the water[5]. The release rate of biocides remains constant until the paint has completely dissolved[24]. However, the toxicant leaching rate is often too high[25].

1.2.2.2 Insoluble matrix paints

In insoluble matrix paints, also referred to as contact leaching paints, the release of biocides is based on the use of a hard, mechanically strong, insoluble matrix that does not erode after immersion in water[26]. A wide range of high molecular weight polymers have been used in this system, and commonly employed binders are insoluble vinyl and chlorinated rubber polymers[25]. The critical biocide concentration is achieved only through the dissolution of biocide particles. The seawater penetrates the thin membrane, the paint film, dissolving some of the pigment[27]. The dissolved species diffuse through the interconnecting pores formed after dissolution of the soluble pigments[12, 27]. The leaching rate of the biocides from these paints decreases exponentially with time, resulting in the surface concentration falling below the minimum value required to prevent fouling[25, 26]. These types of systems are mechanically strong and stable to oxidation and photodegradation[5]. In summary, in the two paint systems, soluble and insoluble, the biocide is released only through the dissolution of the biocide particles.

1.2.2.3 Self-polishing paints

Since the early 1970s, the most effective systems have been the tributyltin self-polishing copolymer (TBT–SPC) based paints. TBT–SPC paints are based on an acrylic polymer

with TBT groups bonded onto the polymer backbone by an ester linkage (Figure 3). The formation of TBT self-polishing copolymer has been reviewed by Omae[28] (Figure 4).

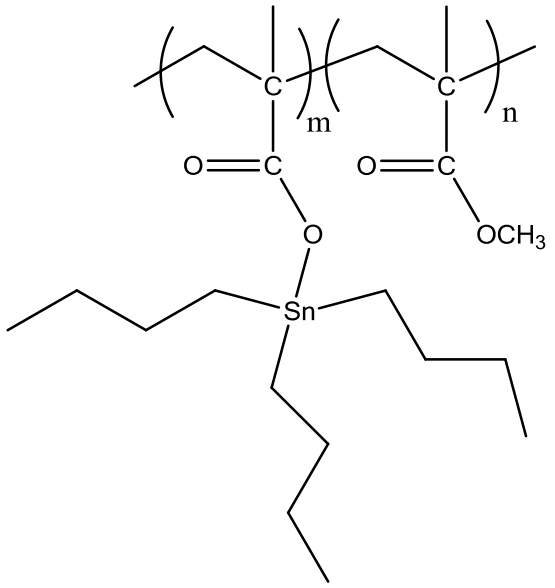
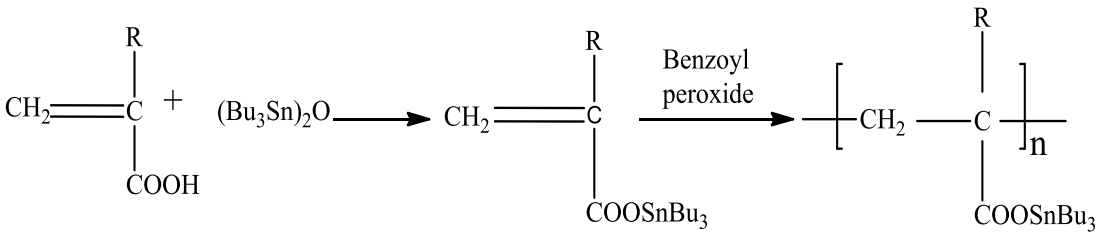


Fig. 3 Chemical formula of a repeating copolymer of tributyltin methacrylate (TBTM) and methyl methacrylate (MMA) (adapted from Ref.[12])



R= H, Me

Fig.4 Formation of TBT self -polishing copolymer (adapted from Ref. [28])

The main working mechanisms of these paints have been explained by Kiil et al.[5, 22]. The self-polishing mechanism of the copolymer begins with the hydrolysis of the

carboxyl–TBT linkage in seawater[29]. This reaction results in the controlled release of a TBT moiety from the copolymer[18,30] (Figure 5).

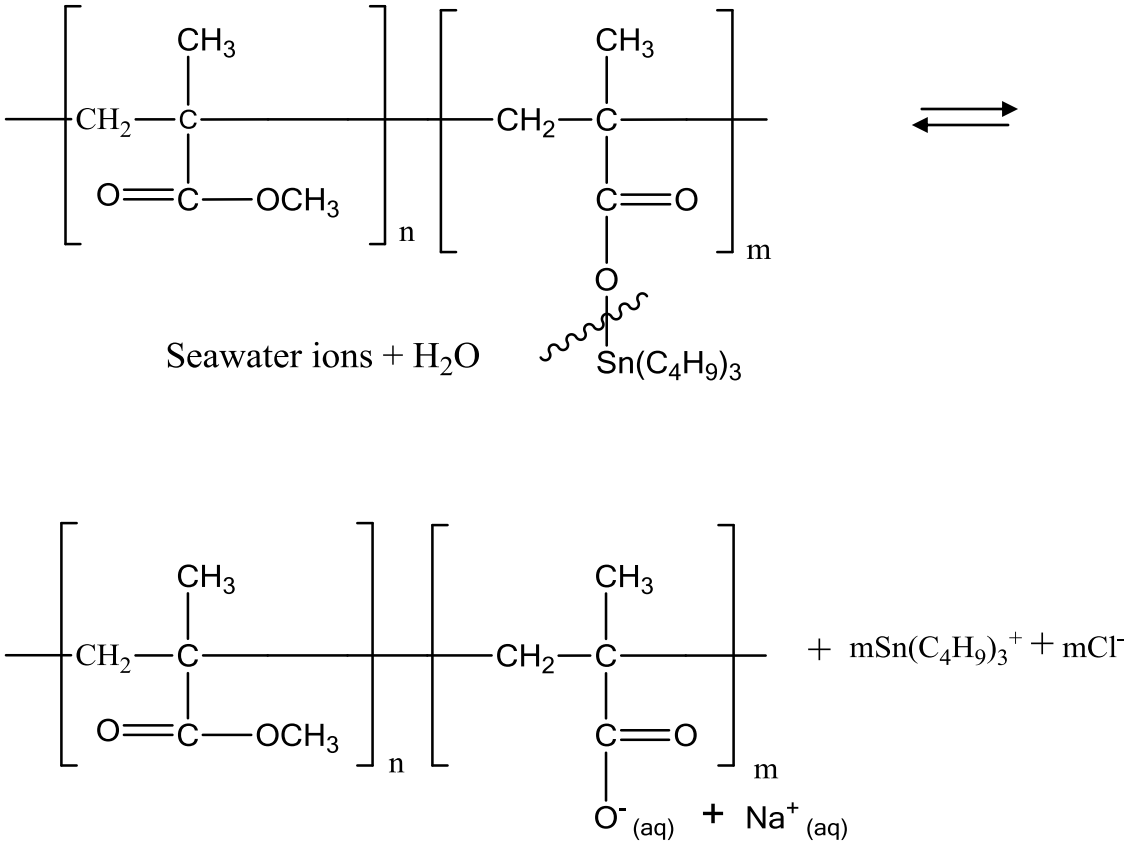


Fig. 5 Controlled release mechanism of TBT copolymer by hydrolysis (adapted from Ref.[12])

With time, the number of TBT moieties released increases and the partially reacted layer becomes weak and easily eroded by the moving seawater. A self-polishing effect occurs, exposing a new layer of paint[29, 30].

$$(Polymer)_m - COONa(s) \rightarrow (Polymer)_m - \underset{+}{COO^-}\ Na^+(aq) \rightleftharpoons (Polymer)_m - COOH \quad \text{Eqn.1}$$

The hydrolysis and erosion mechanism continues until no polymer is left.

1.3 Physico-chemical properties of organotins

Tin has four electrons in its outer electronic shell and thus forms compounds in which tin has oxidation states of +II and +IV[31]. In organotin compounds (OTC), at least one group is attached to tin through an SnC bond. These compounds are represented chemically by the general formula R_nSnY_{4-n}, where n is one to four. R is an alkyl or aryl group (e.g. methyl-, butyl-, ethyl-, phenyl-) and X may be hydrogen, a metal or a group attached to tin through oxygen, sulfur, nitrogen, halogen, etc.[32, 33]. OTC have been synthesized via two broad routes, namely directly from elemental tin or indirectly via tin compounds[34]. The indirect route involves different reactions: the Grignard reaction, the Wurtz recation (tin halides react with alkyl sodium) or the alkylaluminium reaction[35]. These reactions have been used in two approaches. Approach 1, involves the reaction of tin tetrachloride ($SnCl_4$) with excess Grignard to form the tetraalkyltin compound (R_4Sn). This R_4Sn can then be reacted with $SnCl_4$ to form less alkylated organotin chlorides, such as R_3SnCl, R_2SnCl_2 or $RSnCl_3$[33, 36] (Figure 6).

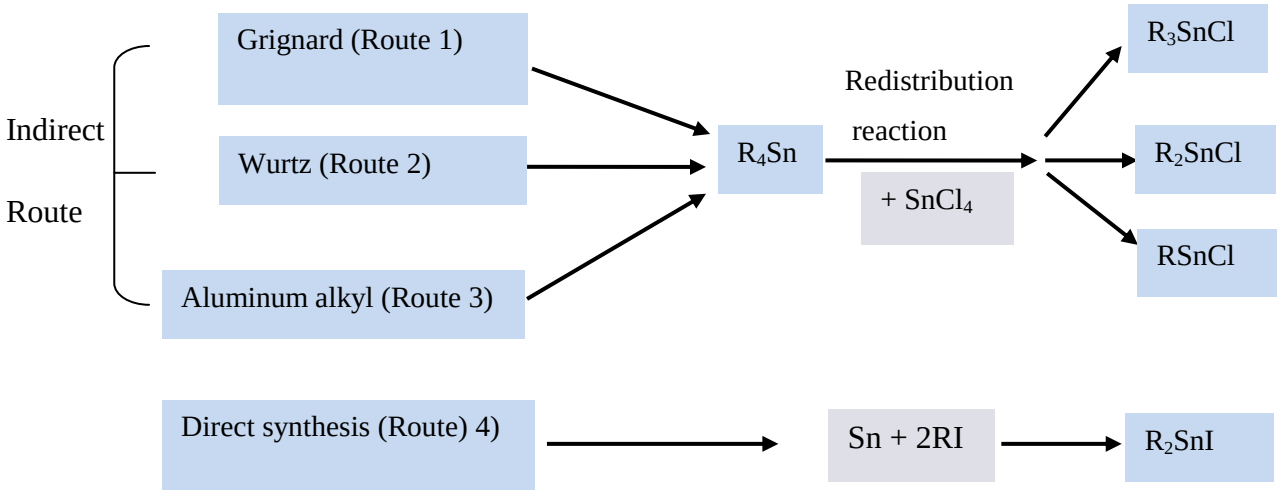


Fig. 6 Synthesis routes for organotin compounds[33]

The number and nature of organic groups attached to tin affect the electronegativity of the tin and thus the physical and chemical properties of its compounds. The Sn-C bond is relatively non-polar and is therefore stable in the presence of air and moisture[37]. In general, organotin compounds have low water solubility and a relatively high affinity (particularly TBT) for particulate matter[28, 38]. It has been reported that the presence of the chloride ion in seawater inhibits the solubility of tributyl- and triphenyltin compounds, probably by association with the cation to form the covalent organotin chloride[39]. The solubility of an OTC in water decreases with the increasing number and length of the organic substitutes. The solubility of an OTC is related to its leaching rate. The higher its solubility in water, the higher its leaching rate from the paint film into the water[22]. The toxicity of OTC is closely related to their speciation. The number and nature of the organic group attached to tin plays an important role in the toxicity[40, 41]. Table 1 gives some detailed information about the physical properties of selected organotin compounds.

Table 1 Physical properties of selected organotin compounds[33]

Compound	Melting temperature (°C)	Boiling point (°C)	Density (g/cm^3)	Solubility (mg/cm^3)
Bu_4Sn	-97	145	1.06	n.i [a]
Bu_3SnCl	-16	172	1.21	50[b]
Bu_2SnCl_2	39-41	135	n.i.	5-17[c]
$BuSnCl_3$	n.i.	93	1.69	n.i.
Me_3SnCl	37-39	154	—	n.i.
Me_2SnCl_2	106-108	188-190	—	20 000[c]
$MeSnCl_3$	48-51	171	—	n.i.

[a] n.i. = no information.

[b] Solubility in seawater.

[c] Solubility in distilled water.

1.3.1 Degradation of TBT

A large number of studies have been carried out on the degradation of TBT in sediment and water[42, 43]. The degradation is the progressive loss of organic groups attached to the Sn atom: $R_3SnX \longrightarrow R_2SnX_2 \longrightarrow RSnX_3 \longrightarrow SnX_4$.

Some studies suggest that TBT degradation kinetics in sediments is a much slower process than in the water phase[44-46]. Investigations of the degradation rate of TBT in the marine environment demonstrated that half-lives of TBT are several days to weeks in water, and from 1 to 10 years in sediments[47-49]. The half-life of TBT depends on the environmental conditions such as, temperature, oxygen content, availability of sunlight and concentration of TBT[50] and this can explain the broad range of half-life estimations. For instance, the rate of TBT degradation has been found to be slower at low temperatures because the colder temperatures inhibit the growth of TBT-degrading microorganisms[51]. The degradation of TBT in environmental samples can occur by chemical, biochemical and photochemical processes[52]. The rate of degradation of TBT is the sum of these processes. While the degradation of TBT in water happens due to photlysis and biological process, only biological degradation occurs in the sediment[53]. Regarding the kinetic aspects, it seems that photolysis by the near- UV component of sunlight is a relatively fast route of degradation for butyltins in water at limited depths[33]. The UV degradation rates are decreased by increasing salinity and humic acid concentrations[54]. Biological processes are the most important mechanism for the degradation of TBT in the marine environment[55]. Also, TBT biodegradation has been reported in fish, crabs, and oysters. However, TBT biodegradation is limited by conditions required by the organisms such as light, temperature and nutrients[56]. Thus, TBT is considered to be less degraded by the bacteria in sediment than by those in more shallow coastal waters[51, 57, 58]. The Sn-C bond is relatively polar in either direction, [Sn (δ+) — C (δ-)] or [Sn (δ-) — C (δ+)][59], therefore, heterolytic cleavages of the Sn-C bond can occur both by nucleophilic and electrophilic reagents such as mineral acids, carboxylic acids and alkali metals.

1.4 Occurrence of organotins in the environment

Organotin compounds have been introduced into the environment mainly as a result of human activity. OTC have a broad range of applications and they have been used extensively for many years. MBT and DBT compounds are used for stabilization of polyvinyl chloride (PVC) and as catalysts in the production of polyurethane foams and silicons. TBT and triphenyltin (TPhT) have been used as insecticides, fungicides, bactericides, wood preservatives and biocides in antifouling paints[32]. Other applications include the use of inorganic or organotin compounds deposited onto hot glass to form tin oxide coatings[31]. As a result of these widespread uses of OTC around the world, they have been detected in numerous environmental samples, including water, sediments, biological tissue and sewage sludge. Among the organotins, TBT is one of the most toxic compounds introduced into the environment. TBT enters the marine environment by four main ways. Firstly, during the widespread use of organotin compounds in antifouling paints used to protect ships, boats, fish nets or fishing tackle[60]. Secondly, by leaching out from paint on the hulls of vessels or any submersed structures[9]. Thirdly, by removing paints from vessels in shipyards and dockyards, where maintenance of boats takes place[38]. Fourthly, by discharging paint waste from shipyards into waterways[61]. Whilst, all these sources have contributed to increase the concentration of TBT, the fourth entrance route is the current major source of unregulated TBT in the marine environment. Polluted sediments act as a natural source of TBT and help to maintain its persistence in the aquatic environment. The affected sediments usually accumulate near dry docks, marinas and harbours[62]. Water movement, boating traffic, rain, the seasons and tidal cycle eventually transport TBT from the affected sediments into the sea[63]. Hence, special attention is given to polluted sediments since they are likely to be responsible for the TBT content of the overlying water.

1.5 Historical distribution of TBT in the world

Once the biological effects of TBT compounds towards non-target organisms in the aquatic environments was demonstrated in the early 1980s, interest in the extent and distribution of TBT in the marine environment has increased. During the past thirty years, several countries have introduced a strict ban prohibiting the use of TBT-based paints in order to reduce inputs into the marine environment. Despite widespread restrictions and the strict ban on its use, concentrations of this compound and its degradation products, dibutyltin DBT and monobutyltin MBT, are still found in water, sediments and sewage sludge, since these compounds persist in the sediments.

A relatively large number of studies have involved surveys of TBT distribution in the water column, sediments, and biota. The ban on TBT use in antifouling paints resulted in progressive decrease of TBT in the marine environment. For example, TBT concentrations in Arcachon Marina in France were 5-10 times lower in 1985 than in 1982. Moreover, one year before the UK ban (1986), the TBT concentration in Worxham Broad was 898 ng/L, but this concentration was reduced to 500 ng/L one year after the ban[9]. A more systematic study was carried out in the UK, covering five places in the Crouch Estuary, Essex over the period 1987 to 2005. This study showed a statistically significant decrease in TBT concentrations from 160 ng/g in 1987 to 20 ng/g in 2005[64] . This declining trend in TBT concentration has been observed in US[65], New Zealand[66] and Australia[67]. Exceptions to this general decline of sediment TBT have been reported at hot spots associated with ports, harbours and marinas. For instance, it has been reported that the concentration of TBT at the UK commercial port of Southampton was 43 ng/L in 1994 and this level was still at 33 ng/L in 2001[68] . Additionally, recent study to assess the contamination levels of TBT in sediments and water samples collected in March 2007 at Port Dundas, Glasgow, UK demonstrated levels of TBT 32 and 850 times higher than the UK Environmental quality target values for TBT in sediments and water samples respectively[69]. Moreover, several studies from around the world have found that TBT is still present in marine sediments at high concentrations. For example, Haynes and Loong[70] (2002) found TBT at 7.50 to 340.00 mg/kg in sediments from a ship-grounding

site on the Greet Barrier. In addition, TBT concentrations *ca.* 573 ng/g were found in sediments of the Gulf of Cadiz (Spain) in 2009 by Garg *et al*[71]. Other exceptions also occur in countries where no regulations have been adopted, such as in Bahrain where concentrations of TBT ranged from 2.29-17.9 ng/L in seawater and 128-1930 ng/g in sediments in samples collected from four coastal stations[72]. Also, in areas where research into TBT is relatively limited, such as the Atlantic Gulf coast, TBT has been found in high levels; 33-1021 ng/g and 66-469 ng/L in sediments and water samples (respectively) collected in 1995[73]. On the other hand, during the last few years, monitoring of organotin contamination has been enforced in developing Asian countries and investigations of TBT concentrations in different areas have been reported. Widespread contamination of TBT was found along the Chinese Bohai coast during the period 2002-2005 with TBT concentrations ranging from 2.5 to 345.6 ng/g in bivalves samples[74]. In Vietnam TBT levels ranged from 0.89-34 ng/g (2004) [75]and in a coastal areas of Malaysia 0.45-174.7 ng /g[76]. In contrast to the majority of countries in the Asia region, Japan showed a strict approach to combating TBT pollution from a very early stage in the debate over the effects of TBT. However, TBT pollution was still apparent in areas of industrial shipping[77]. These high levels of TBT are enough to pose harmful effects to benthic biota and gave evidence of widespread TBT contamination.

1.6 Environmental problems of tributyltin

The toxicity of TBT has been examined closely for some decades. TBT is considered bioaccumulative towards a wide range of organisms even at low concentrations, and the toxicity of TBT may lead to adverse effects in humans and other mammals[78]. It has been reported that bioaccumulation in humans is related to the consumption of contaminated shellfish[79]. However, no clear association between human health risk and TBT has been established. The toxic effect of TBT appears in water and sediments at very low concentrations (1-2 ng/L)[80]. The most common toxicological consequences of TBT are the disruption of the endocrine system of marine shellfish, leading to the development of male characteristics in female species (imposex)[6] (Figure7), impairment of the immune

system of organisms, which develop shell malformations, reduced reproduction and growth retardation of some marine organisms causing a drop in the population of these species[81].

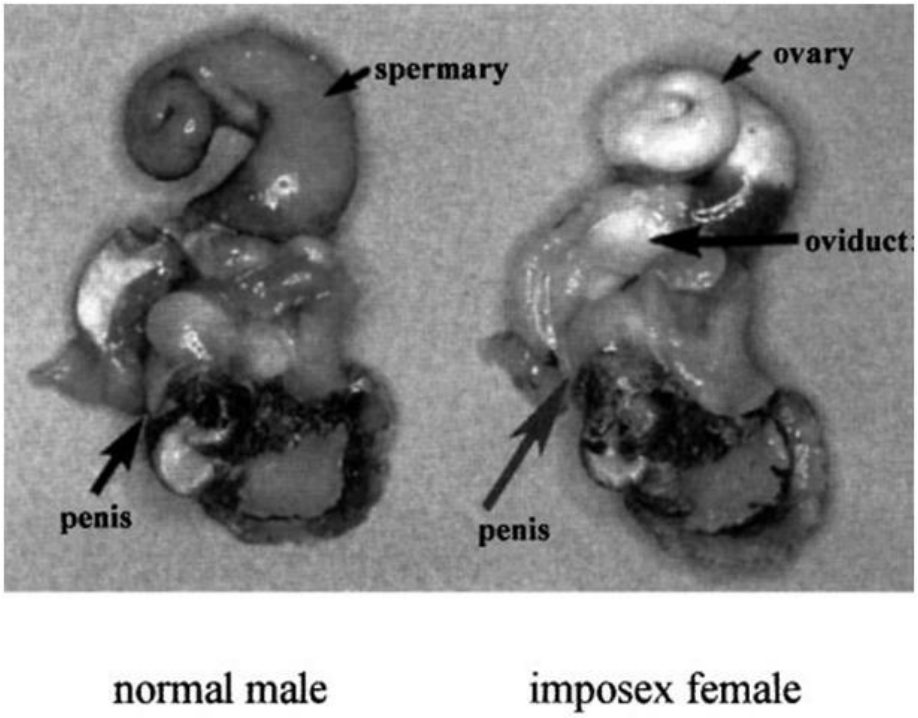


Fig. 7 Imposex in rock shell (Thais clavigera) [28]. With permission of John Wiley and Sons

1.7 The TBT ban

The destruction of the oyster industry in Arcachon Bay, France, resulted in restrictions being placed on the use of TBT[6]. In 1982, the Ministry of the Environment for France enforced a 2-year ban on paints containing approximately 3% of TBT[82]. Over 5 years, the government developed regulations to control the use of organotin antifouling paints. These regulations banned the application of antifouling paints containing TBT on vessels less than 25 m in length and vessels, of any length, which were made of aluminium or aluminium alloy[37]. By mid-1985, UK legislation was introduced. The Environment Minister set up actions for the control of TBT; banning the use of TBT-based paints on all vessels less than 25 m in length, limiting the tin content in co-polymer paints,

controlling the retail sale of organotin-based paints; developing a provisional Environmental Quality Target (EQT) for TBT in water at 20 ng/L and banning the use of TBT on aquaculture cages[10]. The Oslo and Paris Commission (OSPAR) introduced TBT into the list of priority hazardous substance. Furthermore, at the beginning of the 1990s, the environmental agencies in most countries of the world announced a proposed ban on the use of TBT-based paints on ships. Most recently, TBT appeared as a top priority pollutant in the European Union and the US Environmental Protection Agency lists. Also, in water policy in the European Water Framework Directive (WFD), TBT was included in the list of toxic substances[2]. Later, after an international convention held in 2001, the Marine Environmental Protection Committee MEPC of the International Maritime Organisation IMO declared the imminent ban on the use of environmentally harmful TBT-based paint products on ships from 1 January 2003, and complete prohibition of these biocides in antifoulants used on ships by 2008[83]. Overall, the legislation appears to have had a positive environmental effect in reducing TBT levels indicated by assessments of water, sediments and shellfish taken from areas where TBT had previously been detected[2].

1.8 Conclusions

Tributyltin was largely introduced in the natural environment as an anti-fouling agent in paints for the hulls of ships, and other structures to be used in the marine environment. TBT enters the marine environment by leaching out of the paint, by diffusion, directly into the water. Most of it is believed to be adsorbed on the sediment, due to the high affinity of TBT to sediment particles. TBT is very persistent in nature and has a half-life of 1-20 years. TBT has identified as an endocrine disruptor, responsible for imposex syndrome in certain marine gastropods at concentrations in water of a few ng/L. Despite widespread restrictions and bans on its use, the TBT remains a problem aquatic contaminant in most countries and high levels of this compound and its degradation products, DBT and MBT, are still found in water, sediments and sewage sludge.

2 Review on the analysis of butyltin-contaminated sediment and water

2.1 Introduction

Organotin compounds are found in the aquatic environment associated with a variety of ions (carbonates, chlorides, sulphides, hydroxides and polymers) or as oxides. They can interact with the environmental samples in different ways e.g. ionic and/or hydrophobic. The greatest change in analytical methodology that has occurred in field of organtin analysis has been the determination of individual compounds. Speciation analysis of organotin allows the investigation of biogeochemical changes of tin in the environment. Most of the analytical procedures developed for organotin compound speciation are based on the idea that only the organic group is preserved during the extraction step, whereas the counterion is cleaved during extraction or derivatization.[84]

The requirements of an analytical procedure for the determination of organotin compounds in environmental samples are quite strict, requiring detection limits at the ng/ L level for water samples and at the ng/ g level for sediment samples. At the same time, selectivity must be high enough to avoid interferences from the matrix, and simultaneous determination of all relevant organotin species must be possible. To meet these requirements, procedures typically involve the following steps: (a) extraction from the matrix, (b) derivatization of ionic organotin species, clean-up (if required), chromatographic separation and selective detection[85, 86]. Various techniques have been developed for environmental studies, but most are based on separation by gas chromatography (GC).

In the case of a gas chromatographic separation, organotin compounds are converted into volatile compounds normally, either an alkyl or a hydride derivative. Organotin compounds are usually identified by measuring retention times using tin-selective detectors such as atomic absorption spectrometry (AAS),[87] inductively coupled plasma

atomic emission spectrometry (ICP-AES),[88] flame photometric detection (FPD)[89] and more recently pulsed flame photometric detection (PFPD) [90].

2.2 Sampling and storage

Sources of error during sample collection and storage are numerous. Sediments and water may be subject to physical and chemical changes when in contact with air and losses of TBT can occur by adsorption onto the containers[91]. How representative the analysis is of the true concentration in an environmental sample depends upon the collection, pre-treatment and storage of the sample. Precautions have therefore to be carried out prior to analysis to ensure reliability of the data. The choice of suitable containers for sample collection and storage is important. Polyvinyl chloride (PVC) materials have to be avoided as they may contain dibutyltin[92]. Polytetrafluoroethylene (PTFE) has been used successfully. Collection and storage of samples are preferably done in glass containers, particularly for long-term storage[93]. The determination of organotins in environmental samples requires a storage method suitable for preserving chemical forms, because micro-organisms present in the environmental samples may degrade butyltin compounds during storage. Several sample storage approaches have been recommended, such as freezing, wet storage at 4 °C, air drying, freeze-drying and oven-drying[94]. Among these treatments, freeze-drying is usually the best option, since it preserves the concentrations of TBT, DBT and MBT and helps to make the sediment samples sufficiently homogenous[95]. With freeze-drying, sediment samples can be stored for over a year in a freezer (below – 20 °C)[96]. Storage (in the dark at 4 °C) of filtered natural water samples acidified at pH 2 with HCl was demonstrated to be suitable for achieving good stability for over four months[94]. However, the long-term stability of butyltins in water samples appeared to be doubtful, due to possible interactions with particulates and/or microbial communities present in the water samples, resulting in biodegradation of the butyltin species. Many authors analyse organotin compounds in filtered waters. However, some organotins, such as TBT, are strongly adsorbed onto suspended matter. Thus, assessment of the contamination may be inaccurate if only

filtered water samples are analysed[94]. This cause of underestimation could be avoided if the collected water samples are left unfiltered and are freshly analysed.

2.3 Leaching and extraction

Extraction of organotin compounds OTC from environmental samples, such as sediment, water and biological tissues is the most difficult part of an OTC speciation analysis, due to the limited stability of the analyte and the strong association of the analyte and the matrices. Moreover, the choice of the extraction technique is very important because TBT may convert to DBT or MBT if exposed to very rigorous extraction conditions. On the other hand, the extraction will not be complete if the conditions are too mild[97].

To obtain efficient extraction, the binding forces between TBT and its matrices must be diminished and the solubility in the extraction solvent must be maximized. Different extractants have been used for the extraction of OTC from environmental samples. These can be classified according to solvent polarity, sample acidification, the use of enzymatic hydrolysis with protease and lipase for biological samples, and extraction technique[98]. Organic solvents of low to medium polarity such as hexane, benzene, toluene or dichloromethane (DCM) are currently used with or without the addition of a complexing agent[55]. Simple extraction with non-polar solvents is generally sufficient for a trisubstituted alkyltin such as TBT, but more polar species, e.g. DBT and MBT, need complexation or acidification of the sample[98]. A wide variety of acid extraction procedures have been used for sediment analyses. Acidification of the sample is usually performed with hydrochloric, hydrobromic or acetic acid as these have been shown to improve the recovery of organotin species[99].

Extraction of organotin from sediments is more difficult than from water. The most frequently adopted methods for organotin extraction from sediments are leaching with acids or acid-polar solvent mixture[91]. The most important factor within the extraction condition is the acid concentration, too strong acid conditions leading to organotin degradation. The extraction is assisted by mechanical shaking, microwave energy or

sonication[84]. Yang and Lam[100] used a closed-vessel microwave system with glacial acetic acid for the extraction of organotin from sediment without a complexing agent. In extensive study on the extraction of butyltin compounds from sediment Namanic *et al.*[41] compared the efficiency of three different extraction solvents (acetic acid, a mixture of acetic acid with methanol and a mixture of acetic acid, methanol and water) and three different of extraction techniques, (mechanical shaking, ultrasonic, and microwave-assisted extraction) and they found that the ultrasonic extraction with acetic acid was the most efficient extraction method.

Glacial acetic acid has recently been shown to extract TBT from biological samples more efficiently than HCl or HBr acids[101]. The addition of the acid decreases the mineral binding of butyltin compounds, resulting in the release of positively charged ionic butyltin species. Extraction with a polar solvent such as aqueous hydrochloric acid, or acetic acid in a polar solvent (methanol, acetone),[102, 103] performed using mechanical shaking[104] or ultrasonics,[105] is followed by a non-polar solvent extraction (hexane, benzene) prior to derivatization by a Grignard reagent were used widely in OTC analysis.

Against these classical extraction methods, other more recent approaches such as microwave-assisted extraction (MAE),[106] supercritical fluid extraction (SFE),[107] pressurized liquid extraction (PLE),[97] solid-phase extraction (SPE)[108] and solid-phase micro-extraction (SPME)[109, 110] offer a new possibilities in OTC analysis. SFE is one of the most attractive approaches for sample preparation due to the universal effort to reduce consumption of organic solvents and total analysis time. Alzaga and Bayona showed that the use of supercritical CO_2 extraction of tri- and dibutyltin compounds from aqueous matrices reduced analysis time and solvent volume by 50% and 90%, respectively, compared with the liquid–liquid extraction methods[111]. SPME on the other hand simplifies sample handling and manipulation by integrating sampling, extraction, concentration and sample introduction into one step and one device[112,113]. Despite the advantages of these fast extraction techniques, some disadvantages have been observed, such as possible loss of chemical stability of the chemical forms of the analyte during the extraction steps due to the high pressures and/ or temperatures used in SFE, PLE and

MAE[114]. Also, MAE may degrade some butyltins[115]. Finally, SPME has some problems, e.g. some degradation of the fibre occurs during repeated usage[116, 117]. More recently, the use of ultrasound to accelerate the extraction of organotin species from soil samples has been proposed[118].

2.3.1 Using complexing agents

Complexing agents are used to improve the extraction of organotin species into low polarity solvents. Different types of complexing agents, such as tropolone, diethyldithiocarbamate in the form sodium-diethyldithiocarbamate (NaDDC)[119] and diethylammoniumdiethyldithiocarbamate (DEA-DCC) have been used[120]. Tropolone (2-hydroxy-2,4,6-cycloheptatrienone) (Figure 8) has been used extensively to promote the solubility of organotin compounds in non-polar solvents. However, the addition of tropolone has been found to prevent the hydride generation of volatile species of MBT. Extraction efficiencies have been found to be the same using tropolone concentrations from 0.01% to 0.5% (w/v)[121]. Tropolone has the advantage over NaDDC, of exhibiting good stability in organic solvents; NaDDC solutions on the other hand have to be prepared immediately prior to use[122]. Although NaDDC has been used successfully for the extraction of ionic alkyltin compounds from water,[123] its use in extracting sediment is limited by its operating pH (buffered medium pH 5). The sediment must be acidified before extraction, but the acid causes decomposition of the NaDDC[124]. DEA-DCC has been used to improve the solubility of organotins in supercritical carbon dioxide[120]. However, up to now, no systematic study on the role of the complexing agent in the extraction has been reported. Some extraction conditions for organotin compounds are summarized in Table 2.

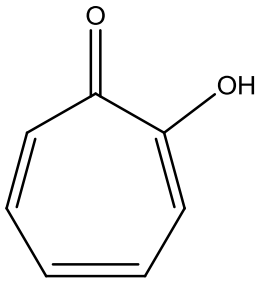


Fig. 8 Structure of the complexing agent tropolone

Table 2 Solvent extraction procedures for organotin species

Species	Extraction conditions	Ref.
TBT, DBT, MBT	HCl, pH 2–3 Extraction with 0.25% (w/v) tropolone in diethyl ether	125
TBT, TPhT	NaDDC complexation Toluene–acetic acid (10:4) extraction	99
MBT, MPhT	Stirring with water–HCl (1:1), extraction with hexane	126
DBT, TBT, TPhT	Reflux, 80 ^{o}C, 30 min with HCl–MeOH (5:95) Extraction with benzene	127
TBT, DBT, MBT	Sonication 30 ^{o}C with 0.08% (w/v) tropolone in MeOH Extraction with hexane	128
TBT, DBT, MBT	Shaking overnight, sonication for 30 min Extraction with pure acetic acid	94
TBT, DBT, MBT, TPhT, DPhT, MPhT	Stirring with water–HBr mixture, 30 min Extraction with 0.04% (w/v) tropolone in Cl_2CH_2	129
TBT, DBT, MBT, TPhT	Shaking with HBr Extraction with 0.07% (w/v) tropolone in pentane	130
Methyl- and butyltin	Sonication 2 h with 6 M HCl at 50 ^{o}C	105
TBT, DBT, MBT	Conc. HCl + conc. HBr Extraction with 0.05% (w/v) tropolone in pentane	131

MPhT, monophenyltin; DPhT, diphenyltin; TPhT, triphenyltin.

2.4 Derivatization

Derivatization of the butyltin species to form volatile compounds is a key component of TBT determination by gas chromatography. Derivatization methods are based on alkylation or hydridization reactions.

2.4.1 Alkylation reactions

The butyltin species are normally converted to fully alkylated forms by a Grignard reagent or by sodium tetraethylborate ($NaBEt_4$)[132-134]. A variety of Grignard reagents (RMgBr) have been used, but they can only be applied in non-polar solvents (hydrocarbons or ethers) and very dry conditions are required. Grignard reagents are usually dissolved in diethylether and are used in an inert atmosphere. Excess Grignard reagent is generally destroyed by adding acid[84]. It is possible to alkylate with any chain length.

$$R_xSn_{(4-x)} + \acute{R}MgX \rightarrow R_xSn\acute{R}_{(4-x)} + H_2 \qquad \text{Eqn. 2}$$

where R and $\acute{R}$ are organic substituents and x ranges from 1 to 3.

Some of the first methods on the use of a Grignard derivatization in tributyltin analysis appeared in the 1970s. Meinema and co-workers[135] proposed a method for butyltin determination in water samples based on solvent extraction, derivatization with a Grignard reagent prior to GC separation and detection by mass spectrometry. The smaller the alkylation group, the more volatile the products of derivatization, and the greater the losses during transfer and work-up[136]. On the other hand, the larger the carbon number of the alkyl group, the longer the residence time on the GC column,[41] and this can increase the total instrument time significantly. Considering these facts, pentyl or hexyl magnesium bromides are commonly used. Hexylalkyltins are sufficiently volatile for gas chromatography and they are the more thermally stable. When hexylmagnesium bromide is used, evaporation losses can be eliminated and separation of the butyltin species is good[91]. In addition, the tetraalkyl derivatives formed with Grignard reagents are stable

for extended periods of time[137]. The concentration of the Grignard reagent has a significant influence on the efficiency of the derivatization. Lower yields were observed using diluted Grignard reagent (1:4, in ether) instead of the concentrated reagent[136]. During the analysis of organotins in sediment samples containing high concentrations of sulphur, interferences can be observed. In this case, elemental sulphur is co-extracted with the organotin and is alkylated during the Grignard derivatization, leading to the formation of dialky mono-, di- and trisulphides[138]. These sulphur species interfere with the final determination of organotin. In this case, the use of a clean-up procedure is necessary to improve organotin recovery from complex sample matrices.

Ethylation of butyltin species with sodium tetraethylborate $NaBEt_4$ is another option for alkylation. Ethylation with this reagent can be performed in an aqueous or a methanolic environment and it can be done together with the extraction step[139]. Derivatization with $NaBEt_4$ is very rapid and takes place at a pH between 4 and 6.

$$R_xSn_{(4-x)} + NaB(C_2H_5)_4 \rightarrow (C_2H_5)_xSnR_{(4-x)} \qquad \text{Eqn. 3}$$

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

Below pH 4, the reagent reacts with H^+ ions and decomposes, while above pH 6 the organotins convert to their hydroxide forms[140]. The amount of buffer employed to control pH must be sufficient, especially if a high concentration of acid has been used during the extraction. The addition of the acid was employed to cleave the Sn-O bond or the tin mineral binding of butyltin compounds in sediment, resulting in the release of ionic butyltin species. This method is particularly successful for aqueous samples,[141] but lower derivatization yields compared with a Grignard reagent are observed in complex matrices containing large amounts of co-extracted compounds[84]. For the direct ethylation of organotin in sediment and biological samples, extra $NaBEt_4$ must be used in order to achieve high yields[142, 143]. The influences of pH, reaction time and concentration of $NaBEt_4$ on the derivatization yields in the determination of butyl- and phenyltin compounds have been investigated. The highest recoveries were observed in the pH

range 4.5–5.0, with 2 to 3 minute reaction time and with concentrations equal to, or higher than, 0.2% (w/v) [136].

2.4.2 Hydridization reactions

The reaction of organotin compounds with sodium tetrahydroborate ($NaBH_4$) to produce tin hydride species has long been known[144]. The reagent works within a pH range of 4–11. The hydridization reaction can take place in aqueous media and simultaneous extraction/derivatzsation can be performed in NaOH-MeOH[145]. This method is less often used for off-line determination, because volatility and reactivity of the hydride derivatives are high. Furthermore, it has been suggested that organotin hydrides may degrade more rapidly than the tetralkyltin compounds[146]. While hydride generation (HG) has been found to be the most appropriate method for the analysis of large volumes of water,[147, 148] interferences from metals, organics and sulphides have been observed in sediment analysis.[149] In particular, the presence of high metal concentrations in harbour sediments can lead to an inhibition of hydride formation[150]. The influence of organic substances on hydridization yields has also been investigated[151]. Various procedures have been used to reduce the interferences in hydride generation for TBT analysis, for instance, the addition of masking agents such as EDTA, KI or ascorbic acid, or the use of chelating resins or the use of separation techniques such as co-precipitation[132].

2.5 Clean-up

Clean-up is often performed to remove compounds that would interfere during the determination stage. Whether a clean-up step must be applied depends on the sample type, separation technique and detection method used. Most of the analytical procedures based on GC determination require a clean-up, usually after the derivatization step. Extraction methods using an organic solvent will co-extract many other compounds from the sample, such as sulphur, oil and lipids, resulting in interference with the butyltin compounds. Therefore, the need for efficient clean-up procedures is necessary. Common clean-up procedures used in organotin analysis consist of an adsorption chromatography

step after derivatization using adsorbents such as silica, aluminum oxide,[152, 153] Florisil (magnesium silicate),[154] alumina,[99] or C_{18}/silica[155] and with hexane as the eluent. Clean-up procedures before the derivatization have also been used[127]. With sediment samples, desulfurization with activated copper has been used. However, this method is not sufficiently efficient to eliminate the interferences from alkylsulphides that are formed during the Grignard derivatization from elemental sulphur[57]. Alternatively, other desulfurization reagents, such as tetrabutylammonium hydrogen sulphate and sodium sulphide have been successfully used to remove this interference[156]. In addition, a desulfurization procedure based on the oxidation of all sulphur compounds with dimethyldioxirane to give the corresponding sulphones has been developed[157]. The clean-up procedure must be carefully monitored to obtain consistent results and a balance must be obtained between clean-up efficiency and analyte recovery[126]. However, whilst clean-up is recommended, it is not necessary in some water sample analysis, because the detection methods are very selective[158].

2.6 Instrumentation

There are important aspects that need to be noted in organotin speciation analysis: (a) the more specific a detector is, the less demanding the extraction process needs to be for the analytical process; (b) the requirements of acceptable limits of detection and the presence of relatively few interferences. The earlier attempts to analyse TBT in environmental samples using a verity of analytical techniques have been reviewed by Attar[159]. These attempts included the use of spectrophotometric methods, combining the organotin compounds with a photoactive ligand to aid detection,[159] but detection limits of these methods were poor, 0.02–10 mg/ L. Anodic Stripping Voltametry has also been used for the detection of tributyltin oxide in water after steam distillation and thin-layer chromatography, but detection limits were again not sufficient for environmental analysis[160]. Detection limits were lowered to µg/ L levels by the use of fluorimetric and polarographic methods[161]. In the past, the atomic absorption spectrophotometric AAS method was used for the determination of tin in environmental samples, However,

because of the unsatisfactory combustion of tin oxide in the flame, the use of graphite furnace atomic absorption spectrometry based on the converted of organotins to their volatile forms (e.g. hydrides) has been developed[162]. These methods are not based on chromatographic separation, but on selective extraction. Most of the methods in use today for the speciation analysis of organotin compounds are based on the use of chromatographic separation techniques. The main separation techniques involve gas chromatography (GC),[163] high-performance liquid chromatography (HPLC),[164] capillary electrophoresis (CE)[165] and supercritical fluid chromatography (SFC) [166].

2.6.1 Gas chromatography

Gas chromatography, coupled with element specific detection methods, is widely used for analytical separation, identification and quantification of organotin compounds. This is due to its high resolution, low detection limits and its ability to resolve many organotin species[60]. The separation of organotin species in all GC-based techniques requires the conversion of the ionic organotins to more volatile derivatives. The GC technique allows the determination of a wide variety of organotin species such as methyl, butyl, ethyl, propyl, phenyl and cyclohexyltin compounds simultaneously in different matrices such as natural waters, sediments and, recently, in biological tissues[102]. Separation in GC technique was achieved using different types of columns. Capillary columns gained acceptance during the 1990s and nowadays they are commonly used rather than packed columns, because they lead to better resolution and more sensitive detection of many organotin compounds. Various injection systems have been developed (split–splitless, cold on-column, temperature programmable), which allow the complete volatilization of up to 5 μL of a sample. A major advantage of GC that it can be coupled with different types of detectors. The following detectors have been used for organotins speciation analysis: atomic absorption spectrometry (AAS)[87, 155, 167] atomic emission detection (AED),[119] microwave-induced plasma atomic emission spectrometry (MIP–AES),[168] inductively coupled plasma–optical emission spectrometry (ICP–OES),[169], inductively coupled plasma–mass spectrometry (ICP–MS),[170] mass spectrometry (MS),[171] flame

ionization detection (FID),[172, 173] electron capture detection (ECD),[174] flame photometric detection (FPD) [175-177], and more recently pulsed flame photometric detection (PFPD)[90, 178].

There have been a number of reports on the use of gas chromatography coupled with inductively coupled plasma mass spectrometer (GC-ICPMS) for the determination of organotin species. This is because of the high sensitivity that GC-ICPMS can provide and it allows simultaneous multi-isotopic detection in a single run[88]. The use of ICP-MS as a detector enables calibration by isotope dilution mass spectrometry as well as providing very low limits of detection (pg-ng range)[170]. Also this approach offers wide linear dynamic range, high speed analysis and greater precision when compared to an external calibration method.

The lack of selectivity and/or sensitivity of some of those detection systems led to their replacement by more sensitive detectors. For example, GC–MS coupling does not provide sufficient sensitivity for the direct analysis of organotin compounds in many environmental samples[179]. Similarly, FPD suffers some interference associated with sulphur species[180]. The use of a suitable optical filter was introduced to minimize the interference effect, but it has not proved to be a very effective solution. The recent introduction of a pulsed flame photometric detector improved sensitivity and selectivity for organotin compounds[181].

2.6.1.1 Pulse flame photometric detection (PFPD)

About 15 years ago, Amirav and Jing[182] reported a new type of flame photometric detector FPD in which the flame is pulsed. The PFPD was initially used for sulphur, phosphorus and nitrogen determinations. PFPD applications have now been extended to enable determination of other elements such as arsenic, selenium, antimony, aluminium, nickel and tin[182]. The first report on the use of the GC–PFPD for organotin speciation was published by Jacobsen *et al*.[90]. The PFPD has been shown to outperform the conventional FPD in terms of sensitivity and selectivity. The use of PFPD in the determination of tin in environmental samples allows the limit of detection to be

decreased 25–50 times compared with those of classical FPD. Detection limits of 0.2 to 0.4 pg of the organotin compounds were achieved[90]. PFPD coupled with GC has been used successfully for the simultaneous determination of organotins in natural waters, sediments and in biological tissues[183]. The PFPD is based on a discontinuous flame and reduced gas flow rate that cannot sustain continuous combustion and allows flame propagation[184]. An air–hydrogen flame is used. A schematic diagram of the PFPD system is shown (Figure 9).

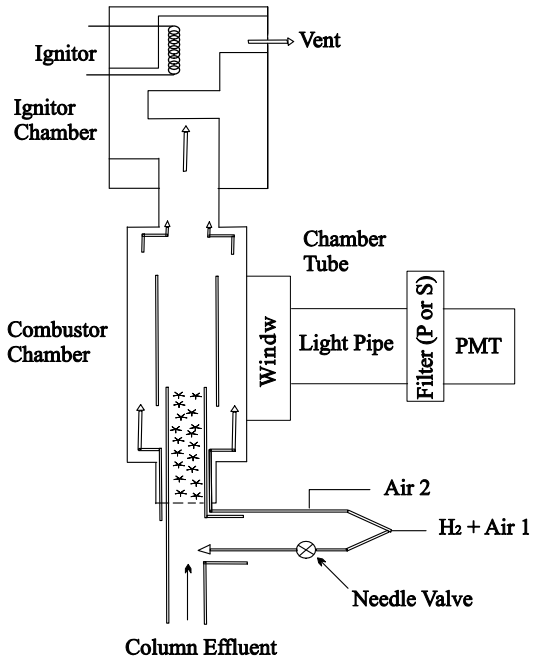


Fig. 9 Cross-section of the pulsed flame photometric detector[182].

In this system the air flow is split into two portions: the first portion of the air is mixed with hydrogen in a small flame chamber[182]; the second portion of air is added to this mixture in the detector to adjust the total ratio air–H_2 and control the ignition rate[184]. The first part of the air–hydrogen stream mixes with the column effluent and flows to a continuously heated Ni/Cr wire igniter. The ignited flame is then propagated back to the gas source[182]. Once all the combustible gas mixtures have burnt, the combustion chamber

then refills and the process repeats itself, typically at a rate of 2-4 pulses per second[182]. During, and after, the flame propagation, light is emitted by excited molecular species in the combustor. This ignition–propagation–termination cycle continues with a pulsed periodic frequency of about 3–4.5 Hz[182, 185]. PFPD consists electronic gate used to select the time period of the emission that is to be integrated to generate the detector signal. Interference is eliminated by adjusting the frequency of the flame, the electronic gate delay and width settings to permit emissions from different atoms to be distinguished[186]. The product emission lifetime differences, combined with the flame's propagation and termination properties, provide both time and spectral information. The thermal properties of the combustion of hydrocarbon molecules are different from those of heteroatom species. Thus, for heteroatom species, flame emissions are delayed, electronically gated, and separated in time from those of hydrocarbons[185, 186]. The advantages of PFPD over the continuous-flame detectors are (a) reduced flow rate of gases necessary to allow flame propagation; (b) the addition of time-dependence information of pulsed flame emission; (c) higher sensitivity; (d) highly improved selectivity against hydrocarbons; and (e) very good long-term stability due to the self-cleaning action of the pulsed flame[184].

2.6.1.2 Operating principles of PFPD

In the flame, organotin compounds have two different emission bands (centres at *ca.* 390 and 610 nm), resulting from the excited organotin species SnC and SnH, respectively[64]. The emission band at 390 nm is more selective and convenient for OTC analysis than the emission band at 610 nm[138, 185]. However, there can be a problem with this emission band, because sulphur emission (due to S_2^*) occurs at the same wavelength[64]. With the classic flame photometric detector, an optical filter is sometimes used to isolate the emission band at 610 nm. With the PFPD an optical filter is used to select the emission band at 390 nm and the interference is eliminated by adjusting the frequency of the flame, the electronic gate delay and width settings[61]. The details and mechanisms of the chemical reactions which are responsible for this emission time behavior are not totally clear. The flame background emission from species, such as CO*, CO_2* and H_2O*,

occurs during the hottest period of the flame pulse and the reaction is fast, highly exothermic and irreversible. Hydrocarbon related emission of CH* radicals emerges as the result of the very exothermic reaction. $OH + C \longrightarrow CH^* + CO$[182]. That include the OH^* radical. OH^* radicals are very reactive and their life time is very short ($< 10^{-4}$ s)[184], and thus they are totally consumed in a few millisecond after ignition and before the complete propagation of the flame. Other radicals that are responsible for the hydrocarbon emission are C_2^* radicals, which depends on the reaction of C atoms. C_2^* radicals are also highly reactive and their chemical life time is very short[185]. Therefore, hydrocarbon related emission (flame background emission) is very short due to energetic reasons and its time scale is shorter than the time of pulsed-flame propagation. On the other hand the emission of sulphur species is created due to the recombination of sulphur atoms or the recombination activation of S_2 by the recombination of hydrogen atoms[138].

$S + S^* \longrightarrow S_2^*$

$H + H + S_2 \longrightarrow S_2^* + H_2$

In hydrogen-rich flames the lifetime of atomic hydrogen is very long. The lifetime of sulphur atoms is also long. This is because the S_2^* radicals weaker bonds with lower energies and it can be formed later during the cooler post-flame period via the reaction of H atoms with H_2S or HS with more reversible reactions[186]. The longer delay time of the maximum of S_2^* emission can be explained by entropy reasons[182] as the equilibrium concentrations of S_2 molecules is very small in the very high temperature pulsed flame zone. Therefore, the delay emission comes from the delay formation of S_2. Organotin related emission is resulted from the excited SnC and SnH species. Tin emission is barely present during the time of the main pulsed-flame OH^*emission and occurs at shorter delay time in comparison with sulphur. The SnH^* emission emerges from the reaction of Sn atoms with hydrogen and this fact rationalizes the time-delayed emission of organotin. Other excited tin species (SnC^*) resulted from the degradation of the alkyl groups attached to tin by HO^*, resulted in the flame[196]. The bond energy of Sn-C is higher than of S_2 (142 kcal/mol versus 100 kcal/mol) and therefore, the concentration of SnC in the high-temperature zone is higher[182]. Thus, some emission of SnC* can be observed on the pulsed flame front time, and the maximum emission time delay is shorter

than that of sulphur. However, the emission delay is still sufficient for the effective time separation from the flame background and hydrocarbon emission. The products' emission lifetime differences, combined with the flame's propagation and termination properties, provide both time and spectral information. The thermal properties of the combustion of hydrocarbon molecules are different from heteroatom species. Thus, heteroatom species flame emissions are delayed, electronically gated, and separated in time from that of hydrocarbons. Therefore, by adjusting the frequency of the pulses, it is possible to separate the flame background and the interfering-species emission from the analyte emission (Figure 10).

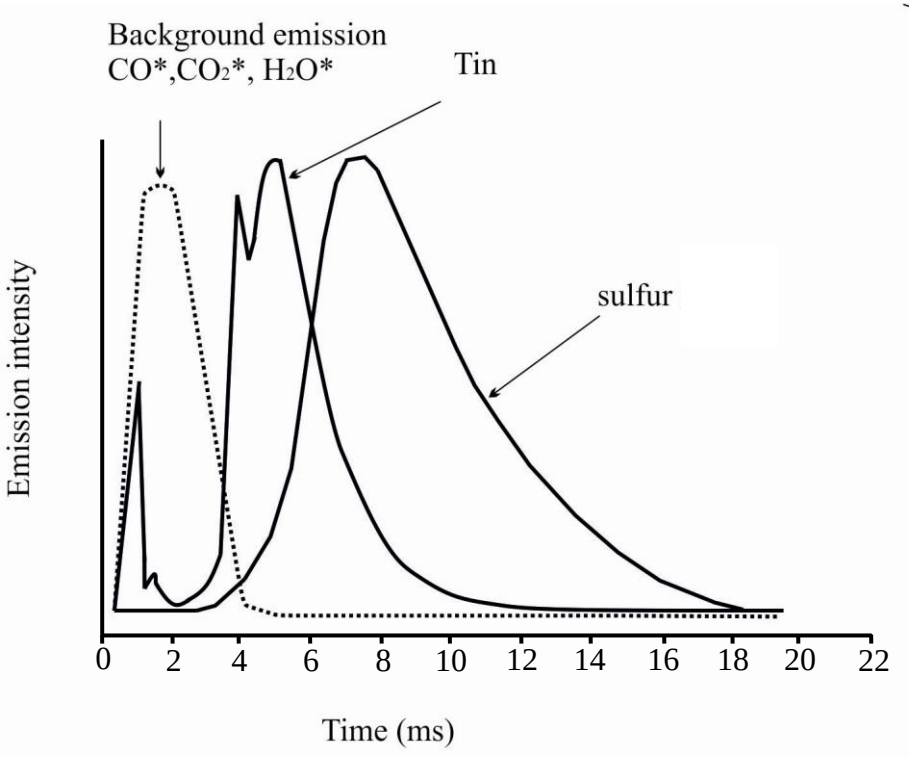


Fig.10 Emission time profile of Sn and potentially interfering species in the PFPD[181].

2.6.2 Liquid chromatography

Another class of widely used techniques in organotin speciation analysis is liquid chromatography (LC) coupled with selective detectors. In contrast to the use of gas chromatography methods, liquid chromatography, and particularly high performance liquid chromatography HPLC, offers the advantage of avoiding the time-consuming step of derivatization and reduces the number of sample manipulation steps, which minimizes possible contamination and analyte loss. The liquid chromatographic methods can be categorized into three main types: (1) ion-exchange chromatography; (2) reversed phase chromatography; and (3) normal phase chromatography[187]. Among the different modes of LC used, ion-exchange has been the most applied[188]. In the case of organotin compounds, ion-exchange chromatography is generally performed in silica-based cation-exchange columns, and the mobile phases consists of a mixture of methanol, or sometimes acetonitrile, and water, containing ammonium acetate or citrate[189]. This approach has been applied to separate TBT and DBT without the use of complexing agent. An alternative approach for separating the three butyltins, TBT, DBT and MBT compounds involves the addition of tropolone to the mobile phase[190]. The reversed-phase mode involves the use of a polar mobile phase with a non-polar stationary phase. The stationary phase usually consists of an alkyl moiety, which is chemically bound to a silica support material. Reversed-phase with an octadecylsilane stationary phase (C_{18}) has been used in the separation of butyltin compounds from sediments ,using a polar mobile phase[191]. Normal phase separation mode involves the use of stationary phases that have a higher polarity and a non-polar mobile phase. The detectors used with LC can be classified into two types: (1) non-element specific ones, such as UV absorption detectors and differential pulse voltametry and (2) element-specific ones, such as atomic absorption[192], atomic fluorescence or ICP–MS[193, 194].

2.7 Conclusions

A series of methods have been developed, validated and comprehensively tested for speciation analysis of organotin in the different environmental samples. Analysis of organotin compounds mainly consists of four steps: extraction, formation of volatile derivatives, separation, and detection. Different extraction techniques have been used for organotin analysis. Liquid-liquid extraction methods have been replaced by other more recent extraction techniques such as microwave-assisted extraction, supercritical fluid extraction solid-phase extraction, and solid-phase microextraction. These techniques methods improve the sample treatment, on the basis of time reduction, sample and reagents consumption. Derivatization methods include formation of alkyl derivatives using a Grignard reagent, formation of ethyl derivatives using sodium tetraethylborate, or formation of hydrides using sodium tetrahydroborate. Derivatized organotin are generally separated by gas chromatogaraphy and detected by the selective techniques such as, atomic absorption spectrometry, flame photometric detector, or pulsed flame photometric detector.

2.8 Project aims and objectives

Extraction of organotins, particularly TBT, from environmental samples is the most difficult step in OTC speciation analysis, due to the limited stability of the analyte leading to possible interconversion between species, low concentration levels in the environment and the strong interactions between the analyte and matrices. All effects decrease the apparent TBT concentration, and therefore TBT is more likely to be under rather than over estimated.

The overall project aims are: (1) to evaluate the present methods for the measurement of TBT and its derivatives DBT, MBT in environmental sediments and waters and to improve upon this measurement by establishing new methods for the determination of TBT, DBT and MBT; (2) to assess the analytical performance of the new procedures and compare it to the performance of other methods; (3) to assess the applicability of the new

procedures by the analysis of environmental sediment and water samples. These overall aims will be complemented by a series of further aims:

- The development of procedures for the measurement of TBT from within paint flakes. This is particularly important due to concern over the ability of current analytical methods to extract TBT from within flakes of paint deposited in sediment during boat refurbishment.
- During the determination of organotin compounds in sediment, the possibility of interferences due to alkylsulphides or elemental sulfphur became evident, leading to some difficulties in TBT quantification. Thus, the identification and removal of sulphur interferences during the determination of TBT in sediment was required.

For the analysis of water samples a new extraction concept will be applied. This is based on the dispersion of sub-micron chemically modified mesoporous silica in water samples. The initial work will be directed toward the synthesis and characterization of the sub-micron modified silica with selective physico-chemical properties (particle size, shape, surface area and surface chemistries). Once this has been achieved, a new analytical procedure will developed for the analysis of butyltin-contaminated water using silica-based nanoscavengers for their extraction and pre-concentration.

The nanoscavenger to be employed is a dual functionalised mesoporous silica surface modified with diol and C18 groups.

3 Determination of butyltins in sediment

3.1 Introduction

Sediments can be an important source of contamination of water and the organisms associated with it. In water a quasi equilibrium is established in which TBT partitions between the water and the sediment. For many sediment phase types this equilibrium is strongly in favour of the solid phase and TBT- laden sediments can remain a long term source of TBT, releasing the contaminant into otherwise uncontaminated waters. TBT-related compounds in sediment can be present as both anthropogenic particles such as paint flakes and adsorbed TBT derived from the water.

The determination of butyltin compounds in sediments has been a major analytical challenge for a number of reasons: (1)- the chemical complexity of the sediment matrices, (2)- the low level of the analyte (a few ng/g), and (3)- the strong adsorption of butyltin compounds, particularly TBT, onto the sediment. Most analytical methods used for the measurement of butyltins employ extraction of the compounds from the sediment matrix then derivatization, chromatographic separation and selective detection. More attention needs to be given to the extraction step as it is one of the main sources of error in the determination of butyltin compounds[41]. In the approach adapted for this study, acidification with acetic acid is carried out followed by hexane/tropolone extraction. The addition of the acid decreases the mineral binding of butyltin compounds, resulting in the release of ionic butyltin species. Tropolone has been used as a complexing agent to promote the solubility of butyltins in hexane by forming stable complexes.

Derivatisation with a Grignard reagent is required in order to achieve more volatile compounds for the chromatographic separation. *n*-Hexylmagnesium bromide is used to hexylate the butyltins (Eqn. 4) as the products are sufficiently volatile for gas chromatography and they are thermally stable.

$$\underline{x}n\text{-HexMgBr} + Bu_{4-x}SnCl_x \xrightarrow{\text{dry hexane}} Bu_{4-x}SnHex_x + \underline{x}MgBrCl \qquad \text{Eqn.4}$$

Low extraction efficiencies and/or low derivatization yields can both lead to an underestimation of the true concentration.

In this study the instrumental analysis was performed by gas chromatography coupled with pulsed flame photometric detection GC-PFPD. This detector has greater selectivity and higher sensitivity than the conventional flame photometric detector. The limits of detection obtained with the PFPD are 5 to 40 times lower than those obtained with the classical FPD, making it possible to quantify organotins down to 0.1 ng Sn g^{-1} in sediment samples[195].

This chapter describes the evaluation of some current methods for the measurements of TBT and its breakdown products DBT and MBT in sediments and identifies improvements that can be made to the procedures.

3.2 The determination of TBT, DBT and MBT in sediment

All materials coming into contact with samples or solutions containing butyltin compounds were decontaminated by immersion in 5% (v/v) aqueous Decon 90 solution overnight. They were then thoroughly rinsed with *ca.* 3M HCl solution, three times with deionised water and then with acetone.

3.2.1 Chemicals and reagents

Deionised water (>5 MΩ-cm at 25 °C) was produced using an ELGA OPTION 4 water purifier, *n*-hexane (99.99%, research grade), anhydrous sodium sulphate was purchased from Fisher, UK (dried, and purified by combustion at 400 °C overnight, laboratory reagent grade). Calcium carbonate was purchased from BDH (UK). Tropolone (98%), *n*-hexylmagnesium bromide (2.0 M solution in diethylether), tri(*n*-butyl)tin chloride (TBTCl, 96%), di(*n*-butyl)tin dichloride ($DBTCl_2$, 96%) and mono(*n*-butyl)tin trichloride ($MBTCl_3$, 95%) were obtained from Aldrich (UK). Tri(*n*-pentyl)tinchloride (TPTCl, 95%) was purchased from ABCR, Germany. Sulfuric acid (98%, analytical reagent grade) and glacial acetic acid (99.99 %) were obtained from Fisher (UK). Tri(*n*-

butyl)mono(*n*-hexyl)tin (MHTBT), di(*n*-butyl)di(*n*-hexyl)tin (DHDBT), used as external calibrants, had been synthesised in-house by C. Warriner and stored in the dark, refrigerated at 4 ºC.

3.2.2 Instrumentation

For the analysis of butyltin compounds a Varian 3800 gas chromatograph (Walnut Creek, CA, USA) was used that was equipped with a PFPD system and a Varian 1079 Programmable temperature vaporizing (PTV) injector. An auto injector unit was used to introduce the prepared solutions / samples. Splitless injection of 1 µL was performed, with a split delay of 1 min and a split ratio of 50. The GC separation was carried out using a non-polar capillary column (Sigma-Aldrich, SA-1 type, fused- silica 30m X 0.25mm, coated with the non-polar stationary phase polydimethyl-siloxane (PDMS, 0.25 µm film thickness)). Nitrogen was used as the carrier gas (flow: 1 ml min^{-1}).

The detector was fitted with a high transmission band filter (320-450 nm; GB 12) and operated at 250 °C. Gas flow rates were: Air_1 17.0 ml min^{-1}, Air_2 10.0 ml min^{-1} and H_2 13.5 ml min^{-1}. The flame ignition frequency was *ca.* 2.6 Hz. The detector gate settings were 3 ms delay and 4 ms width. The operating conditions used for the gas chromatographic determination of butyltin are summarized in Table 3.

Table 3 Operating conditions for the gas chromatographic measurement of butyltin compounds[*]

Carrier gas	Nitrogen (1 ml min-1)
Injector temperature programme	150 °C (1 min hold) to 250 °C at 40 °C/min
Column oven programme	50 °C (1 min hold) to 100 °C at 50 °C/min to 130 °C at 7°C/min to 270 °C at 20 °C/min, hold 15 min.
Gate delay	3 ms
Gate width	4 ms

[*] The column oven temperature programme of the PFPD was adapted from Leermakers *et al*[183], who optimized it for butyltin analysis.

3.2.3 Preparation of standard solutions

Standard stock solutions of individual hexylbutyltin compounds (500 µg Sn/ mL) in *n*-hexane were prepared by dissolving the compounds individually in *n*-hexane. There were kept in the dark, refrigerated at 4 ºC and used for six months. Working standard solutions (2 to 8 µg Sn/ L) were prepared daily by dilution of the stock solutions in *n*-hexane. A standard stock solution of tri(*n*-pentyl)tinchloride ,internal standard (IS), (500 µg Sn/ mL as TPTCl) was prepared in *n*-hexane. This was kept in the dark, refrigerated at 4 ºC.

3.2.4 Sampling and sample preparation

3.2.4.1 Reference material

To evaluate the accuracy of the analytical procedure for the speciation of butyltins in sediments, the certified reference materials PACS-2 was employed. This is a harbor sediment standard developed by the National Research Council of Canada. PACS-2 is certified for TBT and DBT contents, whilst for MBT, an indicative value is given.

3.2.4.2 Spiked sediment sample

An ´uncontaminated´ sediment sample was collected during low tide from the Beaulieu River in Beaulieu village, UK in October 2007. The sample was collected from a location expected to be relatively uncontaminated with tributyltin compounds. The sample was immediately frozen and freeze-dried. Before storing in glass containers in the dark at 4 ºC, large particles were removed by dry-sieving through a 1 mm mesh sieve.

3.2.4.3 Coastal sediment samples

To assess the applicability of the analytical procedure, twenty nine surface sediment samples (top 5 cm) were collected at low tide* from a boatyard on the River Itchen in Southampton, UK in April 2008 (Figure 11). Samples (1 to 6) were obtained at or near a slipway located within the boatyard. This area had been used for the repainting of boats and high TBT levels we expected in that region. Samples (7 to 15) were collected next to the pontoon, below moored boats. Samples (16 to 26) were collected away from the slipway. Samples 27, 28 and 29 were sandier sediments collected further away from the boatyard (approximately 0.5 km upstream away from the boatyard). The samples were immediately frozen and freeze-dried. Before storing in glass containers in the dark at 4 ^{o}C, large particles were removed by dry-sieving through a 1 mm mesh sieve.

*Fig. 11 Approximate location of sampling sites in a boatyard on the River Itchen in Southampton. Samples 27-29 were collected about 0.5 km upstream (location not shown on the map). Source of image: 'River Itchenin Southampton, UK'. 50° 55.523′N and 01° 22.43′W. Google Earth. April 19, 2007. * tide height during the sample collection was 0.8 meter.*

3.2.5 Analytical procedure for determination of TBT, DBT and MBT

The initial method is adapted from the procedure reported by Evgenia-Varvara Gkenakou [196] . It is the core method used, but some parameters were changed after optimization experiments described later in this chapter.

For the analysis of butyltin contaminated sediments, 0.5 g (dry weight) of sediment was accurately weighed in polypropylene centrifuge tubes. 2 mL of water and 2 mL of conc. HCl were added. 5 mL of 0.1% (w/v) tropolone in hexane was added and the mixture was shaken using a wrist action shaker at its highest speed for 1 hour. The mixture was centrifuged at 4000 xg for 10 min, the supernatant hexane layer was placed in a glass sample vial. Na_2SO_4 (anhyd.) was added to remove any water and the supernatant was transferred to a dry glass vial. 1 mL of 2.0 M *n*-hexylmagnesium bromide solution in

diethylether was added to the hexane solution and it was left to react. After 20 min, 2 mL of conc. HCl was added to de-activate the excess *n*-hexylmagnesium bromide and the PTFE-lined cap was screwed on the glass vial. The glass vial was shaken well to ensure complete deactivation of the reagent. After the separation of the layers the aqueous layer was discarded and the hexane layer was dried with Na_2SO_4 (anhyd.) and transferred to a 10 mL volumetric flask. The reaction glass vial was rinsed with 2.5 mL of hexane which was then added to the 10 mL volumetric flask. The solution volume was made up to 10 mL with hexane.

3.2.6 Quantification

The identification of organotin compounds was based on their retention times and quantification was based on peak height. Figure 12 shows a typical chromatogram obtained by GC-PFPD to identify and quantify TBT, DBT and MBT from the certified reference sediment PACS-2. At the beginning of the chromatogram, a negative baseline was observed. This is because the flame cannot ignite just after the injection due to the high solvent content of the detector and this caused the chromatographic signal to drop temporarily.

Quantitative analyses were performed using two different approaches: (a) employing external standards (ES) and (b) an internal standard method (IS). The results obtained from these two methods were compared. In the ES method, calibration curves were constructed daily from the analysis of hexylbutyltin compounds over the range 2 — 8 µg Sn /L. A tri(*n*-hexyl)mono(*n*-butyl)tin standard solution was prepared by hexylation of mono(*n*-butyl)tin trichloride with excess *n*-hexylmagnesium bromide, following the derivatization procedure described in Section 3.2.5. Linear calibration graphs were constructed by least-square regression of peak height versus concentration. Good linearity was found over the concentration range tested (2 — 8 µg Sn /L) (Figure 13). Above this concentration range the response deviated from linearity.

In the IS method, tri(*n*-pentyl)tinchloride TPTCl was used as the internal standard. TPTCl is suitable for use as an internal standard for quantification of other organotin species because of its absence from environmental samples and it does not interfere with the analysis of any of the alkyl tin compounds of interest[197]. A 2 µg Sn (as TPTCl) /L solution of was used as an internal standard to correct the concentration values of analytes for losses during manipulation, extraction inefficiency, incomplete conversion during derivatisation and evaporative losses. 2 µg Sn (as TPTCl) /L (IS) was added in with the butyltins standard solutions (2 — 8 µg Sn /L) and the solutions were analysed daily. Linear calibration graphs were constructed by least-squares regression of the peak height ratio (analyte/IS) vs. concentration (Figure 14). Good linearity was found over the tested concentration range. A blank was generated by using deionized water instead of sediment samples. 2 ml of deionized water was analysed for TBT, DBT and MBT following the analytical procedure described in Section 3.2.5 for actual sediment samples. Procedural blanks were performed for every set of samples.

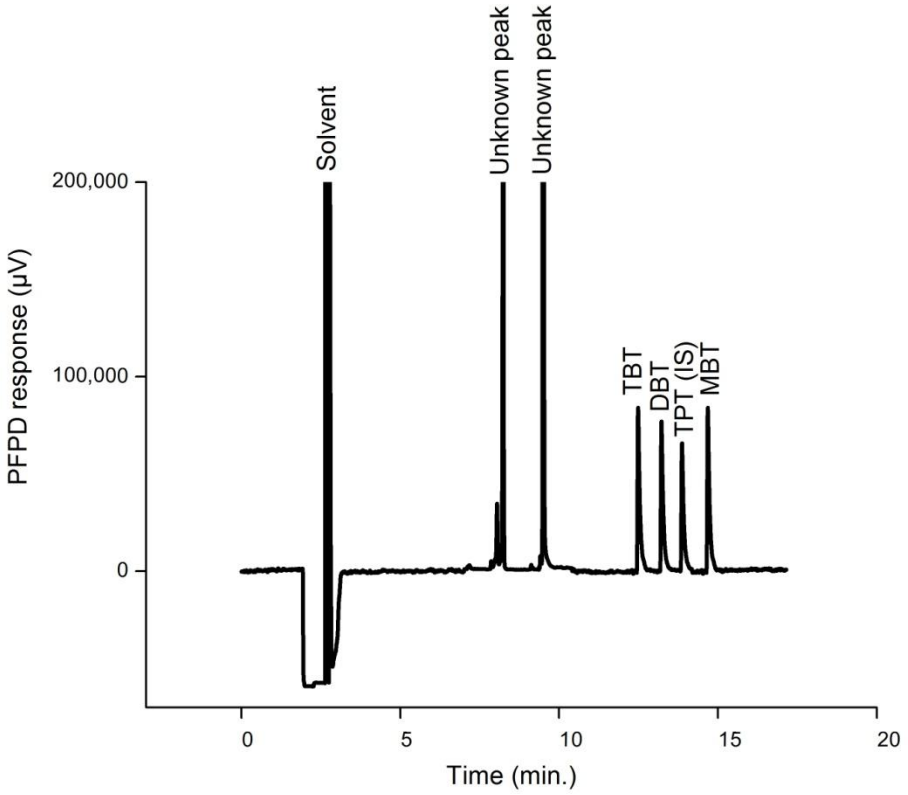


Fig. 12 Typical chromatogram obtained from the hexylated of butyltins derived from the certified reference sediment PACS-2. (Concentrations of TBT, DBT and MBT in the sediment were 0.91, 1.01and 0.88 ng Sn /g dry weight respectively).

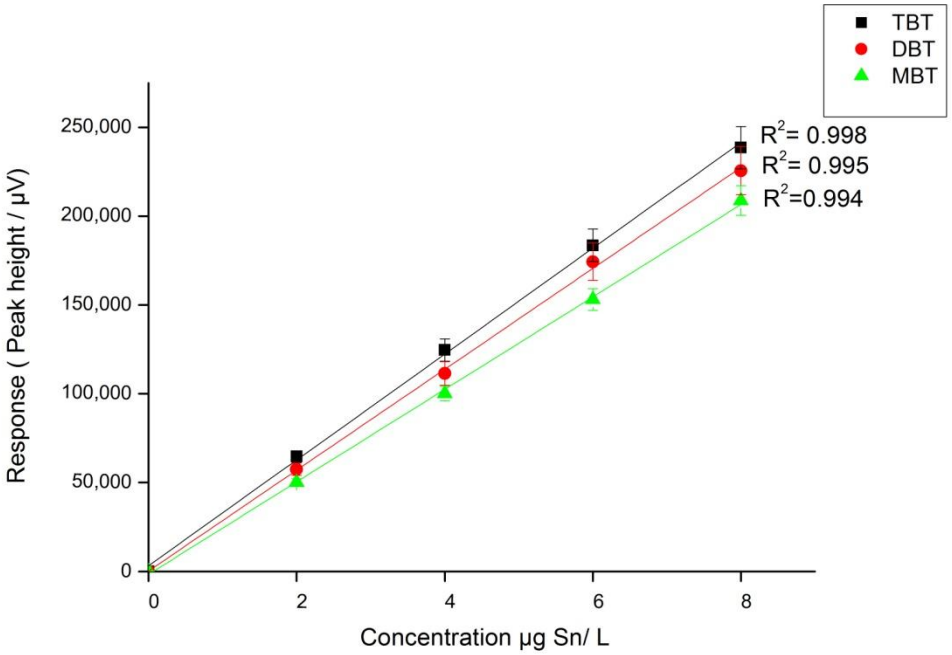


Fig. 13 Linearity of measurements established from peak height, covering the concentration range 2 to 8 µg Sn /L as each compound of TBT, DBT and MBT

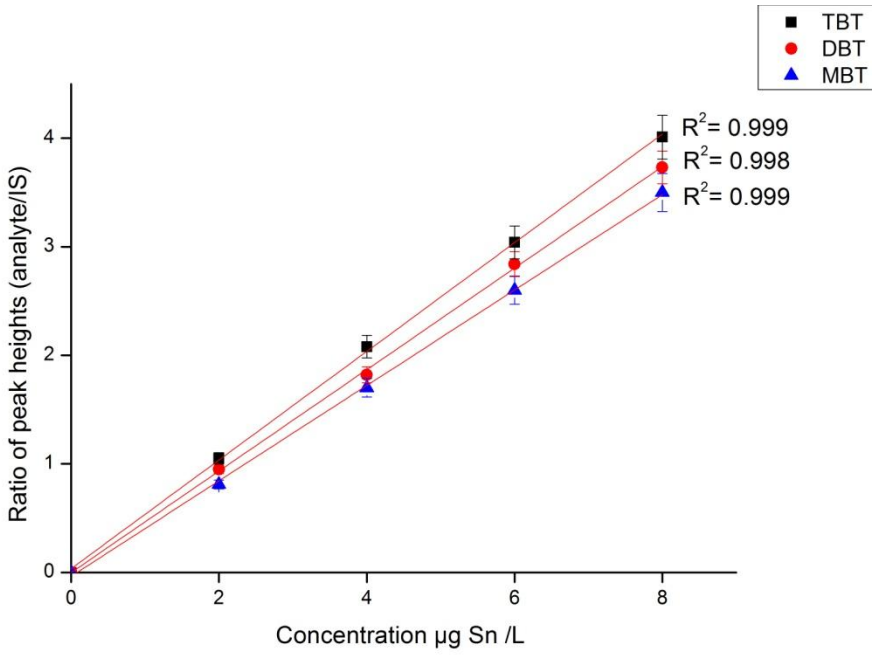


Fig. 14 Linearity of measurements established from the ratio of analyte and IS peak heights, covering the concentration range 2 to 8 µg Sn /L as each compound of TBT, DBT and MBT

3.2.7 Method development for the measurement of butyltins in Sediment

In order to be able to further develop the initially adapted method, optimization experiments have been performed. For most of the analytical steps more than one experiment was tested and the most suitable was adapted thereafter. In the following Sections these are presented.

3.2.7.1 Sequential extraction procedure

In order to assess the number of extraction sequences required to obtain an efficient extraction of TBT from sediment, three 0.5 g (dry weight) subsamples of the Southampton sediment sample (Samples 1) were accurately weighed in polypropylene centrifuge tubes. On each subsample, three sequential extractions were performed using the extraction procedure described in Section 3.2.5. After each extraction the extractant was separated from the solid residue by centrifugation at 2500 x g for 10 min, and derivatized, isolated and analysed following the procedure described in Section 3.2.5. The internal standard method (See Section 3.2.6) was used to calculate the results.

3.2.7.2 Effect of acid type

To evaluate the effect of acid type on butyltin extraction efficiency, hydrochloric (HCl) and acetic acids (HOAc) were selected and the following experiment was carried out. Two 0.5 g (dry weight) subsamples of the certified reference sediment PACS-2 were accurately weighed in polypropylene centrifuge tubes. The use of a reference material ensured that differences between results were not caused by poor homogeneity of the analysed sample. The first subsample was extracted as described in Section 3.2.5 (HOAc-hexane/tropolone approach). The second subsample was extracted using the same procedure except HCl was used instead of HOAc (HCl-hexane/tropolone approach). Each extraction was quantified using the internal standard method described in Section 3.2.6. The experiment was repeated three times for each extraction approach.

3.2.7.3 The addition of a complexing agent

To evaluate the effect of tropolone on the butyltin extraction efficiency, two 0.5 g (dry weight) subsamples of Beaulieu sediment were accurately weighed in polypropylene centrifuge tubes. 1 ml of a mixed standard solution of TBTCl, DBTCl and MBTCl (containing 0.1 µg Sn /mL as each compound) was added to each subsample and 1 ml of a 0.1 µg Sn (as TPTCl)/ mL solution was added as an internal standard. The first subsample was extracted according to the procedure described in Section 3.2.5. The second subsample was extracted following the same procedure except that the tropolone was not added. Each extraction was replicated three times and analysed following the procedure in Section 3.2.5.

3.2.7.4 Effect of Calcium Carbonate

The effect of calcium carbonate on the derivatization reaction was assessed. 0.5 g (dry weight) of ´uncontaminated´ Beaulieu sediment was accurately weighed, spiked and extracted through the extraction procedure as described in Section 3.2.5. Before derivatization, the extracted butyltin solution was divided into two equal portions. 0.5 g of $CaCO_3$ was added to the first portion while the other portion was derivatized directly. The extracts were analysed using the internal standard method. The experiment was replicated three times.

3.2.7.5 Amount of Grignard reagent

The appropriate amount of Grignard reagent required to achieve maximum derivatization efficiency was examined. 0.5 g of the certified reference sediment PACS-2 was extracted for TBT and DBT, according to the procedure described in Section 3.2.5. The extracted butyltin solution was divided into three equal portions. 0.2, 0.5 and 1 ml of Grignard reagent were added to the three portions. After 40 min, 2 ml of aqueous sulphuric acid 5% (v/v) was added to destroy the excess of Grignard reagent. The hexane layer was isolated and analysed following the procedure outlined in Section 3.2.5.

3.2.7.6 Derivatization reaction time

The time required to obtain quantitative derivatization of the butyltin chlorides was investigated. Six 0.5 g (dry weight) subsamples of the certified reference sediment PACS-2 were accurately weighed in polypropylene centrifuge tubes and were extracted according to the procedure described in Section 3.2.5. 0.5 ml of Grignard reagent was then added to each butyltin extract and the derivatization was allowed to proceed for different reaction times (10, 20, 30, 40, 50 and 60 min). At the end of each derivatization, 2 ml of aqueous sulphuric acid 5% (v/v) was added to destroy the excess Grignard reagent. After isolation the hexane layer was analysed for butyltins according to the procedure described in Section 3.2.5.

3.2.8 The Finalised procedure

After changes had been made to the initial analytical procedure (Section 3.2.5), the new analytical procedure was established as follows:

The experiment was carried out in two stages: Stage-1 involved the determination of butyltins in unspiked Beaulieu sediment to establish the concentrations of TBT, DBT and MBT in the sediment before spiking. Stage-2 involved analysis of the Beaulieu sediment after spiking with butyltin compounds. For the unspiked Beaulieu sediment analysis, 0.5 g (dry weight) of Beaulieu sediment was accurately weighed in polypropylene centrifuge tubes. 2 mL of deionised water and 2 mL of glacial acetic acid were added to the sediment mixture to promote the release of ionic butyltin species from sediment. 5 mL of a 0.1- 0.2% (w/v) solution of tropolone in hexane was added to the mixture; the tropolone was present to promote the solubility of the butyltins in the hexane. The mixture was then shaken using an wrist action shaker for 1 hour on high speed. The hexane layer was separated from the solid residue by centrifugation at 2500 x g for 10 min. The extraction was repeated three times on each sediment sample and the organic extracts were combined. The combined hexane layers were transferred to a clean glass sample vial. 0.5 g of sodium sulfate Na_2SO_4 (anhyd.) was added to the hexane layer to

remove water. This was followed by 0.5 g of calcium carbonate $CaCO_3$ to remove traces of acid. The dried hexane layer was removed from the Na_2SO_4 and $CaCO_3$. Under an atmosphere of nitrogen, 0.5 mL of 2.0 M *n*-hexylmagnesium bromide solution in diethylether was added to the dried hexane layer to convert the butyltins to their corresponding hexylbutyltins and the reaction was left to proceed for 40 min. 2 mL of aqueous sulphuric acid (5% v/v) was then added to destroy the excess hexylmagnesium bromide and the glass vial was shaken well. The hexane layer was isolated, dried with Na_2SO_4 (anhyd.) and then transferred to a 25 mL volumetric flask. After making up to 25 mL with hexane, 1 µL of the hexane solution was analysed by gas chromatography.

For the analysis of the spiked Beaulieu sediment, 0.5 g (dry weight) portions of Beaulieu sediment were accurately weighed in polypropylene centrifuge tubes. 1 ml of a mixed standard solution of TBTCl, DBTCl and MBTCl (containing 0.1 µg Sn /mL as each compound) was added into the sediment (corresponding to 0.2 µg/g of TBT, DBT and MBT in the sediment). 1 ml of a 0.1 µg Sn (as TPTCl)/ mL solution was added as an internal standard into the sediment mixture. The sediment mixture was then extracted, derivatized, isolated and detected using the analytical procedure described above.

3.2.8.1 Extraction recoveries

To investigate the recovery of TBT, DBT and MBT obtained for the new procedure described in Section 3.2.8, an experiment was carried out in two stages. Stage-1 involved the analysis of unspiked ´uncontaminated´ Beaulieu sediment for TBT, DBT and MBT, as described in Section 3.2.8. In Stage-2, six 0.5 g (dry weight) subsamples of the ´uncontaminated´ Beaulieu sediment were accurately weighed in polypropylene centrifuge tubes. 1 ml of a mixed standard solution of TBTCl, DBTCl and MBTCl (containing 0.1 µg Sn /mL as each compound) was added to each sediment subsample. 1 ml of a 0.1 µg Sn (as TPTCl)/ mL solution was added as an internal standard into the sediment mixtures. The mixtures were then analysed for TBT, DBT and MBT according

to the procedure described in Section 3.2.8. The results were calculated using the internal standard method.

3.3 Comparison of the external and internal standard methods of quantification

3.3.1 Introduction

Quantitation in environmental analysis is mainly performed by two methods, the external standard (ES) and the internal standard (IS) methods. In the ES method, the peak height or area from the analyte is compared with the peak height or area from a series of analyte standard solutions. In the IS method, a compound (the internal standard) which is not present in the sample, is added in known proportion to both the sample and the standard solutions. The ratios of the peak heights or areas from the standard and IS are calculated. In the present study, ES and IS methods for the quantitative determination of TBT, DBT and MBT in sediment samples were compared for: (1) linearity of the butyltin calibration curves and (2) for their precisions as assessed by examining repeatability and reproducibility of the detector response using the two methods.

3.3.2 Linearity of the ES and IS method

Linearities of the calibration curves obtained by the ES and IS methods were assessed according to the procedure described in Section 3.2.6

3.3.3 Repeatability and reproducibility of the ES and IS methods

Repeatability and reproducibility of the external and internal standard methods was assessed. In the case of the ES method, six 0.5 g subsamples (dry weight) of ´uncontaminated´ Beaulieu sediment were accurately weighed in polypropylene centrifuge tubes. 1 ml of a mixed standard solution of TBTCl, DBTCl and MBTCl (containing 0.1 µg Sn /mL as each compound) was added to each sediment subsample.

The mixture was extracted, derivatized and analysed according to the procedure outlined in Section 3.2.5. The results obtained from this experiment were quantified using the external standard method described in Section 3.2.6.

In the case of the IS method, six 0.5 g subsamples (dry weight) of ´uncontaminated´ Beaulieu sediment were accurately weighed in polypropylene centrifuge tubes. 1 ml of a mixed standard solution of TBTCl, DBTCl and MBTCl (containing 0.1 µg Sn /mL as each compound) was added to each sediment subsample. 1 ml of a 0.1 µg Sn (as TPTCl)/ mL solution was added as an internal standard into the sediment mixture. The mixture was extracted, derivatized and analysed according to the procedure outlined in Section 3.2.5. The results obtained from this experiment were quantified using the internal standard method described in Section 3.2.6.

3.4 Determination of total tin in sediment

3.4.1 Introduction

Tin and its compounds are introduced into the environment naturally, as well as from a variety of human activities. Whilst tin in sediments could be associated with TBT-releasing paints, tin can also occur in variety of other forms. It can occur as a constituent of bedrock, or leached from other materials such as the alloy bronze[2]. The toxicities of inorganic and organic tin compounds differ greatly [40]. The determination of inorganic tin in environmental samples will provide insights into the natural behaviour of tin in the environment. Moreover, determination of total tin and its species allows the investigation of biogeochemical changes of tin and the relationships of tin with butyltin compounds in the environment. Therefore, the analysis of total tin was planned to assess whether, in very specific areas having a low natural tin background, total tin measurements might be used to predict the distribution of organotin contamination.

3.4.2 Instrumentation

For the measurement of total tin a Philips MagiXPro fully automatic, sequential, wavelength dispersive X ray fluorescence spectrometer (XRF) was used. The system includes an automatic 144 place sample changer. For generating the probe X-ray, a Rhodium (Rh) anode is used in the X-ray tube, which can be operated at up to 60 kV and a current up to 125 mA, at a maximum power level of 4 kW. The Philips MagiXPro is equipped with three detectors (Ar-Methane flow and a sealed Xe-filled proportional detector in tandem and a scintillation detector in parallel) and a range of beam filters. The system software is Philips SuperQ v4.0 which is used for data collection, calibration and analysis

3.4.3 Sample preparation

The twenty nine surface sediment samples (See Section 3.2.4) were analysed for total tin by XRF. XRF analysis and the elements data was carried out in the National Oceangraphy Center, Southampton University by Professor Ian Croudace. The preparation of the samples involved freeze-drying, grinding and sieving through a 0.5 mm mesh sieve to form a powder. The XRF requires a flat sample surface hence the ground sediment samples were placed in an aluminum die (40 mm in diameter) and pressed (25 tonne) using a HERZOG HP40 hydraulic press to produce a pellet. Samples were analysed using a calibration set based on a number of international reference samples. Accuracy and precision are nominally between 1% and 5% (2 sigma).

3.5 Results and Discussion

Three important experimental parameters have been investigated: a) – PFPD parameters b) - extraction and c) - derivatization.

The investigation of the PFPD parameters has been carried out for two reasons: (1) contamination of the instrument during the instrumental analysis is a major source of error especially in trace analysis leading the analyte to be overestimated (2) repeatability is a critical parameter that is often determined by the stability of an instrument; poor instrumental repeatability leads to low measurement precision.

Evaluation of the extraction variables is particularly important due to concern over the ability of current extraction procedures to quantitavely extract butyltin compounds from within components of the sediment and this is leads directly to an underestimation of the true concentration.

Investigation of the derivatisation variables is important since low derivatization yields and losses of analytes can easily occur at this stage, again leading to an underestimation of analyte content in the samples.

3.5.1 Instrumentation

3.5.1.1 Instrument blank

To assess possible cross contamination between sample injections, pure *n*-hexane was injected, under the same instrumental conditions (See Section 3.2.2), before and between standards or samples. No tin derived peaks were observed, showing the instrument to be clear of ghosting effects resulting from analyte retention in the system.

3.5.1.2 Repeatability of the instrument

Repeatability of the instrument for the butyltin compounds (TBT and DBT) was checked by eight replicate injections of the same standard solution (4 µg Sn (as MHTBT and DHDBT) /L). The RSD values for the MHTBT and DHDBT peak heights were 5% and 6% respectively.

3.5.2 Extraction

The most important extraction variables that have been investigated are: (a)- the number of extractions to be applied to each sample (b)- the effect of acid type and (c)- the addition of the complexing agent tropolone to enhance recoveries.

3.5.2.1 Repeated extraction of samples

The number of extractions required to extract TBT from sediment samples efficiently has been assessed using the procedure described in Section 3.2.7.1. There was a significant amount of TBT remaining in the sediment after the first extraction (Table 4). The incomplete extraction was attributed to the presence of slow-release TBT paint flakes in the sediment, leading to poor extraction of the TBT from the sediment at the first extraction. By repeating the extraction, more TBT was released from the paint flakes in the sediment. For all sub samples, a fourth extraction was performed and it was analysed individually in order to confirm that no more TBT can be extracted from the sediment subsamples. No significant amount of TBT could be extracted from the sediment in the fourth extraction indicating that three sequential extractions had released the available TBT from the sediment efficiently. In summary, the results showed that there is an easily extractable phase at the start and much difficult later.

Table 4 TBT concentrations measured after the 1st, 2nd, and 3rd extraction of the Southampton coastal sediment (Sample-1).

	1st extraction	2nd extraction	3rd extraction	Calculated Total
Average extracted amount (µg Sn /g)[a]	4.02	1.9	0.78	6.70
% RSD	5	7	11	14[b]
% extracted[c]	60%	25%	15%	

[a] Single values are mean concentrations (on a dry weight basis) of three subsamples.

[b] Combining experimental errors = $\sqrt{(\%\, e_1)^2 + (\%\, e_2)^2 + (\%\, e_3)^2}$.

[c] Based on total quantity recovered by 3 extraction.

3.5.2.2 Effect of acid type

The effect of acid type on the extraction efficiency has been investigated by analyzing the certified reference sediment PACS-2 for TBT and DBT by the experiment described in Section 3.2.7.2. Hydrochloric and acetic acids (HOAc) were selected since they can displace the different to counterions occurring in the sediment, forming either chlorides or acetates. Two different extraction approaches were performed: a)- HCl-hexane/tropolone, b)- HOAc- hexane/tropolone. The comparison of the two acids indicated that acetic acid yielded better recoveries (93% ± 2%. and 96% ± 1%) for TBT and DBT respectively than hydrochloric acid, which also was confirmed by *t*-test at 95% confidence) (Figure 15). This result is attributed to the highly acidic conditions in hydrochloric acid resulting in protonation of the tropolone (pKa= 7)[198] diminishing its complexing ability.

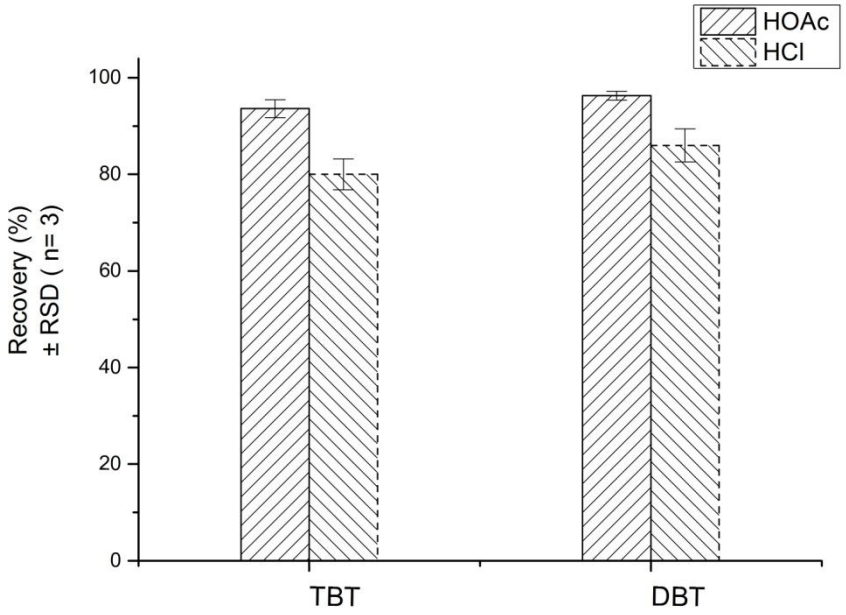
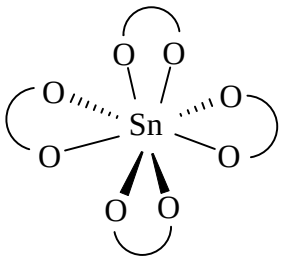


Fig. 15 Effect of acid type on the extraction efficiency of butyltins. Mean values and Standard Deviation RSD (n = 3) are represented

3.5.2.3 Effect of a complexing agent

Complexing agents are often added in the extraction step to promote the extraction of organotin species into low polarity solvents such as hexane. One of the most common complexing agents used is tropolone (2-hydroxy- 2, 4, 6-cycloheptatrienone). The effect of this complexing agent on the extraction efficiency was assessed using the experiment outlined in Section 3.2.7.3. The results of two parallel procedures are presented in Figure 16. For TBT no significant difference was evident irrespective of whether the tropolone was present or not (confirmed by t-test at 95% confidence). The recoveries of DBT and MBT however were improved by the addition of tropolone. These results can be explained by the chelation by tropolone of the butyltins. In the complex of tropolone with Sn(IV), four tropolonato ligands are equivalently coordinated to the Sn atom forming an 8-coordinate complex. The tin atom is coordinated by the eight oxygen atoms of four tropolone ligands[199].



As the degree of alkylation increases there are less metal coordination positions available to coordinate with the tropolone ligands. Steric constraints may therefore limit the Bt_3Sn-tropolone complex formation but the intrinsic hydrophobicity of the TBT acetate may enhance its partition into the extractant when composed to the less alkylated compounds. The less alkylated tin compounds on the other hand might be expected to exhibit poorer partition characteristic in the absence of tropolone, but as they readily complex with tropolone the extraction efficiency of MBT and DBT would be expected to be enhanced by the addition of the complexant.

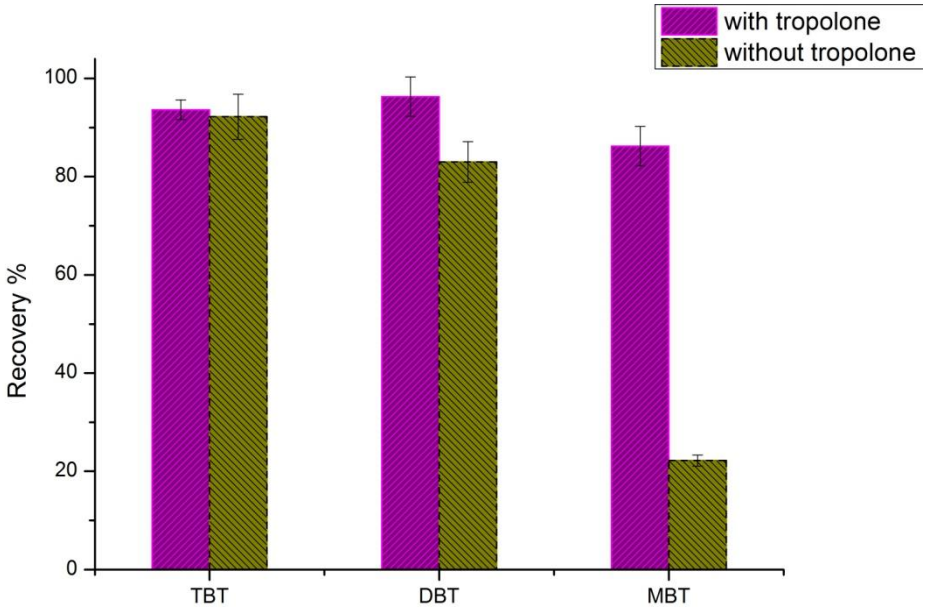


Fig. 16 Comparison of TBT, DBT and MBT recoveries using: (a) extraction with tropolone (b) extraction without tropolone. Mean values and RSD (n = 3) are represented

3.5.2.4 Extraction recoveries

Analysis of the unspiked ´uncontaminated´ Beaulieu sediment showed the sample to contain less than the detection limits (0.005 µg Sn (as TBT)/g, 0.007 µg Sn (as DBT)/g and 0.01 µg Sn (as MBT)/g. On spiking to a level of 0.2 µg Sn/g of butyltin compounds recoveries of 93% ± 2%, 96% ± 1% and 87% ± 2% were obtained for TBT, DBT and MBT respectively (experiment described in Section 3.2.8.1).

3.5.3 Derivatization

The most important derivatization variables that have been investigated are: (a) removing traces of residual acid using calcium carbonate prior to the addition of the Grignard reagent (b) - the effect of the amount of Grignard reagent and (c) – the effect of the derivatization reaction time.

3.5.3.1 The addition of calcium carbonate

The effect of calcium carbonate ($CaCO_3$) on the derivatisation reaction was examined (See Section 3.2.7.4). This step is employed to remove traces of acid before the addition of the acid/water sensitive Grignard reagent. The use of $CaCO_3$ increased the recoveries of TBT, DBT and MBT significantly, confirmed by *t*-test at 95% confidence (Table 5).

Table 5 Recoveries of butyltin compounds with and without $CaCO_3$. (Mean values and RSD (n= 3) are represented).

analyte	with $CaCO_3$	without
TBT	93% ± 2%	88% ± 3%
DBT	96% ± 1%	91% ± 2%
MBT	87% ± 2%	82% ± 3%

3.5.3.2 Effect of the amount of Grignard reagent added

Altering the quantity of Grignard reagent (0.2, 0.5 and 1 ml) showed lower yields when 0.2 ml of the reagent was employed, but no significant improvement was obtained using more than 0.5 ml (Table 6). 0.5 ml was therefore used for subsequent work (experiment described in Section 3.2.7.5).

Table 6 Recoveries of butyltin compounds obtained with different amounts of Grignard reagent (Mean values and RSD (n= 3) are represented).

	Amount of Grignard reagent		
	0.2 ml	0.5 ml	1 ml
TBT	89.3% ± 2.3%	92.6 % ± 1.8%	92.7% ± 1.5%
DBT	90.7% ± 1.1%	96.1% ± 1.3%	95.3% ± 1.6%
MBT	79.7% ± 3.2%	84.5% ± 2.1%	84.9% ± 1.9%

3.5.3.3 Derivatization reaction time

The time required to achieve quantitative derivatization of butyltin chlorides was investigated (Section 3.2.7.6). Maximum recovery was achieved within 40 min and no further improvement was observed after that time (Figure 17).

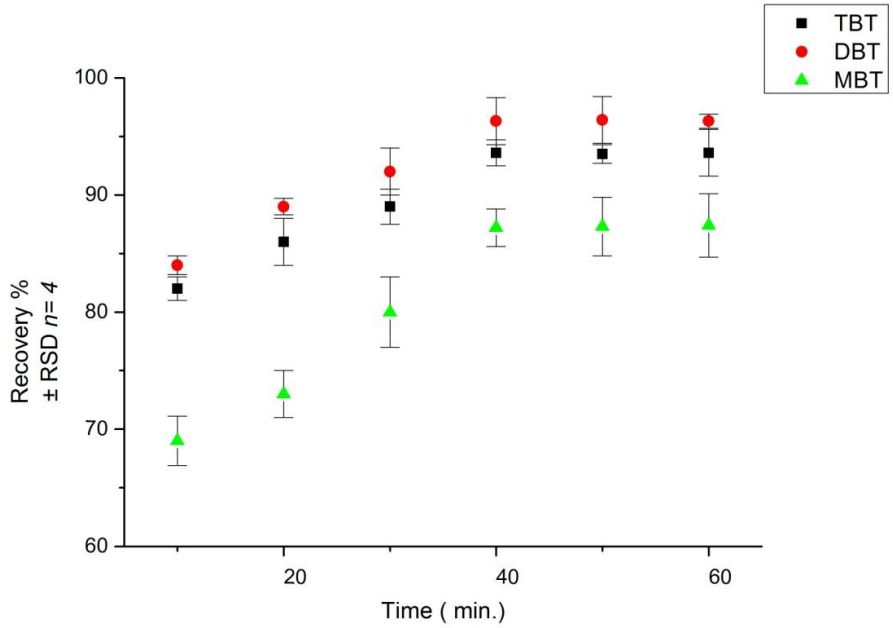


Fig. 17 Recoveries of butyltins achieved with different derivatization times

3.5.4 Performance data

3.5.4.1 Limit of detection (LOD)

The limit of detection was assessed as the lowest concentration of analyts that could be reliably distinguished from the background noise with 95% confidence. This was found by identifying the concentration that yielded a signal three times the standard deviation of the base line noise from a blank. A blank was measured four times per day over 4 days, yielding an average noise of 300 ± 120 µV. However, the concentration detection limit depends on the sample size, the concentration of the extract, and the amount injected into the GC. In the present study, 0.5 g of Beaulieu sediment was extracted, and the extract was diluted to 25 mL then 1 µL was analysed. The detection limits obtained for the three different species were: for TBT 5 µg Sn/ kg, DBT 7 µg Sn/ kg and MBT 11 µg Sn/ kg. These detection limits are comparable with other similar analytical techniques[178, 200], and more than adequate for the UK environmental quality target, set at 1-2 mg Sn / kg[201].

3.5.4.2 Comparison of the external and internal standard methods

The two methods of quantification (ES and IS) were compared for: (a) the linearities of the butyltin calibration curves and (b) the precisions of the two methods. Precision was assessed by examining repeatability and reproducibility of the detector response by the external and internal methods. The linearities of both the ES and IS methods were assessed using the procedure outlined in Section 3.2.6, by measurement of the correlation coefficients (R) values. Good linearity was found for both methods at the concentrations range tested (2 — 8 µg Sn /L), with coefficients greater than 0.98 being obtained for all butyltin compounds (Table 7). Slightly better results were however achieved using the internal standard. This is because the specific responses of the detector to the analyte relative to that of the internal standard remain constant even if the detector sensitivity varies from run to run. As importantly, improved correlation will occur as a result of the IS method compensating for variability in injected sample volume. Repeatability was assessed as the variability of the measurements obtained by the analysis of six spiked subsamples of ´uncontaminated´ Beaulieu sediment consecutively under the same analytical conditions.

Reproducibility was assessed as the variability of the measurements obtained by the analysis of six spiked subsamples of ´uncontaminated´ Beaulieu sediment on three different days. The repeatability and reproducibility of the two quantification methods were compared (as described in Section 3.3.2). In the measurements of butyltins in spiked Beaulieu sediment samples relative standard deviation (RSD) better than 5% was obtained for all butyltin compounds using the external calibration method and better than 3% repeatability was obtained with the internal standard calibration method. In terms of reproducibility, values better than 15% were obtained for all compounds using external standards. In contrast, values better than 8% were obtained with the use of an internal standard (Table 8). These results indicate that the IS method is more precise than the ES method (confirmed by an F-test). This is because the mass of internal standard relative to that of the analyte remains constant during the procedure; the same fraction of internal standard and analyte being lost during any step of the procedure.

Table 7 Correlation coefficients of calibration curves obtained by the use of external and internal standard methods at concentrations range (2 — 8 µg Sn /L).

Analyte	Internal standard	External standard
TBT	0.999	0.998
DBT	0.998	0.995
MBT	0.999	0.994

Table 8 Repeatability and reproducibility of butyltin measurement of spiked Beaulieu sediment containing 0.2 µg Sn /g as TBT, DBT and MBT.

	External standard		Internal standard	
	Repeatability (%)	Reproducibility (%)	Repeatability (%)	Reproducibility (%)
TBT	2.2	8	1.2	4.0
DBT	3.1	11	1.5	5.1
MBT	4.9	14	2	7.1

3.5.5 Validation of the new method

To confirm that the new method, described in 3.2.8 is suitable for its intended use, it was validated by analyzing the sediment certified reference material PACS-2. Six 0.5 g (dry weight) subsamples of PACS-2 were analysed using the procedure described in Section 3.2.8 with quantification using the internal standard method (as described in 3.2.6). The concentrations of TBT and DBT in the certified reference material recovered during the analysis were very close to the certified values. A comparison of the experimental mean value with the certified value was carried out using the Student's *t* equation (1.3):

$$t_{calculated} = |(\bar{x} - \mu)|\sqrt{n/s} \quad (1.3)$$

Where $\bar{x}$ = sample mean, μ = certified value s = sample standard deviation and n = number of replicates. Equation (1.3) is written with absolute value signs so that $t_{calculated}$ is always positive.

The calculated *t*-values for the analytes were = 0.67 and 0.89 for TBT and DBT respectively. Since the calculated *t* values were less than the tabled *t* value (critical) at *n*=6 and 95% level of confidence, set at 2.57, no significant differences were present between the obtained and certified values (Table 9).

Table 9 Statistical evaluation of the differences between the measured and certified values of butyltins in PACS-2 (n=6).

Compound	Obtained value	Certified value	Calculated t	Tabled t
	(ng (as Sn)/ g dry weight ± S.D.)			(95% confidence)
TBT	0.84 ± 0.21	0.89 ± 0.11	0.67	2.57
DBT	1.01 ± 0.01	1.05 ± 0.05	0.89	2.57

3.5.6 Analysis of Southampton coastal sediments

To assess the applicability of the new method, it has been used to determine TBT, DBT and MBT in Southampton coastal sediment samples.

3.5.6.1 Butyltin species in Southampton coastal sediments

The TBT, DBT and MBT concentration in the Southampton coastal sediments were determined by the new method, described in Section 3.2.8. All obtained values are expressed as the mean of three injections of each extract ± standard deviation. Table 10 shows the concentrations of TBT, DBT and MBT in Southhampton coastal sediments. TBT concentrations ranged from 0.27 to 6.70 µg Sn/ g with an average concentration of 1.65 ± 1.1 µg Sn/ g, the DBT from 0.1 to 1.02 µg Sn/ g with an average concentration of

0.28 ± 1.70 µg Sn/ g and the MBT ranged from 0.3 to 0.5 µg Sn/ g with an average concentration of 0.4 ± 0.1 µg Sn/ g. The average level of TBT in these coastal sediments is approximatly equal to the UK environmental quality standard (EQS) value for TBT in sediments, set at 1-2 mg Sn/ kg[201].

High values of TBT compared with the EQS value were found in the sediment near the slipway located within the boatyards (sampling points 1 to 6). This area had been used for the maintenance of boats and high TBT levels are therfore expected in that region. However, samples 15 and 16 were collected from points about 0.1 km upstream from the slipway, yet high levels of TBT were still found (3.2 and 1.4 µg Sn/ g respectively). These values can be explained by tidal transport of resuspended sediments from a highly contaminated areas to other areas.

The distribution of TBT concentrations in Southampton coastal sediments shows three different zones of TBT contamination within the studied area. The first zone is the heavily polluted slipway area (sampling points 1 to 7) having an average TBT concentration 4.04 ± 2.3 µg Sn/ g. The second zone, the pontoons and the area near them (sampling points 8 to 26) is moderately polluted with average TBT concentration 0.97 ± 0.7 µg Sn/ g. The third zone (sampling points 27 to 29 and about 0.5 km upstream from the boatyard) is less contaminated with an average TBT concentration 0.41 ± 0.5 µg Sn/ g (Figure 18). The TBT hot spot identified in the slipway area had an average TBT concentration was twice than the UK environmental quality target. It is believed that this TBT hot spot are created by the historical use of organotin compounds and the tidal transport of the effected sediment. The results obtained shows that historic TBT contamination is still present in the studied area due to the stripping of old paint from boat hulls and repainting using paint-based organotin compounds. This lead to the direct deposition of TBT as TBT-containing paint particles leading to the long residence time of TBT in this area. Therefore, remedial treatment is needed in this area to reduce the source of butyltins into the estuarine sediments and water.

Table 10 TBT, DBT, and MBT concentration (µg Sn/g) in the Southampton coastal sediments (based on dry weight of sediment)

Sampling points[b]	Butyltins (µg Sn/g ± S.D.,*n*=3[a]) TBT	DBT	MBT	Sampling Points[b]	Butyltins (µg Sn/g ± S.D.,*n*=3[a]) TBT	DBT	MBT
1	6.70 ± 1.1	0.85 ± 0.32	0.3 ± 0.1	16	1.41± 0.7	0.10 ± 0.04	< DL
2	4.50 ± 1.0	1.02 ± 0.54	< DL[c]	17	0.74 ± 0.3	0.18 ± 0.1	< DL
3	5.20 ± 0.7	0.60 ± 0.2	0.5 ± 0.1	18	0.71 ± 0.1	0.15 ± 0.1	< DL
4	0.42 ± 0.1	0.55 ± 0.1	< DL	19	0.78 ± 0.03	0.18 ± 0.04	< DL
5	2.40 ± 0.6	0.28 ± 0.2	< DL	20	0.52 ± 0.2	0.27 ± 0.2	< DL
6	5.00 ± 0.3	0.43 ± 0.7	< DL	21	0.84 ± 0.1	0.19 ± 0.7	< DL
7	3.50 ± 0.5	0.21 ± 0.1	< DL	22	0.76 ± 0.2	0.15 ± 0.2	< DL
8	0.88 ± 0.2	0.20 ± 0.2	< DL	23	0.65 ± 0.1	0.11 ± 0.05	< DL
9	0.65 ± 0.2	0.28 ± 0.1	< DL	24	0.52 ± 0.2	0.28 ± 0.05	< DL
10	0.73 ± 0.1	0.24 ± 0.4	< DL	25	0.59 ± 0.2	0.18 ± 0.1	< DL
11	0.29 ± 0.1	0.21 ± 0.1	< DL	26	0.27 ± 0.2	0.15 ± 0.1	< DL
12	0.53 ± 0.2	0.15 ± 0.1	< DL	27	0.43 ± 0.05	< DL	< DL
13	0.88 ± 0.2	0.10 ± 0.04	< DL	28	0.47 ± 0.1	0.14 ± 0.2	< DL
14	1.00 ± 0.2	0.72 ± 0.2	< DL	29	0.32 ± 0.1	0.13 ± 0.3	< DL
15	3.20 ± 0.8	0.17 ± 0.04	< DL				

[a] Three subsamples.

[b] Sampling points are shown on the map Fig. 11.

[c] Values are below the detection limit of the developed method (5 µg Sn (as TBT)/ kg, 7 µg Sn (as DBT)/ kg and 11 µg Sn (as MBT)/ kg).

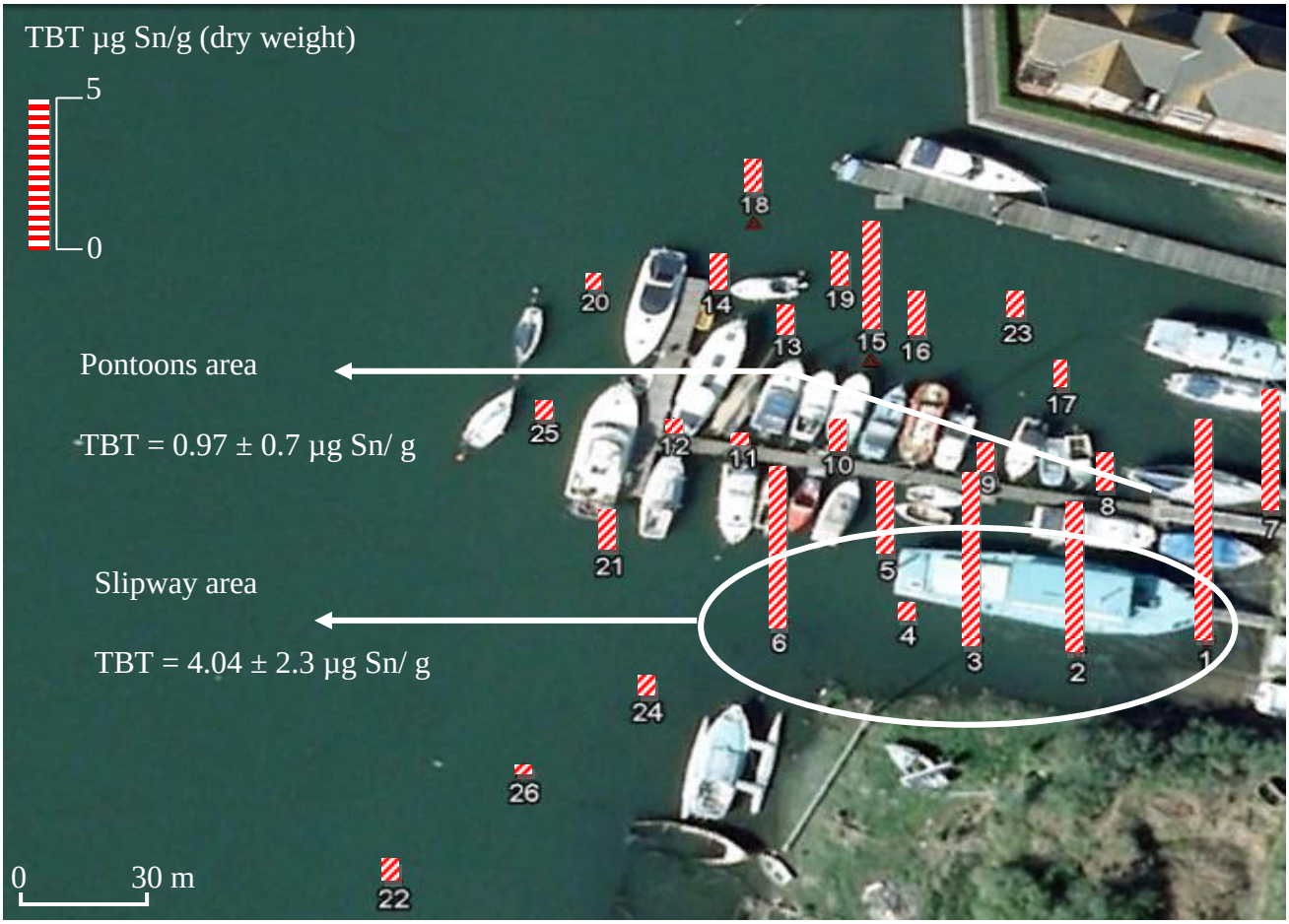


Fig. 18 Concentrations of TBT in the Southampton coastal sediments (sampling points 1-26). Samples 27-29 were collected about 0.5 km upstream (location not shown on the map). The heights of the red and white bars at each sample location indicates the TBT level

3.5.6.2 Total tin in Southampton coastal sediment

Environmental levels of total tin depend both on natural sources and anthropogenic inputs. Natural sources include inorganic tin that occurs in the Earth's crust. However, higher levels can occur in areas of high tin deposits. Total tin was measured in the Southampton coastal sediments (See Figure 11) using XRF as described in Section 3.4.2. Total tin concentrations ranged from 26 to 208 mg/kg with an average concentration of 53.8 mg /kg. Total tin concentrations as butyltin species (TBT + DBT + MBT) in the Southampton coastal sediments ranged from 0.32 to 7.60 µg Sn/ g dry weight. Table 11 shows the total tin and butyltin species concentrations (µg Sn/ g dry samples) in the Southampton coastal sediments and the percentage of the total tin present as butyltin species (∑BTs). The highest percentage was 9.70 % at sampling point 1. In order to understand if high Sn levels indicate TBT contamination, it is important to investigate the TBT and total Sn levels at each sampling point individually. From the 29 samples tested here, only 11 had a Sn concentration > 40 mg/kg. Six of those samples (sampling points 1 to 6) also had a high TBT content (> 1.0 mg Sn/kg) . High levels of tin are to be expected in the slipway location, particularly if organotin compounds persist there for substantial periods of time. From the nine highly TBT-contaminated samples (concentration >1.0 mg Sn/kg), six are from areas that are Sn-contaminated as well. In five other highly Sn-contaminated areas (Samples 24, 25, 27, 28 and 29) the TBT levels are below 1 mg/kg. In Samples 5, 14 and 16 high TBT levels are observed but the Sn levels are low. Other aspects for comparison involve the changing of Sn and TBT concentrations with the distance from the slipway and boatyard areas. While, TBT concentrations generally decreased with the increased distance from the slipway high Sn concentrations appear to occur randomly. These results suggest that other factors, such as the presence of tin from mineral particles or from sources other than anti-fouling paints or as natural component of the sediment can cloud the interpretation of total tin data. No clear overall correlation between total tin levels and TBT concentrations was observed but upon inspection of Figure 19, it is possible to identify four clusters. The first cluster (Set A) involves samples have high TBT and Sn concentrations, located generally in the

slipway area. The second cluster (Set B) samples have high TBT concentrations while the Sn concentrations are low. The third cluster (Set C) samples have low TBT and Sn concentrations and the fourth cluster (Set D), samples with high concentrations of Sn and low TBT concentrations. As there is no clear correlation between TBT and Sn contents in this area, it is believed that Sn hot spot is created due to different sources: natural sources and leached from other materials e.g. (bronze alloy where Sn is also used). Therefore, total tin measurements in this area did not provide some insight into the distribution of organotin contamination.

Table 11 Percentage of the total concentrations of butyltin species (BTs) respected to total tin in the studied sediment

Sampling points	Total Sn	∑BTs*	∑BTs/Sn (%)**	Sampling points	Total Sn	∑BTs*	∑BTs/Sn (%)**
1	78	7.60	9.70	16	29	1.52	5.11
2	190	5.51	2.91	17	29	0.82	2.81
3	208	5.82	2.82	18	26	0.86	3.30
4	29	0.97	3.32	19	28	0.86	3.14
5	38	2.70	7.14	20	28	0.59	2.12
6	105	5.41	5.21	21	27	0.93	3.41
7	62	3.60	5.82	22	35	0.91	2.61
8	33	0.97	2.90	23	27	0.76	2.80
9	32	0.73	2.32	24	43	0.60	1.42
10	32	0.77	2.40	25	41	0.67	1.63
11	39	0.37	0.95	26	28	0.32	1.14
12	30	0.68	2.31	27	67	0.63	0.94
13	28	0.98	3.52	28	71	0.51	0.72
14	28	1.72	6.13	29	94	0.56	0.59
15	57	3.30	5.70				

* ∑BTs : tributyltin + dibutyltin + monobuyltin (mg Sn/ Kg).

** Percentage of BTs respect to total Sn in the studied sediment = ∑BTs/Sn x 100.

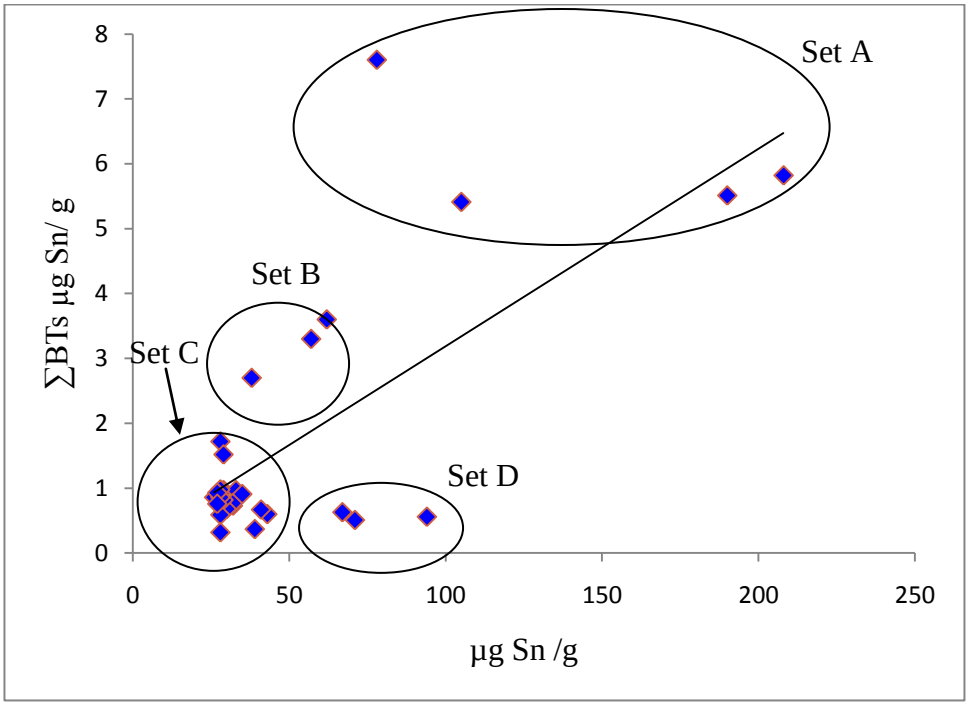


Fig. 19 The correlation between total Sn and butyltins concentrations in the Southampton coastal sediment

3.6 Conclusions

An analytical procedure for the determination of butyltin compounds (TBT, DBT and MBT) in sediment has been developed by investigating extraction variables (acid type and the presence of complexing agent) and the most important derivatization variables (amount of Grignard reagent and derivatization reaction time). Acetic acid-hexane/tropolone based extraction was found to give better recoveries of all butyltin compounds than HCl-hexane/tropolone. External and internal standard methods of TBT quantitatiation were compared, evaluating the precisions and linearities of the two methods. Precision was assessed by examining the repeatabilities and reproducibilities of the two methods. Better linearity and precision were observed with the IS method. Quantitative recoveries were obtained of target analytes from the spiked sediment using the developed method. The detection limits achieved in the present study were 5 µg Sn/ kg, 7 µg Sn/ kg and 11 µg Sn/ kg for TBT, DBT and MBT respectively. This limit is below the UK environmental quality standard (EQS) value for TBT in sediments, set at 1-2 mg Sn/ kg[201]. Using this method it would be therfore possible to establish if the UK environmental quality target (EQS) is met or not.

The method developed was validated using the sediment certified reference material PACS-2. All the results obtained fall within the certified value.

The method developed was then applied to the determination of TBT, DBT and MBT in sediment collected from a boatyard on the River Itchen in Southampton. The average concentration of TBT in the sediments was approximatly equal to the UK environmental quality target (EQS) value for TBT in sediments. However, near the slipway area TBT levels were twice the UK EQS; levels that can be attributed to the historical use of organotin compounds, mainly relating to boat-repairing in this area.

In the third part of this study, total tin contents of Southampton coastal sediment were measured by X-ray fluorescence spectrometry. Total tin concentrations in the studied area are variable from one site to another and show no particular trend. The correlation between TBT and Sn contents in this area was investigated and no clear correlation of

TBT with Sn was observed. It is believed that Sn hot spots in this area are created due to different sources: naturally and leached from other materials such as bronze alloy rather than derived from antifouling-TBT paints. Therefore, XRF analysis of Sn alone does not appear to accurately predict the areas where highly TBT-contaminated sediment is found. Further work is necessary to explore relationships with other elements such as, Cu, Zn, Pb, Ni and Cr, which are easily measured using XRF, and to use multivariate statistical analysis to determine whether a correlation of TBT with other elements can be found.

4 The extraction of tributyltin from within flakes of paint

4.1 Introduction

Organotin compounds, especially tributyltin, have been widely used as paint ingredients to prevent biofouling on ships' hulls. The main source of TBT in the marine environment is leaching from TBT-based paint. During boat maintenance and cleaning, antifouling paint particles, which contain a high concentration of TBT, are discarded into semi-enclosed marine systems, such as harbours, marinas and estuaries. Large quantities of antifouling paint particles are generated in boatyards and shipyards where the maintenance of vessel hulls takes place (Figure 20). The paint removal method used (i.e. scraping, stripping and blasting) has an effect on the size of paint particles produced[202]. TBT is directly released from the paint surface into the water and its residence in the area depends on its physico-chemical properties and the conditions of the environment into which it is released[68]. In some cases, TBT is present in sediments as TBT-containing paint particles and these particles increase the TBT persistence in that area[203]. The rate of TBT degradation in sediment has been found to be much slower if TBT is present in the sediment as TBT-containing paint particles than if the TBT is associated directly with the sediment particles[204]. Once paint particles have become combined with bottom sediments, the fate of their biocidal component is uncertain. Understanding the behaviour of paint particle-associated contaminants is necessary in order to assess the risk they pose to the environment.

During the determination of TBT in environmental samples, the major consideration is not the determination itself but the extraction of TBT from within flakes of paint deposited in sediment during boat refurbishment. Various extraction methods have used for the determination of TBT in environmental samples[99] but no systematic study of the extraction of TBT from within flakes of paint deposited in sediment has been reported.

Therefore, improvement in the extraction methods for TBT determination in paint particles is necessary.

This chapter describes the improvement of the extraction efficiency of some current methods for the determination of butyltin compounds in aged paint particles deposited in sediment during boat refurbishment and identifies improvements that can be made to the procedures.

Fig. 20 Discarded paint particles on the hard-standing used for boat repair in a boatyard on the River Itchen in Southampton 1cm

4.2 Experimental section

The following experiment outline describes: (1) the characterization of aged paint particles deposited in Southampton coastal sediment using X-ray fluorescence (XRF), and (2) the determination of TBT from within the aged paint particles deposited in the sediment.

4.2.1 Reagents and standard solutions

All reagents were of analytical reagent grade unless otherwise stated. Reagents described in Section 3.2.1 were used to extract TBT from within the aged paint particles. With the addition of dichloromethane (DCM) (analytical reagent) which was purchased from Rathburn Chemicals (UK). Standard stock solutions (500 µg Sn/ mL of tri(*n*-butyl)mono(*n*-hexyl)tin (MHTBT) in hexane were prepared by dissolving the compound in hexane and there were kept in the dark, refrigerated at 4 °C and used withen six months. Working standard solutions (2 to 8 µg Sn as (MHTBT)/ L) were prepared daily by dilution of the stock solution in hexane.

4.2.2 Instrumentation

For the determination of a wide range of elements, a Philips MagiXPro fully automatic, sequential, wavelength dispersive X ray fluorescence spectrometer (XRF) was used. XRF operating conditions for the analysis of total elements in the aged paint particles have been described in Section 3.4.2.

For the determination of TBT, a Varian 3800 gas chromatograph was used that was equipped with a PFPD system. The operating conditions used for the gas chromatographic determination of TBT in the aged paint particles have been described in Section 3.2.2.

4.2.3 Sampling and sample preparation

Particles of aged paint were removed manually from coastal sediment samples, which had been collected from the boatyard on the River Itchen in Southampton, UK as described in Section 3.2.4.3. For total element analysis, the aged paint particles were classified into three groups based on their colours (red, blue and green). Figure 21 shows a microscopic image of a red paint particle collected from the Southampton coastal sediment.

Preparation of the paint particles involved grinding by mortar and pestle and sieving through a 0.5 mm mesh sieve to yield a powder. The XRF requires a flat sample surface hence the ground paint was placed in an aluminum disc (40 mm in diameter) before being pressed (25 tonne) using a HERZOG HP40 hydraulic to produce a pellet. Samples were analysed using a calibration set based on multiple international reference samples. Accuracy and precision are nominally between 1% and 5% (2 sigma) for trace elements and better than 1% for major elements. The ground and sieved paint particles were also analysed for TBT.

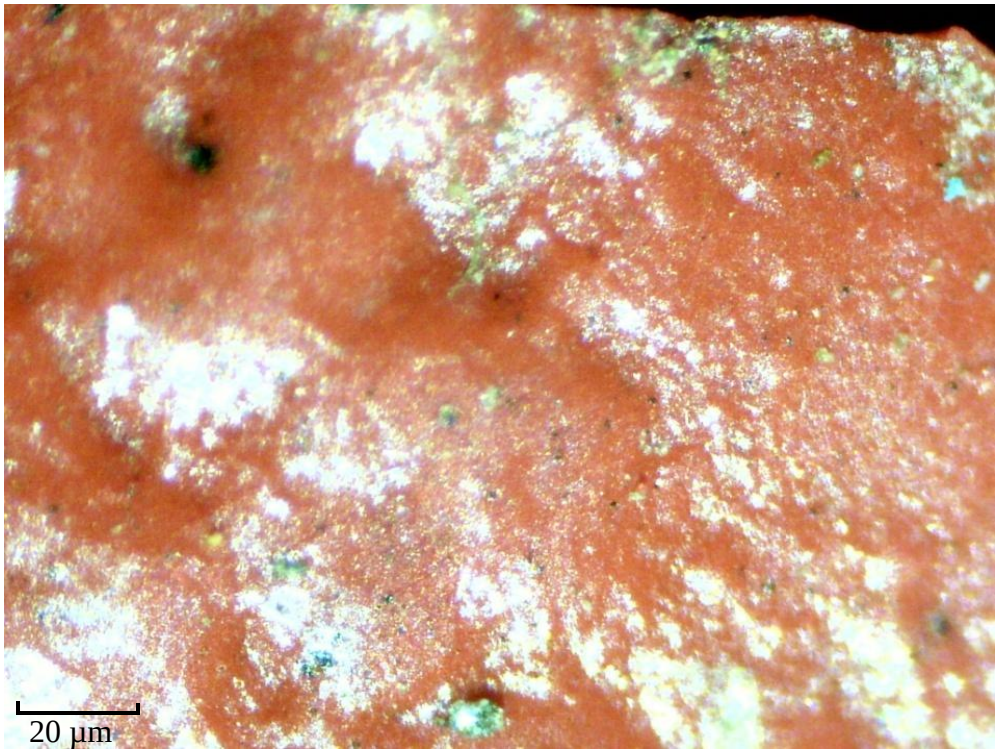


Fig. 21 Microscopic image of a red paint particle collected from boatyard on the River Itchen in Southampton (light microscopy image, 500 x magnification)

4.2.4 The determination of TBT in aged paint particles

4.2.4.1 Introduction

Due to concern over the ability of current analytical methods to extract TBT from within flakes of paint, alternative approaches to the analysis were assessed. Dichloromethane was chosen as the extracting solvent as it is known to be a 'swelling solvent', which causes polymer structures to open out and the TBT compound to be released from within the centre of paint flakes[205].

4.2.4.2 Analytical procedure for determination of TBT in aged paint particles

The initial method for determination of TBT in the aged paint particles was described in Section 3.2.8. Attempts to improve the recovery of TBT involved: a) treating the aged

paint particles with dichloromethane (DCM) and b) extracting the treated sample with hexane/ tropolone as described in Section 3.2.8.

0.2 g (dry weight) of the paint was accurately weighted in a polypropylene centrifuge tube. 3 ml of dichloromethane (DCM) was added and the mixture was sonicated for 30 min at room temperature to open out the polymer structure and release TBT from within the center of the particles. The mixture was then dried using a constant flow of N_2, in order to avoid its reaction with the Grignard derivatisation agent. 2 ml of glacial acetic acid was added to the dry residue to promote the release of ionic tributyltin from the paint. 5 mL of a 0.1% (w/v) solution of tropolone in hexane was added to the mixture and the mixture was shaken, using an wrist action shaker, for 1 hour on high speed. The hexane layer was then separated from the solid residue by centrifugation at 2500 x g for 10 min. The extraction was repeated three times on each paint sample and the organic extracts were combined. The hexane extract was derivatized, isolated and analysed following the procedure described in Section 3.2.8 except the final volume of the hexane extract was diluted to 100 ml with hexane.

4.2.5 Quantification

TBT quantitative analysis was performed using the external standards with calibration curves constructed daily from the analysis of tri(*n*-butyl)mono(*n*-hexyl)tin (MHTBT) over the concentration range 2—8 µg Sn (as MHTBT)/ L. (See Section 3.2.6). The identification of the tributyltin was based on its retention time and quantification was based on peak height (Figure 22).

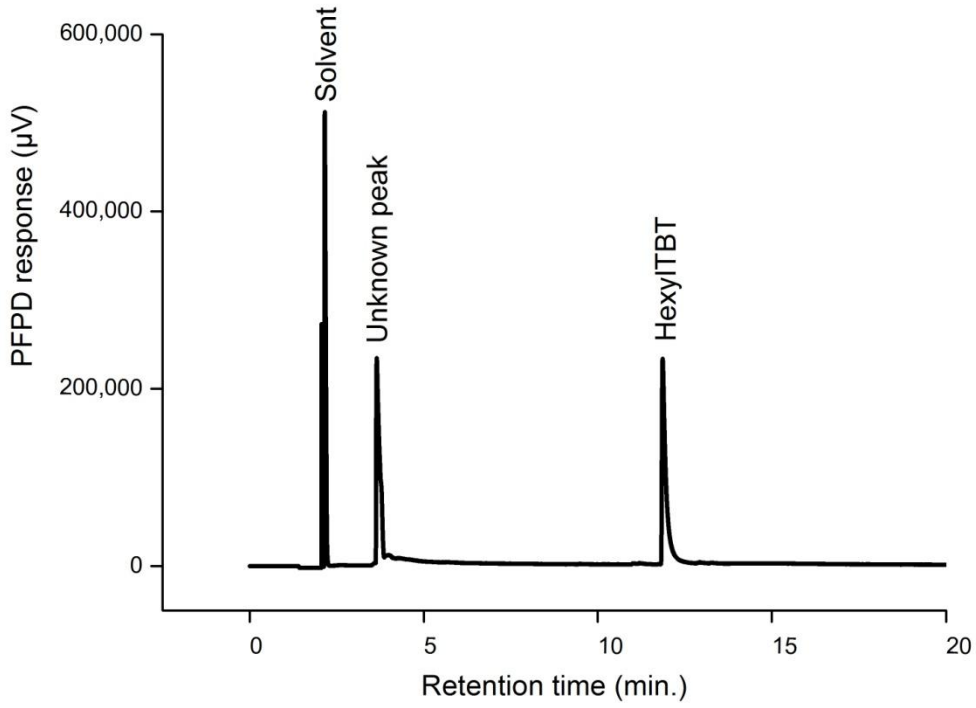


Fig.22 Hexylated tributyltin from an aged paint sample from Southampton coastal sediment. (Concentration 0.75 ng Sn (as TBT)/g)

4.2.6 Improvement of the analytical procedure

In preparing of the method described in Section 4.2.4.2 for use, improvement experiments have been performed.

4.2.6.1 Effect of DCM treatment

In order to assess the effect of DCM on the TBT extraction efficiency, six 0.2 g (dry weight) subsamples of the ground and sieved aged paint particles (red particles) were accurately weighed in polypropylene centrifuge tubes. The six subsamples were divided into two groups: Group-1 (Samples 1, 2 and 3), and Group-2 (Samples 4, 5 and 6). The Group-1 subsamples were treated with DCM as described in Section 2.4.2. For each of the subsamples, three sequential extractions were performed using the procedure

described in Section 3.2.8. After each extraction the extractant was separated from the solid residue by centrifugation at 2500 x g for 10 min, derivatized, isolated and analysed following the procedure described in Section 3.2.8. The Group-2 subsamples were analysed for TBT without DCM treatment. For each of the subsamples in Group-2, four sequential extractions were performed using the procedure described in Section 3.2.8. After each extraction the extractant was separated from the solid residue by centrifugation at 2500 x g for 10 min, derivatized, isolated and analysed following the procedure described in Section 3.2.8.

4.2.6.2 Evaporative TBT losses during DCM removal

Losses of TBT during the evaporation step were suspected. In order to assess the effect of the evaporation step on the overall yield of TBT, four TBTCl solutions (5 ml in DCM) were spiked at 0.04 µg Sn (as TBTCl)/mL. 5 mL spiked solutions in DCM were prepared from 4.8 mL of DCM and 0.2 mL of 1 µg Sn (as TBTCl)/mL solution in hexane. The solutions were evaporated to dryness using a constant N_2 flow. After the evaporation 5 ml of hexane was added to each solution and then the derivatization was carried out.

4.2.6.3 Amount of DCM

In order to assess the effect of the amount of the dichloromethane used, four 0.2 g (dry weight) subsamples of the paint particles (red particles) were accurately weighed in polypropylene centrifuge tubes. They were then mixed with different amounts of DCM (1, 2, 3, 4 and 5 ml). Each mixture was ultrasonicated for 30 min at room temperature. After evaporating the DCM the samples were analysed for TBT following the procedure described in Section 3.2.8.

4.2.6.4 The effect of sonication time

A sonication step is necessary to enhance the movement of the DCM into the paint structure. To evaluate the effect of sonication time, four 0.2 g (dry weight) subsamples of

the paint particles were accurately weighed in polypropylene centrifuge tubes. Then they were mixed with 3 ml of DCM and sonicated at room temperature for different sonication times (10, 20, 30, 40 and 50 min.). After evaporating the DCM the samples were analysed for TBT following the procedure described in Section 3.2.8.

4.2.6.5 The use of Soxhlet extraction

Soxhlet extraction was used as alternative extraction approach to extract TBT from the aged paint particles. The Soxhlet extraction was used without DCM pretreatment of the paint samples. A conventional Soxhlet extraction apparatus was used, consisting of a condenser, a Soxhlet chamber, and an extraction flask. The extraction thimble was a glass thimble with 1 cm internal diameter and 4 cm external length. Six subsamples of the aged paint particles (red particles) were accurately weighed (each 0.1 g dry weight) into glass thimbles. The glass thimble was then placed in the Soxhlet apparatus. 50 ml of a 0.1% (w/v) solution of tropolone in hexane was used as the extractant. The extraction was carried out at 70 ºC for 18 h. The tropolone/hexane solution was then transferred to a 100 mL volumetric flask. After making up to 100 mL with hexane, 1µL of the solution was analysed by gas chromatography. The amounts of TBT extracted from the paint particles by Soxhlet extraction were compared with those extracted with DCM extraction approach described in Section 4.2.4.2.

4.3 Results and discussion

4.3.1 Total elemental analysis

Characterization of the aged paint particles removed from the Southampton boatyard (Section 3.2.4) was carried out using X-ray fluorescence spectroscopy. Table 12 shows some of the results from the elemental analysis of the aged paint particles. Cu, Zn, Sn, Ti, and Pb were considered to be important as these elements have been widely used in paint manufacture. Total Cu and Zn concentrations ranged from 3010 to 6634 mg/ kg and from 2788 to 4331 mg/ kg respectively. Copper is the major biocidal element in all samples analysed, consistent with its use as the main biocidal pigment (Cu_2O) of most contemporary antifouling formulations[12]. Variations in the concentrations of Cu were observed. This variability of Cu levels comes from different sources. 1)- each sample has different numbers of paint layers and each layer has different amount of Cu and 2)- each sample has different rust and sediment contents. Inorganic zinc, as zinc oxide or zinc acrylate, is often used in combination with copper to increase the toxicity of the formulation or to aid the leaching process[206]. Also it is used massively in plating to prevent corrosion[207]. Such chemical characteristics are similar to those of paint particles previously detected[202, 206, 208].

Most paints contain titanium dioxide as the main component in white pigment[209]. Therefore, the presence of titanium dioxide is an important indicator for characterizing paint particles. Concentrations of titanium dioxide up to 8% were detected in these particles.

The levels of total Sn ranged from 322 to 973 mg/ kg (dry weight). The presence of Sn may reflect traces of old tributyltin-paint formulations in the composite, presumably from historic applications removed concurrently with newer paint layers (Figure 23) or it can occur as a constituent of sediment, or leached from other materials such as the alloy bronze[2].

Other trace metals such as Ba, Cr, Ni and Pb have been detected and may be constituents of other paints contained in the composite. For example, Pb has been used in paint as a dryer and to provide corrosion resistance[210, 211]. Quantities of these elements have been reported in fresh antifouling paint formulations[206, 212] demonstrating that the presence of these elements is an important indicator for characterizing paint particles. Also, analyses of the aged paint particles are similar to those data reported by Turner and Parks[202, 208]. Levels of Fe ranged from 16.54-71.61 were found in the studied samples. This was attributed to being surface rust which occurs when the paint surface has been broken. Si, Al and Ca were found in all paint samples. This to be expected as these elements are common constituents of marine sediment and the paint samples were collected from the sediment.

Table 12 Total element concentrations (in mg/ kg dry weight) obtained from the aged paint particles collected from the boatyard

Element	Red paint	Blue paint	Green paint
Cu	301	589	663
Zn	433	278	131
Ti	310	131	162
Sn	973	322	881
Ba	790	257	176
Ni	46.2	33.2	21.8
Cr	39.9	45.4	54.5
Pb	607	239	562
Fe	23.4	49.3	33.6
Si	65.1	71.7	33.6
Al	11.6	28.4	15.4
Ca	22.2	47.5	31.8

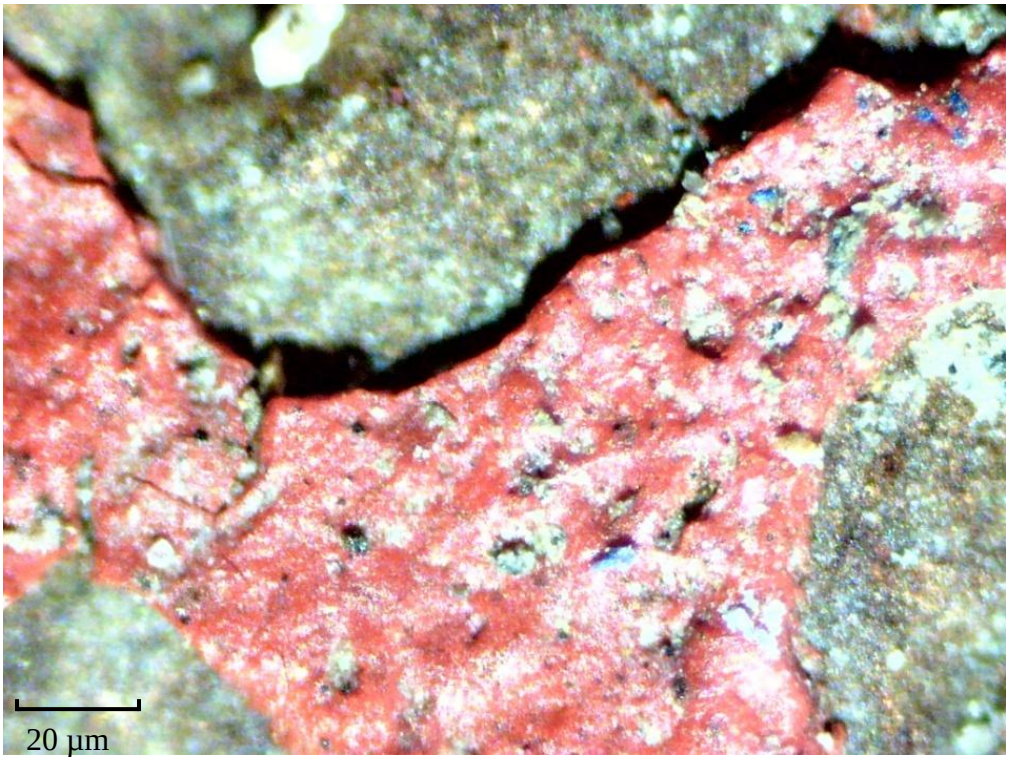


Fig. 23 Example of a multi-layered paint particle (light microscopy image, 500 x magnification)

4.3.2 Effect of DCM treatment

The effect of DCM on TBT extraction efficiency was assessed following the procedure in Section 4.2.6.1. Three sequential extractions were carried out resulting in 453 ± 90 µg Sn/g (Table 13). A fourth extraction was then performed in order to confirm that no more TBT could be extracted from the samples. No significant amount of TBT was extracted by this fourth extraction, indicating that three sequential DCM extractions had released the available TBT from the aged paint particles efficiently. Without DCM treatment, four sequential extractions were performed. The total amount of TBT extracted by four sequential extractions was 366 ± 23 µg Sn/g (Table 14). A fifth extraction was then performed and no significant quantity of TBT was found. Four sequential extractions had therefore released the available TBT from the aged paint particles. These results show that the number of sequential extractions required to release the available amount of TBT from the aged paint particles was reduced by DCM pretreatment due to DCM swelling of

the paint causing the polymer structure to open up and at the same time TBT leaches out. The quantity of TBT extracted is also significantly increased by DCM pretreatment (confirmed by t-test at 95% confidence).

Table 13 TBT extracted following DCM pretreatment

	1st extraction	2nd extraction	3rd extraction	Calculated total
Average extracted amount (µg as Sn/g)[a]	295	113	45.3	453
% RSD	17	20	11	28[b]
% extracted[c]	65%	25%	10%	

[a] Single values are mean concentrations (on a dry weight basis) of three subsamples.

[b] Combining experimental errors = $\sqrt{(\%\, e_1)^2 + (\%\, e_2)^2 + (\%\, e_3)^2}$.

[c] Based on total quantity recovered by 3 extraction.

Table 14 TBT concentrations extracted without DCM treatment

	Extraction sequences				Calculated total
	1st	2nd	3rd	4th	
Average extracted amount (µg Sn/g)[a]	220	76.9	25.6	18.3	366
% RSD	5	4	4	2.3	7.9[b]
% extracted[c]	60%	21 %	14 %	5 %	

[a] Single values are mean concentrations (on a dry weight basis) of three subsamples.

[b] Combining experimental errors = $\sqrt{(\%\, e_1)^2 + (\%\, e_2)^2 + (\%\, e_3)^2}$.

[c] Based on total quantity recovered by 4 extraction.

4.3.3 Evaporative TBT losses during DCM removal

Evaporative TBT loss during DCM removal was investigated by following the procedure described in Section 4.2.6.2. The recovery of TBT in the 0.04 µg Sn (as TBTCl)/ml solution was 65% ± 23%. This is because the N_2 flow used for evaporation was not well-controlled and might have been causing droplet formation or deposit the analyte on the glass wall or some of the analyte will evaporate during this process and this increased the TBT losses and variability of the results.

4.3.4 Amount of DCM

The amount of DCM required to release TBT from within the center of the paint particles was studied using the procedure described in Section 4.2.6.3. The volumes of DCM investigated in this study were between 1 and 5 ml. A lower extracted TBT concentration was obtained when 1 ml of the DCM was employed. The extracted amount of TBT and the variability of the results increased by increasing the volume of DCM. Therefore, a compromise has been made between extraction efficiency and variability of the results. 3 ml DCM pre-treatment gave maximum extraction efficiency with acceptable variability of the results (Table 15).

Table 15 Effect of the DCM volume on extraction efficiency

Volume of DCM (ml)	Average extracted amount (µg Sn (as TBT)/g)[a]	% RSD
1	375	11
2	487	17
3	530	23
4	511	26
5	499	35

[a] Single values are mean concentrations (on a dry weight basis) of three subsamples.

4.3.5 The effect of sonication time

The effect of sonication time (at room temperature) was investigated, as described in Section 4.2.6.4. The extracted amounts of TBT increased with the increasing times of sonication up to 30 min. After that time, amount of TBT recovered decreased (Table 16). Despite the fact that sonication enhances the diffusion of the DCM into the paint structure, it was believed that degradation process of TBT, which convert TBT to DBT, can be happen by the longer sonication times decreasing the extracted amount of TBT. Such effect of the sonication time on TBT recovery has been reported[213].

Table 16 Effect of sonication time on extraction efficiency

Sonication time (min.)	Average extracted amount (µg Sn (as TBT)/g)[a]	% RSD
10	323	15
20	475	14
30	532	17
40	500	16
50	488	22

[a] Mean concentrations (on a dry weight basis) from three subsamples.

4.3.6 The use of Soxhlet extraction

The use of the Soxhlet extraction technique to extract TBT from within the aged paint particles was carried out following the procedure described in Section 4.2.6.5. The extracted amounts of TBT obtained using the Soxhlet extraction were compared with those obtained by the DCM extraction method (Figure 24). Soxhlet extraction was found to extract less TBT than the DCM extraction method. The average amount of TBT extracted by Soxhlet extraction was 309.8 ± 30 µg Sn/g whilst the average amount of TBT extracted by the DCM extraction method was 432.5 ± 81 µg Sn/g. The Soxhlet extraction efficiency was less than that of the DCM extraction method. This is believed to be due to the ability of DCM to penetrate the paint flakes releasing more TBT.

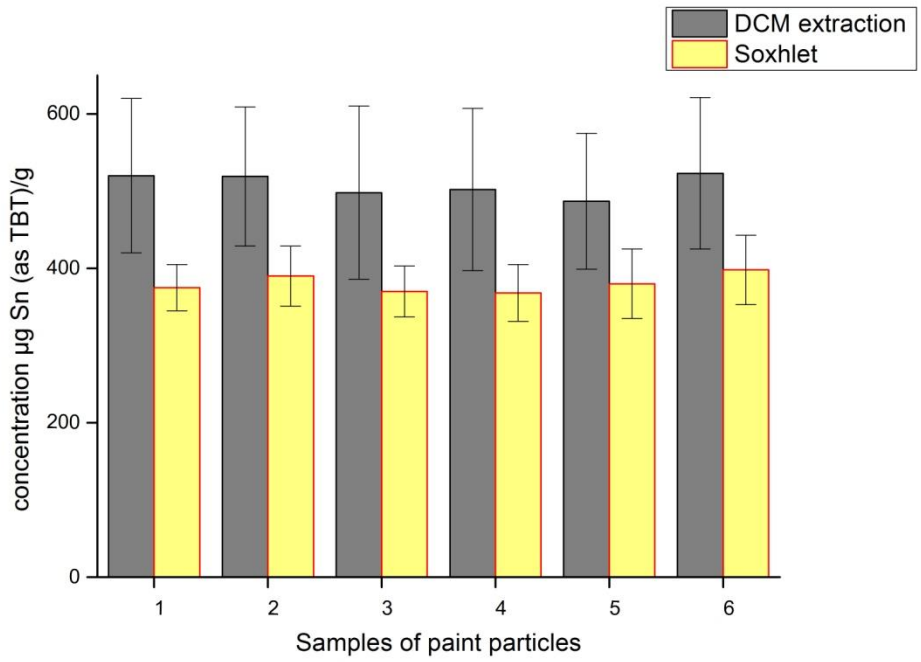


Fig. 24 Comparison the amount of TBT (Sn µg (as TBT)/g) extracted by (a) the DCM extraction (b) Soxhlet extraction with hexane

4.3.7 Tributyltin extracted from the aged paint particles

The TBT in the aged paint particles removed from the Southampton coastal sediment was determined by the analytical procedure described in Section 4.2.4.2 (Table 17). TBT concentrations ranged from 232 to 456 µg Sn (as TBT)/ g. The presence of high levels of tributyltin in the aged paint particles reflects a historical use of antifouling TBT-based paints, mainly related to boat repair in the slipway area. Moreover, this result indicates that the persistence of TBT in the environment is very long when it is introduced as TBT-containing paint particles. Comparison of TBT levels with the total amount of Sn measured by XRF demonstrates that extracted TBT is 50% of the total Sn. This is expected because the total Sn in the samples is not only derived from TBT. Also, the TBT in the samples is not completely extracted and there are evaporative losses. The highest value of TBT obtained from a Southampton coastal sediment (Sample-1) was 6.70 µg Sn/ g (See Section 3.5.6.1) while the highest value of TBT extracted from the aged paint particles removed from the same Southampton coastal sediment was 456 µg

Sn/ g. The amount of TBT extracted from the paint was therefore about 70 times higher than that found in its parent sediment (Sample-1). This result illustrates that paint particles containing TBT remain a potential source of future TBT environmental contamination.

Table 17 TBT concentrations (mg Sn /kg) in the aged paint removed from the Southampton coastal sediment (Sample-1). Mean values and RSD (n = 3) are represented [a]

Red paint particles	Blue paint particles	Green paint particles
456 ± 23 %	388 ± 20%	232± 17 %

[a] Single values are mean concentrations (on a dry weight basis) of three subsamples.

4.4 Conclusions

This chapter has covered the characterization of aged paint particles collected from a small boatyard in the Southampton, UK. A wide range of elements were determined by X-ray fluorescence spectroscopy of isolated naturally-aged paint samples. Significant concentrations of Cu, Zn, Sn, Ti and Pb were found, elements which have been commonly used in paint manufacture. Si, Al and Ca in the samples reflected the presence of residual sediment in the samples. The characterization results were in close agreement with published data[208]. In order to improve the extraction of TBT from within aged paint particles, pretreatment procedures were developed based on pretreatment of the paint with dichloromethane and ultrasonicating the mixture for 30 min followed by hexane/tropolone extraction. Several experiments were carried out to explore alternative approaches to improve the extraction efficiency. Maximum TBT recovery was achieved with 3 ml of DCM and with 30 min sonication time. Three sequential extractions were found to be necessary to maximize the extraction of available TBT from the paint. The total quantity of TBT extracted from the paint was significantly increased by DCM pretreatment. Following this treatment the DCM had to be removed in order to avoid its reaction with the Grignard derivatization agent. Evaporation to dryness was however suspected of contributing significantly to the high variability of the results. Up to 40 % TBT loss was found during DCM removal. Similar TBT losses have been reported elswhere[91]. Improved control of TBT losses is required during the evaporation step. The use of Soxhlet extraction was also assessed, but this did not improve the overall yield of TBT. The concentrations of TBT in the aged paint particles isolated from Southampton coastal sediment (Sample-1) were about 70 times higher than that found in the parent sediment sample (Sample-1). This indicates the persistence of TBT when it is released into the environment within antifouling paint particles. For a better understanding of the distributions, hazards and effects of antifouling paint-based TBT particles, further research is required into the physical or chemical means of identifying and quantifying fine paint particles in the marine environment.

5 Identification and removal of sulphur interferences

5.1 Introduction

Elemental sulphur (S^0) is a common component of marine sediment, present in anoxic sediment due to biogeochemical and microbiological processes that convert sulphates and sulphides to elemental sulphur.[214] Various sulphur forms occur in soils and sediment: (a) Inorganic sulphur forms include sulphates (SO_4^{2-}), elemental sulphur(S^0), metal sulphides (e.g. FeS) and pyrites (FeS_2). In-between the sulphate and the sulphides, several intermediate oxidation state sulphur species be formed, such as bisulphide (S_2^{2-}, oxidation state -1), thiosulphate ($S_2O_3^{2-}$, $+2$) and dithionite ($S_2O_4^{2-}$), $+3$)[215]. (b) Organic sulphur generally occurs in sediment in two main forms: (1) a carbon-bonded sulphur such as in alkyl sulphide compounds; (2) a carbon–oxygen–sulphur bond as in sulphated polysaccharides.

The primary inorganic form of sulphur in soils is sulphate , but sulphide and elemental sulphur are the main forms of inorganic sulphur in anaerobic sediments. The average level of total sulphur in sediments has been estimated to range from 0.1 to 0.5 g/ kg[215]. During the determination of organotin compounds in sediment samples, high levels of sulphur-containing compounds, which are abundant in sediment, can impair detection and quantitation of tributyltin by eluting with a retention time very close to that of TBT or by forming alkyl sulphide compounds during the derivatization step. The explanation of sulphur interferences that occur during organotin determinations using flame photometric detection is based on the emission spectra of sulphur and tin in the flame. In the flame, organotin compounds give rise to excited organotin species SnC and SnH , which emit in the blue region of the spectrum at 390 nm and in the red at 610 nm[181]. The emission band at 390 nm is up to 100–1000 times more intense than the emission band at 610 nm. However, at 390 nm, the main-interfering sulphur species (S_2^*) also emits[216]. This interference of sulphur species with organotin compounds has been previously documented[138, 168, 217]. However, no systematic study has been carried out on sulphur interferences with tin species after hexylation with a Grignard reagent.

A few clean-up procedures have been used in organotin analysis. The use of activated copper[218], tetrabutylammonium sulphite[219] or mercury[220] allows for elemental sulphur elimination, while alkyl sulphides remain in the extract. $AgNO_3$-coated silica is able to remove alkyl sulphides from an organic extract[221], but this method is not completely useful for organotin speciation, since phenyltin compounds are irreversibly adsorbed on to the $AgNO_3$-silica[157]. Oxidation of all sulphur species with dimethyldioxirane (DMD) to sulphones or sulphur oxides, followed by removal of the reaction products by adsorption on a Al_2O_3 column, seems to be the most effective procedure at the moment[222]. However, DMD is commercially unavailable and must be synthesized on site. Therefore, an effective, simple and commercially available method for the elimination of elemental sulphur and organosulphur compounds is mandatory for organotin speciation analysis.

This chapter describes: (1) the identification of unknown peaks seen during the determination of TBT in sediment samples; (2) the development of an efficient clean-up procedure to remove the sulphur interferences during TBT determination in sediment samples; and (3) validation of this method using a certified reference sediment sample.

5.2 Experimental section

The following experiment outlines describe: (1) identification of interfering matrix compounds; (2) removal of sulphur interferences by developing an efficient clean-up procedure.

5.2.1 Materials

All reagents and acids were of analytical reagent grade unless otherwise stated. Chemicals and standard solutions that have been described in Section 3.2.1 and 3.2.3 were used. With the addition of copper powder (<75 μm, 99%), elemental sulphur (S_8, 97%) and *n*-hexanethiol (HexSH, 95%) were obtained from Sigma-Aldrich (UK). Di-*n*-hexylsulphide (*n*-Hex_2S, 97%) and di-*n*-hexyldisulphide (*n*-Hex_2S_2, 80%) were purchased from Alfa-Aesar (UK). Activated copper was prepared by washing the copper powder under sonication (3–5 min) three times each with HCl (25 % v/v), rinsing with hexane then with acetone and leaving to dry). Non-end capped 500 mg/10 mL Isolute C_{18} silica cartridges (61 μm particle diameter, 54 Å pore size) were purchased from Kinesis (UK).

5.2.2 Instrumentation

For the analysis of butyltin compounds, a Varian 3800 gas chromatograph with PFPD system detection was used. Operating conditions used for the gas chromatographic detection of butyltin, which was described in Section 3.2.2, have been optimized to obtain a better separation from the sulphur interferences (Table 18).

Table 18 Optimized operating conditions for the gas chromatographic measurement of butyltin compounds

Column	Sigma-Aldrich, SA-1 type, fused silica – 30 m x 0.25 mm. 0.25 µm film thickness
Injection system	PTV
Carrier gas	Nitrogen (1 ml min^{-1})
Injector temperature programme	150 °C(1 min hold) to 250 °C at 40 °C/min
Oven programme	50 °C (3 min hold), to 100 °C at 30 °C/min, to 130 °C at 7 °C/min, to 270 °C at 11 °C/min, hold 13 min.
Gate delay	4 ms
Gate width	3 ms

5.2.3 Preparation of standard solutions

For butyltin solutions, standard stock solutions of individual hexylbutyltin compounds were prepared at a concentration of 500 µg Sn/ mL in hexane and they were kept in the dark, refrigerated at 4 ºC and used for 6 months. A mixed organotin working solution was prepared weekly by diluting the stock solution with hexane in the range 2–8 µg Sn/ L. A series of standard solutions of a tri(*n*-pentyl)tinchloride (TPTCl) internal standard (IS) were prepared over the range 2– 8 µg Sn/ L (See Section 3.2.3).

For the sulphur compounds, elemental sulphur and hexylsulphide (*n*-HexS, di-*n*-HexS and di-n-$HexS_2$) stock standards were prepared at a concentration of ca. 500 µg Sn/ mL in hexane. A working solution was prepared daily by diluting the stock solutions with hexane to cover the range 0.05–2.5 µg S/ mL.

5.2.4 Reference material and samples

To evaluate the accuracy of the analytical procedure for the speciation of butyltins in sediments, the certified reference material PACS-2 was employed. This is a harbour sediment standard developed by the National Research Council of Canada. PACS-2 is certified for TBT and DBT contents, whilst for MBT an indicative value is given.

For spiking procedures, an ´uncontaminated´ sediment sample was collected during low tide from the Beaulieu River in Beaulieu village, UK in February 2009. The sample was collected from a location expected to be relatively uncontaminated with tributyltin compounds. The sample was immediately frozen and freeze-dried. Before storing in glass containers in the dark at 4 ºC, large particles were removed by dry-sieving through a 1 mm mesh sieve.

To evaluate the applicability of the analytical procedure, five of the 29 surface (top 5 cm) sediment samples (Samples 1,2,3,4 and 5) that had been collected at low tide from a boatyard on the River Itchen in Southampton in April 2008 (Figure 11 in Section 3.2.4.3) were investigated. The choice of these sediment samples was made on the basis that these samples showed high sulphur content, making the quantification of TBT difficult. Prior to the analysis, Southampton sediment samples were prepared as described in Section 3.2.4.3.

5.2.5 Quantification

The identification of the organotin compounds in Southampton coastal sediment was based on the retention time and qualified based on peak height. For quantification purposes, an internal standard method was performed. A 2 µg Sn/L solution as Tri (*n*-pentyl)tinchloride (TPTCl) was prepared and used as internal standard. The calibration graphs of the internal standard method were constructed by adding 2 µg Sn /L of TPTCl (IS) to the butyltin standard solutions covering the range 2– 8 µg Sn /L and the solutions were analysed daily (Section 3.2.6).

5.2.6 Defining the interference problem

During the determination of butyltins compounds in the Southampton sediment samples, unknown peaks were observed (Figure 25). In this chromatogram, four intense unknown peaks (labelled 1*, 2*, 3* and 4*) having retention times of 4.9, 11.85, 14.0 and 15.07 min, respectively, can be observed. The retention times of at least two of these peaks (labelled 3* and 4*) lie very close to those of tributyltin and dibutyltin. Identification and removal of these peaks seems to be essential for accurate butyltin determination.

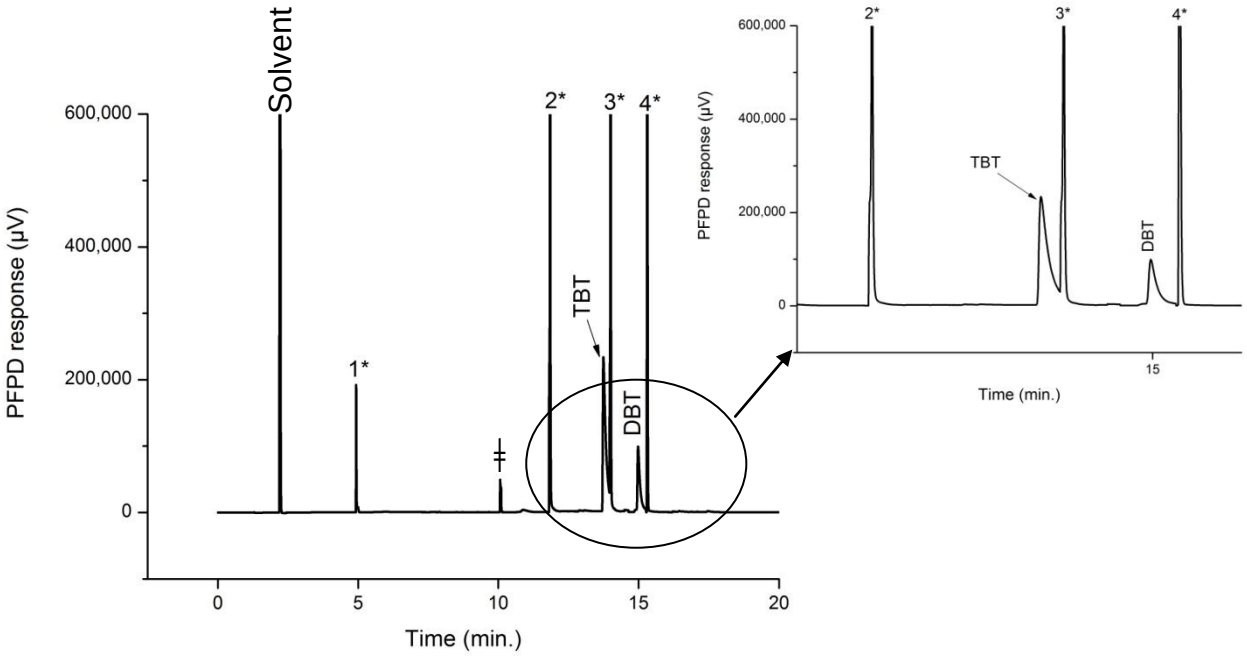


Fig. 25 Hexylated extract from Southampton coastal sediment (Sample-1)

ǂ*Unknown peak, not related to Sn, as unidentical by the tin peak shape*

5.2.7 Analytical procedure for the determination of butyltins in sediment

The initial analytical procedure for the determination of TBT, DBT and MBT was described in Section 3.2.8. It is the core method used, but some parameters were changed after development experiments described later in this chapter.

5.2.8 Method development for the measurement of butyltins in sediment

In order to resolve the co-elution problem, a re-optimization of the GC-PFPD parameters was carried out: firstly, the column oven temperature programme and secondly, the gate settings of the PFPD. To reduce the effects of inferences, different approaches to the use of activated copper and C_{18} solid phase extraction were investigated.

5.2.8.1 Column oven temperature

The column oven temperature was optimized to improve the separation of TBT, DBT, n-Hex_2S_2 and elemental sulphur (S_8). The initial temperature programme was similar to that recommended by Leermakers *et al*[183] , who had optimized it for butyltin analysis, but good resolution of TBT and DBT from n-Hex_2S_2 and S_8 had not been obtained (Figure 25 in Section 5.2.6). Optimization of the temperature programme involved reducing the rate of temperature increase, from 50 °C/min to 30 °C/min at the start of the oven temperature programme and again reducing the rate of temperature increase, from 20 °C/min to 11 °C/min at the end of the temperature programme (Table 18 in Section 5.2.2). 0.5 g (dry weight) of Southampton coastal sediment was analysed following the procedure described in Section 3.2.8. 1µL of the final hexane solution was injected into the GC with the initial temperature programme and with the optimized temperature programme. The obtained chromatograms were compared.

5.2.8.2 Gate setting of the PFPD detector

Gate settings of the PFPD were optimized to improve the detector selectivity to tin over that of sulphur. The optimization was carried out using a 0.04 µg/mL mixed standard solution of TBT and elemental sulphur. Different delay times and gate settings were assessed (3 ms, 4 ms), (3 ms, 5 ms), (4 ms, 4 ms) and (4 ms, 3 ms). For each setting, the same standard was injected three times and the average values of peak heights were reported.

5.2.8.3 Clean-up using activated copper

In order to investigate the use of activated copper for sulphur removal, 0.5 g (dry weight) of Southampton coastal sediment sample was accurately weighed in a 50 ml polypropylene centrifuge tube. The dry sediment was extracted for TBT, DBT and MBT following the procedure described in Section 3.2.8. Before the derivatization step, Two 1 g portions of activated copper were sequentially added to the hexane extract and the mixture was then sonicated for 10 minutes each time. The hexane was removed from the activated copper by centrifugation and the solution was then derivatized, isolated and analysed using the analytical procedure described in Section 3.2.8.

5.2.8.4 Clean-up using a C_{18} solid phase extraction column

The effectiveness of a C_{18} solid phase extraction column in removing alkylsulphides was assessed. 0.5 g (dry weight) of Southampton coastal sediment sample was accurately weighed in a 50 ml polypropylene centrifuge tube. The dry sediment was extracted following the extraction procedure described in Section 3.2.8. A C_{18} solid phase extraction cartridge was then conditioned with 3 mL of hexane, 3 mL of methanol and 4 mL of deionized water at a flow rate of 1.1 mL/ min sequentially. The hexane extract was then slowly passed through the cartridge under gravity (flow rate *ca.* 0.2 ml/min). The eluted solution was then collected in a clean glass vial and

derivatized, isolated and analysed using the analytical procedure described in Section 3.2.8.

5.2.8.4.1 Sample flow rate

In order to optimize the flow rate of the hexane extract through the C_{18} solid phase extraction column, six 0.5 g (dry weight) subsamples of the ´uncontaminated' Beaulieu sediment were accurately weighed in a polypropylene centrifuge tubes. 1 ml of a mixed standard solution of TBTCl, DBTCl and MBTCl (containing 0.1 µg Sn/mL as each compound) was added to each sediment subsample (corresponding to 0.2 µg/g of TBT, DBT and MBT in the sediment) and the samples were divided into two groups: Group-1 (Samples 1, 2 and 3) and Group-2 (Samples 4, 5 and 6). After extraction of each subsample, the hexane extracts of the subsamples in Group-1were slowly eluted by gravity (flow rate *ca.* 0.2 mL/min) through the C_{18} solid phase cartridges. The hexane extracts of the subsamples in Group-2 were pumped through the C_{18} solid phase cartridges using a peristaltic pump at a rate of 1.9 ml/min. In each case, the eluted solution was collected in a clean glass vial and derivatized, isolated and analysed using the analytical procedure described in Section 3.2.8.

5.2.9 The finalised procedure

After changes had been made to the initial analytical procedure (Section 3.2.8), the new analytical procedure was established as follows:

0.5 g (dry weight) of the Southampton coastal sediment sample was accurately weighed in a 50 ml polypropylene centrifuge tube. The dry sediment was extracted following the procedure described in Section 3.2.5. Before the derivatization step, 1 g of activated copper was added to the hexane extract and the mixture was sonicated for 10 min. The hexane was removed from the activated copper. A C_{18} solid phase extraction cartridge was then conditioned with 3 mL of hexane, 3 mL of methanol and 4 mL of deionized water at a flow rate of 1.1 mL/ min. The hexane extract was then slowly eluted by gravity (flow rate *ca.* 0.2 ml/min) through the C_{18} solid phase cartridge. A second elution was carried out using 5 mL of a 0.1% (w/v) solution of tropolone in hexane to ensure all butyltins had been eluted. The eluted solution was

collected in a new glass vial and treated again with 1 g of activated copper, and then the mixture was sonicated for 10 min. The extract was removed from the activated copper and then derivatized, isolated and analysed using the analytical procedure described in Section 3.2.8.

5.2.10 Extraction recoveries

To investigate the recovery of TBT, DBT and MBT obtained by the new procedure (Section 5.2.9), an experiment was carried out in two stages. Stage 1 involved the analysis of unspiked ´uncontaminated' Beaulieu sediment for TBT, DBT and MBT, as described in Section 5.2.9. In stage 2, six 0.5 g (dry weight) subsamples of the ´uncontaminated' Beaulieu sediment were accurately weighed in polypropylene centrifuge tubes. 1 mL of a mixed standard solution of TBTCl, DBTCl and MBTCl (containing 0.1 µg Sn/mL as each compound) was added to each sediment subsample. 1 mL of a 0.1 µg Sn (as TPTCl)/ mL solution was added as an internal standard into the sediment mixtures. The mixtures were then analysed for TBT, DBT and MBT according to the procedure described in Section 5.2.9. The results were calculated using the internal standard method.

5.2.11 Repeatability of the new method

In order to assess the repeatability of the new method, six 0.5 g (dry weight) portions of ´uncontaminated' Beaulieu sediment were accurately weighed in polypropylene centrifuge tubes. 1 mL aliquots of a mixed standard solution of TBTCl, DBTCl and MBTCl (containing 0.1 µg Sn/mL as each compound) was added to each sediment subsample. 1 mL of a 0.1 µg Sn (as TPTCl)/ mL solution was added as an internal standard into the sediment mixtures. The mixtures were then analysed for TBT, DBT and MBT according to the procedure described in Section 5.2.9.

5.3 Results and discussion

5.3.1 Identification of interfering matrix compounds

In order to reduce the effects of interferences, it was important to understand their origins. Since elemental sulphur and alkyl sulphide compounds are the most interfering compounds during the determination of butyltins in sediment, this suggested that this element and its organic derivatives could be responsible for the observed interferences. As commercial standards of elemental sulphur and hexylated sulphur compounds were available, direct identification (based on retention times) was possible. A mixed elemental sulphur and hexylsulphides solution (containing 1 µg S/mL as each compound) was injected directly into GC-PFPD. Figure 26 shows the GC-PFPD chromatogram obtained from this standard solution.

By comparing the chromatogram in Figure 25 (Section 5.2.6), obtained from a hexylated extract of Southampton coastal sediment (Sample 1) and the chromatogram in Figure 26, the first peak, eluting at 4.9 min (marked as 1*), was identified as hexanthiol. The second peak, emerging at 11.85 min (marked as 2*), was identified as di-*n*-hexylsulphide. The third peak, eluting at 14.0 min (marked as 3*), was identified as di-*n*-hexyldisulphide. The fourth peak, emerging at 15.07 min. (marked as 4*), was identified as being elemental sulphur (S_8).

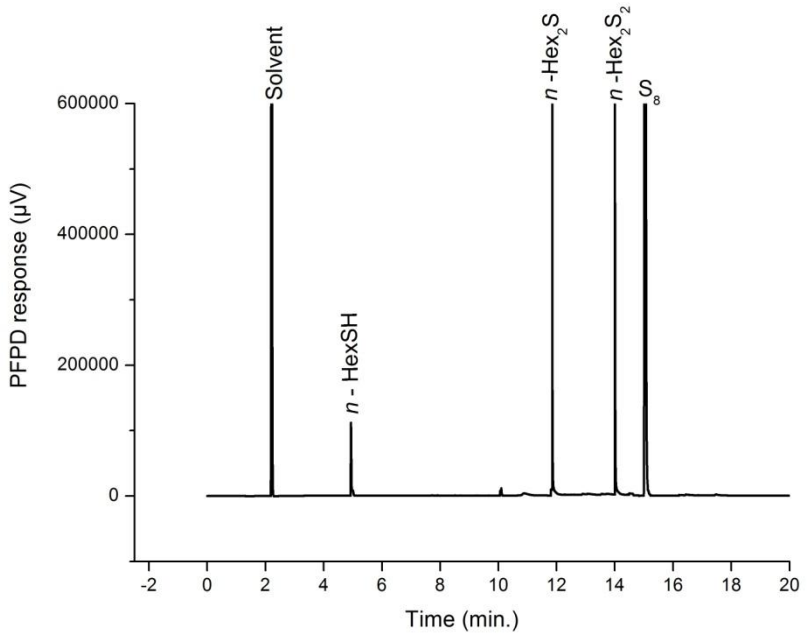


Fig. 26 Mixed standard solution of elemental sulphur and hexylsulphide compounds. (Concentration of 1 µg S/ mL)

5.3.2 Origin of the hexylated sulphur compounds

The origin of the hexylated sulphur compounds generated during the derivatization step was investigated. 0.5 µg/mL of elemental sulphur and sodium sulphide standard solutions in hexane were derivatized individually following the procedure described in Section 3.2.8. 1 µl of each derivatization product was injected into the GC. The sulphur derivatives resulting from the alkylation of elemental sulphur were hexanethiol, di-n-hexylsulphide and di-n-hexyldisulphide; as found in the hexylated extract of the sediment sample (Figure 27). On the other hand, no peaks were observed after the derivatization of the sodium sulphide solution, indicating that the hexylsulphide compounds are products of the reaction of the Grignard reagent with the elemental sulphur in the sediment.

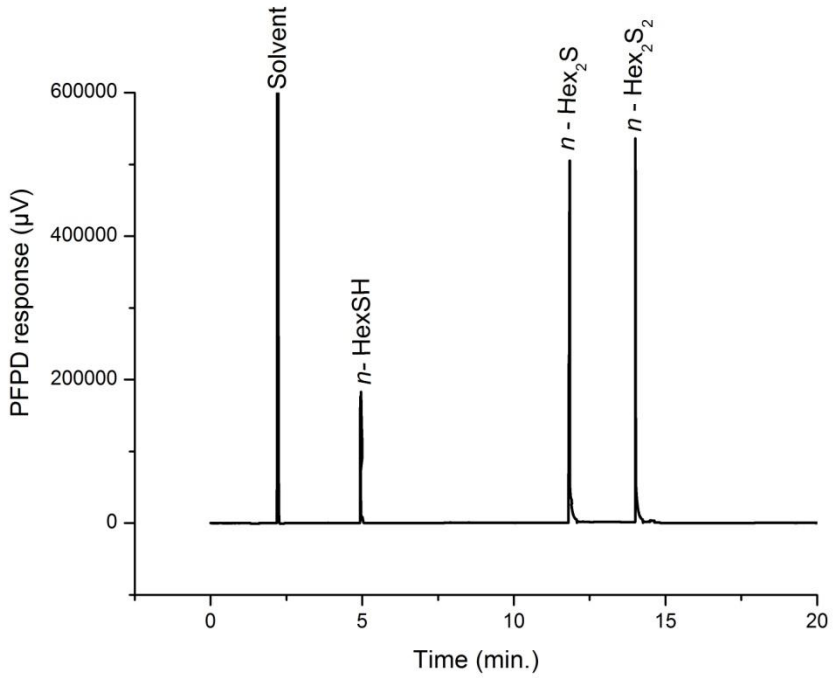


Fig. 27 Hexylated of elemental sulphur solution (concentration of S_8 solution 1 µg/mL)

5.3.3 Improvement of the column oven temperature of the PFPD

The problem of the TBT peak overlapping the di-n-hexyldisulphide peak and the DBT peak overlapping the elemental sulphur (S_8) peak was investigated. To overcome this problem, improvements were made to the column oven temperature programme, as described in Section 5.2.8.1. By comparing the chromatograms obtained in both cases (Fig. 28a and b), very good separations of the two pairs of interfering peaks was obtained, making the quantification of TBT and DBT more reliable.

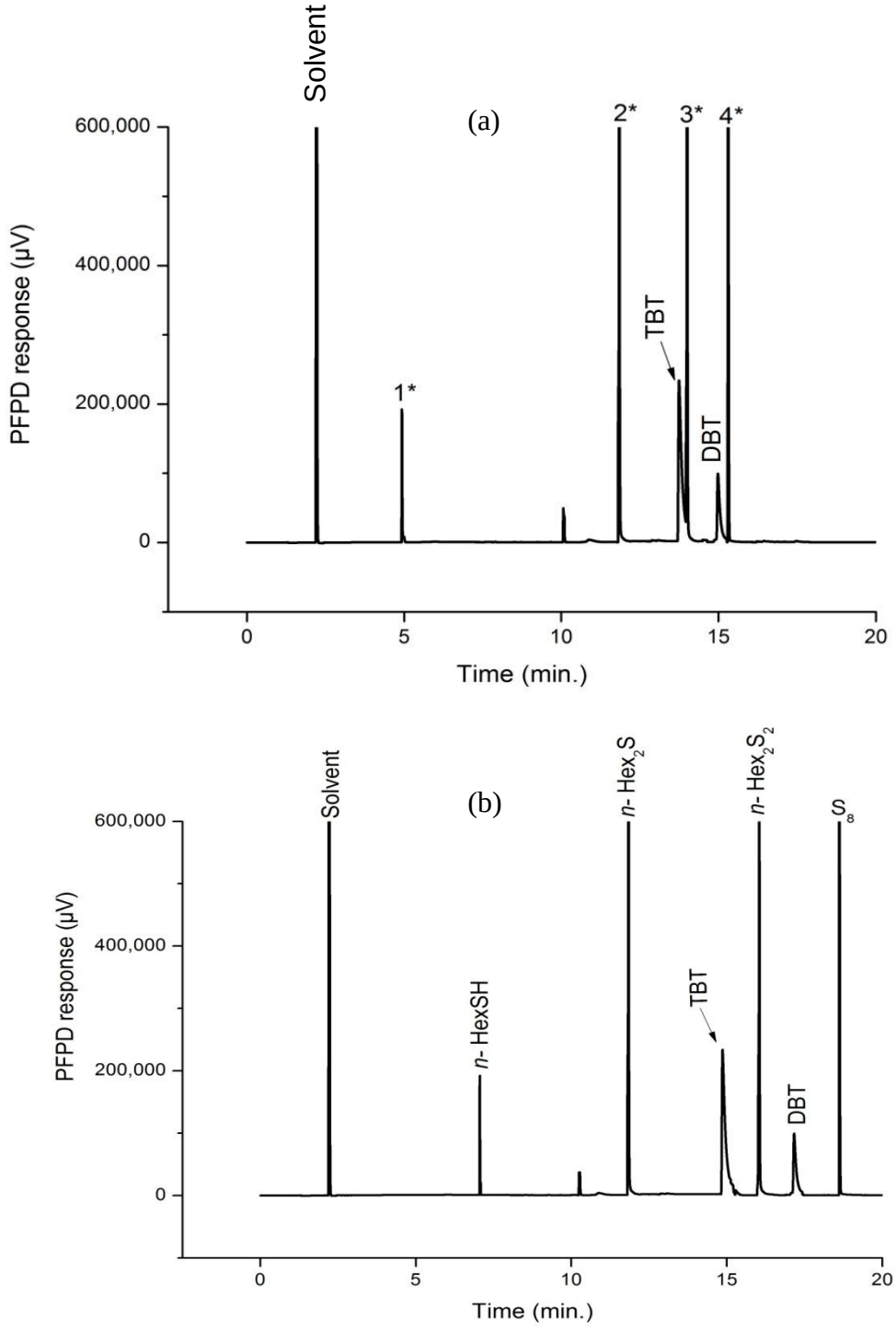


Fig. 28 Hexylated extract of Southampton coastal sediment (Sample-1) using (a) the initial temperature programme (b) the optimized temperature programme

5.3.4 Gate settings of the PFPD

PFPD gate settings were optimized as described in Section 5.2.8.2 and the results are shown in Table 19. Generally, to avoid interferences, the gate width should be as small as possible. Firstly a 3 ms delay and 5 ms width setting were tested, as recommended by the supplier (Varian) for organotin determination. Increasing the gate delay from 3 ms to 4 ms and decreasing the gate width from 5 ms to 4 ms had very little effect on the peak heights from TBT, and no effect on the peak heights from the elemental sulphur. Decreasing the gate width to 3 ms greatly reduced the peak height for elemental sulphur. Hence, 4 ms delay and 3 ms width are the preferred gate settings.

Table 19 Optimization of PFPD gate settings (Peak height in µV)

Gate setting (delay time, width)	TBT [a]	Elemental sulphur [a]
3 ms, 4 ms	75500 ± 90	13200 ± 76
3 ms, 5 ms	80300 ± 75	14400 ± 101
4 ms, 4 ms	82400 ± 63	14300 ± 97
4 ms, 3 ms	83900 ± 65	49400 ± 40

[a] Single values are mean peak height of three injections ± S.D.

5.3.5 Clean-up approaches for the removal of sulphur interferences

Three different approaches were taken to the use of activated copper and C_{18} solid phase extraction columns for the removal of elemental sulphur and alkyl sulphides from sediment extracts: (1) using activated copper (see Section 5.2.8.3) ; (2) using a C_{18} solid phase extraction column (Section 5.2.8.4); (3) combining the activated copper with the C_{18} solid phase extraction column (Section 5.2.9). The elimination of elemental sulphur with activated copper is based on the formation of copper sulphide. The use of activated copper reduced the elemental sulphur, but no effect was observed on the hexylsulphide species (Figure 29a). This result is in agreement with

published data[223]. A C_{18} (octadecyl) bonded silica solid phase extraction column is expected to hold compounds by hydrophobic interactions between the solutes and the stationary phase[224]. C_{18}-silica has non-polar characteristics due to the octadecyl groups on the surface and residual silanol groups that are present allow polar and ionic secondary interactions between the adsorbent and the solutes[225]. The differing affinities of butyltins and alkylsulphides for the C_{18} SPE materials allows the separation of the compounds. The use of C_{18} SPE was found to eliminate the organosulphur compounds completely, but elemental sulphur remains in the extract (Figure 29b). This result is in agreement with a previous study[226]. Combining the activated copper with the C_{18} solid phase extraction column effectively removed elemental sulphur and organosulphur compounds from the sediment extract, allowing the detection of butyltin compounds to be accurately made (Figure 29c)

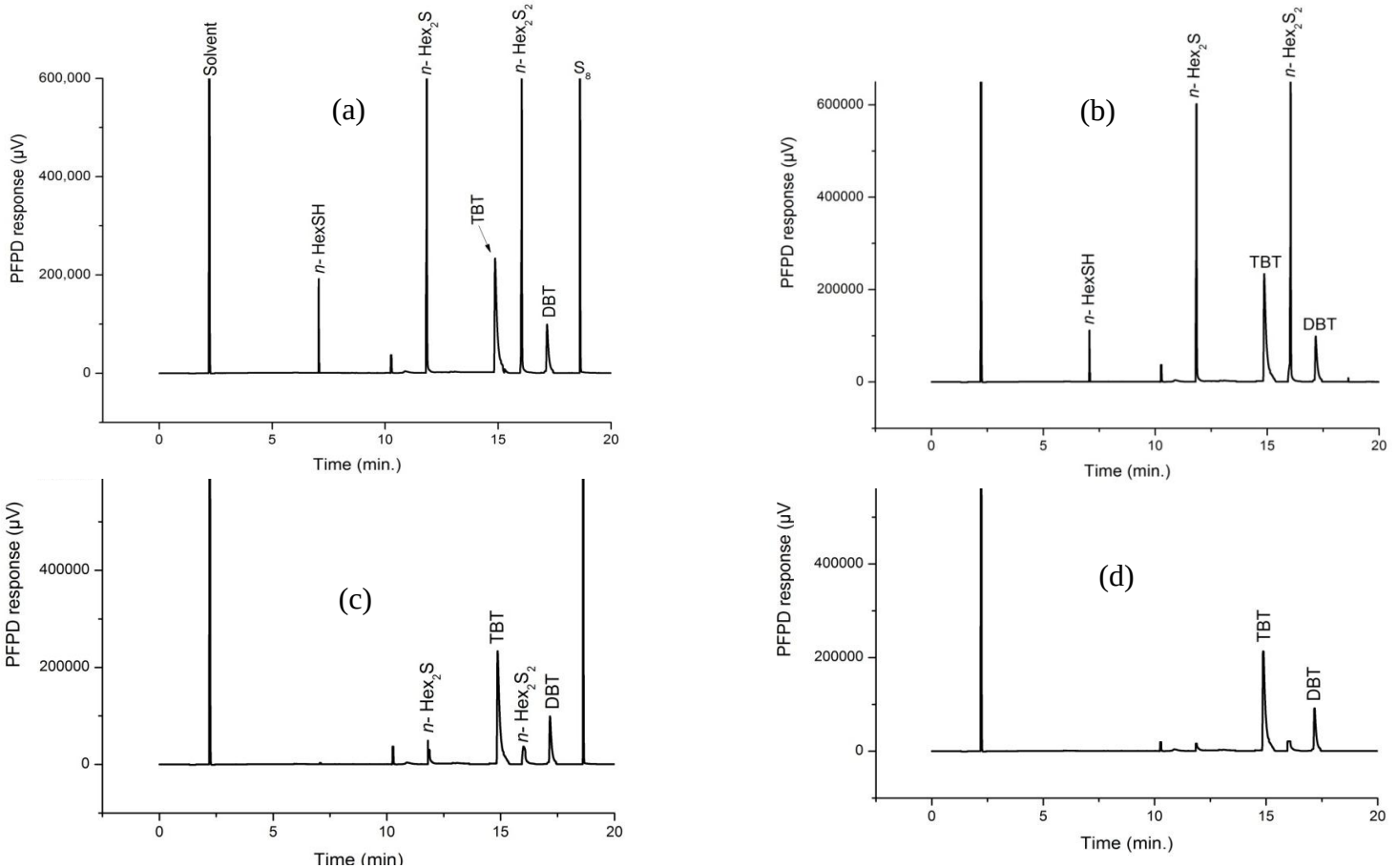


Fig. 29 Hexylated extract of Southampton coastal sediment (Sample-1) (a) before applying the clean-up procedure (b) using activated copper (c) using the C_{18} solid phase extraction columns (d) combining the activated copper with the C_{18} solid phase extraction columns

5.3.6 Extraction recovery

Analysis of the unspiked 'uncontaminated' Beaulieu sediment showed the sample to contain less than the detection limits (5 µg Sn (as TBT)/ kg, 7 µg Sn (as DBT)/ kg and 11 µg Sn (as MBT)/ kg. On spiking with butyltin compounds to a level of 0.2 µg Sn/g as each compound, recoveries of 90.1% ± 1.9%, 91.2% ± 2.1% and 81.3% ± 2.3% were obtained for TBT, DBT and MBT, respectively (experiment described in Section 5.2.10).

5.3.7 Repeatability of the new method

Repeatability of the new method was assessed as the variability of the measurements obtained by the analysis of six spiked subsamples of 'uncontaminated' Beaulieu sediment (experiment described in Section 5.2.11). The RSD values for the TBT, DBT and MBT peak heights were 7%, 5% and 8% respectively (confirmed by an F-test) .

5.3.8 Validation of the new method

To confirm that the new method is suitable for its intended use, it was validated by analysing the sediment reference material PACS-2. Six subsamples of PACS-2 were analysed using the procedure described in Section 5.2.9 with quantification using the internal standard method. However, sulphur interferences have not been reported in the analysis of this sediment sample. To solve this problem, the hexane extract was spiked with excess amounts of sulphur interferences to evaluate the ability of this method to quantify butyltins in the presence of sulphur interferences. The concentrations of TBT and DBT in the certified reference material recovered during the analysis were very close to the certified values. A comparison of the obtained mean values with the certified values was carried out using the Student's *t* test as described in Section 3.5.5. The calculated *t*-values for the analytes were 0.62 and 0.67 for TBT and DBT, respectively. Since the calculated *t* values were less than the tabled *t* values (critical) at n = 6 and 95%

level of confidence, set at 2.57, no significant differences were present between the obtained and certified values (Table 20).

Table 20 Statistical evaluation of the differences between the measured and certified values of butyltins in PACS-2 (n=6).

Compound	Obtained value (ng Sn/ g dry weight)	Certified value (ng Sn/ g dry weight)	Calculated t	Tabled t (95% confidence)
TBT	0.61 ± 0.3	0.89 ± 0.11	0.49	2.57
DBT	0.72 ± 0.2	1.05 ± 0.05	0.67	2.57

5.3.9 Application to Southampton coastal sediment

The TBT, DBT and MBT in the five sediment samples (top 5-cm) were determined by the developed method, described in Section 5.2.9 (Table 21). Before applying the clean-up procedure, it was difficult to quantify the butyltins in the sediments as these samples contain high levels of sulphur compounds and low levels of butyltins. After applying the clean-up procedure, sulphur interferences were completely removed allowed the accurate identification and quantitation of butyltins. TBT concentrations ranged from 0.23 to 4.61 µg Sn/g with an average concentration of 2.82 ± 1.1 µg Sn/g, the DBT from 0.22 to 0.93 µg Sn/ g with an average concentration of 0.53 ± 0.5 µg Sn/g and the MBT ranged from 0.2 to 0.3 µg Sn/g with an average of 0.25 ± 0.05 µg Sn/g.

Table 21 TBT, DBT and MBT concentration (µg Sn/g ± S.D., n=3[a]) in the Southampton coastal sediments (based on dry weight of sediment)

Sampling points[b]	TBT	DBT	MBT
1	4.61 ± 1.1	0.61 ± 0.3	0.2 ± 0.1
2	3.21 ± 0.9	0.93 ± 0.5	< DL[d]
3	4.45 ± 0.8	0.45 ± 0.2	0.3 ± 0.2
4	0.23 ± 0.1	0.42 ± 0.3	< DL
5	1.41 ± 0.5	0.22 ± 0.1	< DL

[a] Three subsamples of each sample were analysed.

[b] sampling points are shown on the map Fig. 1.

[d] values are below the detection limit of the developed method (5 µg Sn/ kg, 7 µg Sn/ kg and 11 µg Sn/ kg for TBT, DBT and MBT, respectivley).

5.4 Conclusions

During the determination of butyltin compounds in the Southampton sediment samples, unknown peaks were observed. Direct identification of the main matrix effects was carried out based on their retention times. Elemental sulphur was found to be mainly responsible for these effects. During the derivatization of the sediment extract, alkylation of the elemental sulphur occurs producing alkylsulphides. Elemental sulphur and alkylsulphides interfere with the TBT and DBT compounds making quantification difficult. The solution for this problem involved: (a) re-optimization of the GC-PFPD parameters and (b) development of a new clean-up procedure. Re-optimization of the GC-PFPD parameters involved: (1) optimization of the column oven temperature programme to obtain good separation of the interfering peaks and (2) optimization of the gate setting of PFPD to improve the detector selectivity to tin over that of sulphur. In order to decrease the matrix effects and increase the selectivity of the butyltin extraction, clean-up procedures were incorporated. Several clean-up approaches were explored to improve the extraction selectivity: firstly, the use of activated copper. Secondly, the use of a C_{18} solid phase column. Thirdly, the activated copper and C_{18} solid phase steps were both employed. The use of activated copper removed the elemental sulphur from the extract, but had no effect on the alkylsulphides. The use of a C_{18} solid phase column removed the alkylsuphide successfully, but elemental sulphur was still found in the extract. By combining both approaches, complete elimination of the elemental sulphur and alkysuphide was achieved, improving the quantification of the butyltins. As well as this procedure being effective in removal of elemental sulphur and alkylsulphides without butyltin losses during the clean-up step, it is rapid and simple. The improved method was validated by analysing the sediment reference material PACS-2. The results obtained for TBT and DBT are in good agreement with the certified values for these compounds. The improved method was applied successfully to sediment samples collected from a small boatyard in Southampton that were highly contaminated with sulphur.

6 Determination of butyltins in water samples

6.1 Introduction

Butuyltin compounds enter the marine environment through the leaching of tributyltin compounds from antifouling paints that are used mainly on vessel hulls. The presence and behaviour of butyltin compounds in water is controlled by their physico-chemical properties. In general, the solubility of organotin compounds ranges from 5–50 mg/L[28, 33]. The larger the number of organic groups attached to the tin atom, the lower the compound's solubility in water and the higher its solubility in organic solvents. The solubility of organotin compounds is greatly inhibited by the presence of the chloride ion which is abundant in seawater and associates with the hydrated cation of butyltin to form covalent organotin chloride[227].

Adsorption of TBT onto sediment particles is considered to be an important mechanism for removal of this compound from the water column due to the high sediment/water partition coefficients of TBT[62]. However, the biochemical[228] and photochemical[229] degradation of tributyltin in water also contributes to TBT removal by converting it to less toxic dibutyltin DBT , monobutyltin MBT, and inorganic tin species. Legislation introduced at the end of the 1980s was aimed at reducing further inputs of TBT, and successfully reduced TBT concentrations in the marine environment. However, the high affinity of TBT for aquatic sediment and the long-term persistence of TBT in sediment results in a high concentration of this compound in marine sediment and the affected sediments continue to release TBT into the water column.

The partitioning of TBT between water and sediment is an important environmental process in our understanding of the transport and the fate of these compounds in the estuarine environment[230]. Different mechanisms explain the adsorption of TBT onto sediment: TBT in its cationic form may be attracted to negatively charged minerals, oxides and hydroxides present in sediment and be retained in the diffuse layer of cations surrounding the negatively charged surface[231]. Also, adsorption of TBT onto sediment

occurs mainly via hydrophobic interaction of TBT with the organic matter content of sediment[30]. In addition, adsorption of TBT onto sediment may occur via specific interactions between the Sn atom and surface ligands on sediment minerals and polar functional groups on organic materials[232]. Meador[233] reported laboratory studies in which TBT in both seawater and freshwater was partitioned with sediments. A correlation was found between the total organic carbon content of the sediment and its uptake of TBT. TBT has a relatively strong tendency to become incorporated into estuarine sediment, which has a larger organic content.

The partitioning of TBT between sediment and water is difficult to calculate in an open marine system, as the water acts as an infinite sink for the TBT, and equilibrium therefore will not be achieved[94]. Partitioning of TBT between the two phases is influenced by a number of factors such as salinity, pH, suspended solids and sediment composition[62, 234]. For example, the adsorption of TBT increases with increasing salinity[62]. Studies of the effect of pH on partitioning behaviour have indicated that the adsorption of TBT decreases sharply towards both higher and lower pH values over the range studied (pH 4.5–10) [62].

In natural waters, TBT will form complexes with inorganic ions such as chloride or hydroxide as well as with organic compounds. According to Arnold[234], 50% of the TBT is in its ionic form at pH 6.25 but in seawater conditions (pH = 8, ionic strength = 0.5 M) 93% of the TBT in solution occurs in a hydroxide form.

The TBT degradation rate is another mechanism that affects the concentration of TBT in water. This rate varies in different types of water. While the half-life of TBT in seawater is between 5 and 20 days[235] , in freshwater it can be 4 to 5 months and in freshwater/sediment mixtures the half-life is more than 11 months[229]. However, the degradation may not always result in de-contamination. In general, a good understanding of the physico-chemical properties of TBT as well as characterization of the properties of the water, sediment and the organisms present may help to predict the distribution and behaviour of TBT in natural water.

The determination of butyltin compounds in water samples has been a major analytical challenge for a number of reasons: (1) the low level of the analyte (a few ng/g); (2) the limited stability of butyltins in water samples; (3) variability of butyltin levels over the same site due to the tidal cycle; and (4) the absence of certified reference materials for water.

In order to measure the low concentrations of these compounds that occur in water samples, most of the methods developed so far apply different extraction and pre-concentration techniques such as liquid–liquid extraction[236, 237], solid phase extraction[108, 238] solid phase microextraction[173, 239] and supercritical fluid extraction[213, 240]. More recently the extraction and pre-concentration of analytes from aqueous solution has been carried out by solid phase dispersion extraction (SPDE). This method was originally introduced by Anastassiades *et al.*[241] as a clean-up step. A small amount of solid phase extraction sorbent was initially dispersed in an extract to remove interfering materials and it was then be removed by centrifugation. Silicas modified with different chemical groups have been used as sorbents in SPDE[242, 243].

In 2005, a novel extraction approach based on the SPDE technique was developed by Howard and Khdary[244] that employed carefully size-selected modified Stöber-type spherical silica particles functionalized with different chemical groups (extracting agent). The idea of this method is based on the partitioning of the analyte between a sub-micron solid and a liquid phase as a colloidal sol. By tailoring the size of the particles to approximately 250 nm diameter, they can be easily dispersed in aqueous solution, without the need for any mechanical or hand shaking and the solid can then be readily recovered, together with the analytes, by simple filtration or centrifugation[244]. The size of particles has a great effect on their sedimentation velocity and surface area. When the particle size reduces, the sedimentation velocity rapidly decreases to a point where sedimentation is balanced against repulsive forces and Brownian motion[244]. The specific surface area will increase by decreasing the particle size and this will improve its capacity to hold the analyte by adsorption, complexation, etc. The word ‘nanoscavenger’ has been used to describe a sub-micron material that can be dispersed in a colloidal sol to

selectively scavenge analytes. Such particles must however be large enough to allow them to be simply recovered. In the approach adapted for this study, extraction is performed by the dispersion of surface modified nanoscavenger silica in aqueous solution. The nanoscavenger silica used was doubly functionalized mesoporous silica with diol and C_{18} alkane surface groups (HOC_{18}-nanoscavenger). This is a water-wettable material that can be employed for the collection of hydrophobic materials such as the TBT. Once collected the tributyltin species are derivatized using a Grignard reagent or sodium tetraethylborate and quantified by gas chromatography with pulsed flame photometric detection.

This chapter is divided into two main parts: Part 1 describes the synthesis and characterization of the HOC_{18}-nanoscavenger. Part 2 describes the development of a new procedure for the SPDE- based determination of butyltin-contaminated water using the HOC_{18}-nanoscavenger.

6.2 Synthesis of a HOC_{18}-nanoscavenger

6.2.1 Introduction

Synthetic silicas are generally classified into three groups: silica gels, pyrogenic silicas and precipitated silicas. The sol–gel process involves the transition of a solution system from a liquid 'sol' (mostly colloidal) into a solid 'gel' phase. The silica is most commonly synthesized from alkoxide resources such as tetraethoxysilane (TEOS) and tetramethoxysilane (TMOS)[245]. The sol–gel process follows a number of steps: (1) hydrolysis of the silica precursor using a base or acid-catalysed process[246], (2) condensation (which occurs simultaneously with the hydrolysis) producing water, alcohol and siloxane bonds[246]. (3) ageing and drying to remove liquid from the pores of the gel and to increase the linkages of within a network (Silica Aerogels)[247]. Pyrogenic silica is silica that is synthesized at high temperature by steam hydrolysis of silicon tetrachloride or by higher temperature fusion of sand. This type includes fumed silica, arc silica and plasma silica. Fumed silica is synthesized by hydrolysis of a silica source

(silicon tetrachloride) in a hydrogen/oxygen flame producing silicon dioxide which condenses to form particles[248]. Arc silica is produced from high purity sand (quartz), which is first converted to silicon monoxide at high temperature, and then oxidized in the air to form silicon dioxide[249]. Plasma silica can be synthesized by conversion of quartz to a plasma state in nitrogen or an inert gas at very high temperature. This gives very fine particles (around 1 nm in diameter)[249].

In 1968 Stöber *et al.* successfully synthesized spherical silica particles of nanometre dimensions by the hydrolysis of alkyl silicates (TEOS) followed by condensation of the resulting silicic acid ($Si(OH)_4$ in the presence of ammonia and alcohol[250]. This type of silica is non-porous. Microporous silica was obtained by combustion of the silica or by using a template. Spherical silica particles of 50–2000 nm in diameter have been obtained; most being from modifications of the Stöber method[251, 252].

In 1992, a new class of aluminosilicate materials was discovered by Kresge *et al*[253]. These materials were mesoporous, having regular pore sizes in the range 2–50 nm. Silica-based mesoporous materials of a nano-scale size and a large surface area have received particular attention because of their potential applications in many areas, such as catalysis, adsorption, separation, chromatography, chemical sensors and bioscience[254]. In general, the synthesis of mesoporous silica is based on the use of organic template molecules around which the inorganic precursor can condense[253]. The choice of precursor and reaction conditions controls the final form of the silica. Many research groups have reported various mesoporous silica particles from hundreds of nanometres to tens of micrometres in diameter [255-257].

In this study, synthesis of the HOC_{18}-nanoscavenger involved four individual steps: (1) synthesis of sub-micron spherical mesoporous silica particles, *ca.* 250 nm in diameter, having a narrow size distribution, using dodecyltrimethylammonium bromide (C_{12}TMABr) as a template and tetramethoxsysilane (TMOS) as a silica precursor, ethylene glycol as a co-solvent and NaOH as a base[258]. (2) modification of the surface of the mesoporous silica with 3-glycidoxypropyl groups. (3) modification of the glycidoxypropyl-mesoporous silica with octadecyl groups. (4) opening of the epoxy

ring in aqueous acid to produce diol groups in an attempt to increase the wettability of the particles. The modification of the silica surface takes place in dry conditions. At the first step, the surface of the silica was modified with the 3-glycidoxypropyl groups due to the interaction between Si-OH and Si-OCH_3 to form Si-O-Si bonds and eliminate CH_3OH. At the second step, the surface was modified with octadecyl groups due to the interaction between Si-OH nd Si-Cl to form Si-O-Si bonds and eliminate HCl. The hydrolysis of the other methoxsy and Chloride groups will take place at the third step due to the presence of water. Also, the water / acid will open the epoxy ring and produce diol gropus on the surface of silica (Figure 30).

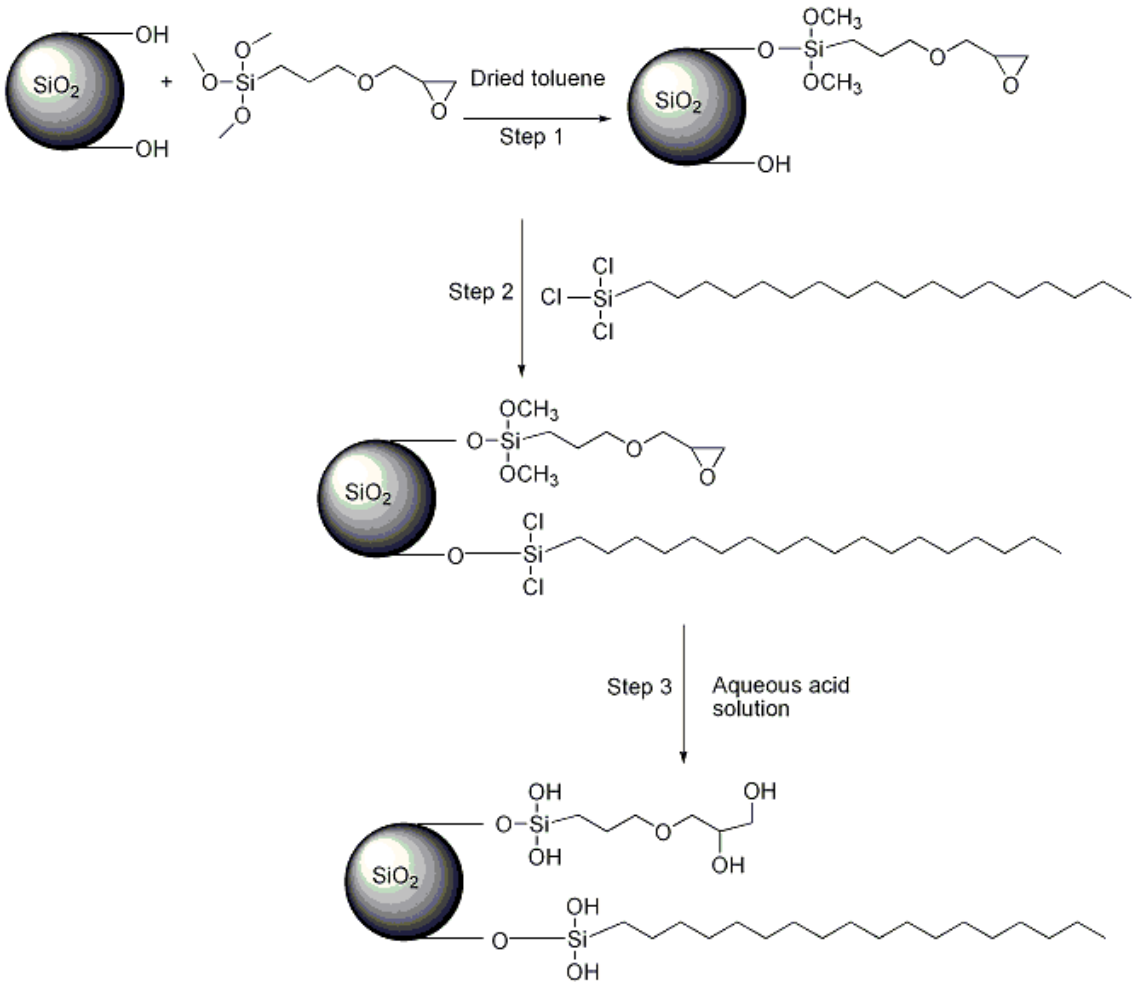


Figure 30 highly theoretical representation of the preparation of a HOC_{18}-nanoscavenger (assuming the modifier attaches the surface from one point)

6.2.2 Experimental

The synthesis of the HOC_{18}-nanoscavenger was adapted from the procedure described by Mohammed Algaradah[259].

6.2.2.1 Materials

All glassware was washed with detergent and water, rinsed with deionised water and then dried in an oven. Before using the glassware, it was rinsed with acetone and finally with hexane, and left to dry at room temperature. Deionised water (>5 MΩ cm at 25 °C) was produced using an ELGA OPTION 4 water purifier. *n*-toluene (99.99%, HPLC grade) was purchased from Fisher (Loughborough, UK), (dried by fractional distillation under N_2 from over P_2O_5). Ethylene glycol (99%, analytical grade), sodium hydroxide (laboratory grade) and hydrochloric acid (37% w/v, laboratory grade) were purchased from Fisher Scientific (Loughborough, UK). Tetramethoxysilane (TMOS, 98%), octadecyltrichlorosilane ($C_{18}SiCl_3$, 90%) and glycidoxypropyltrimethoxysilane (GMOS, 98%) were obtained from Aldrich (Gillingham, UK). Dodecyltrimethylammonium bromide (C_{12}TMABr) (99%) was purchased from Alfa Aesar (Lancashire, UK).

6.2.2.2 Synthesis of mesoporous silica

310 mL of deionised water, 90 mL of ethylene glycol, 3 mL of NaOH (aqueous, 1M) and 1.68 g of C_{12}TMABr were mixed in a 1000 mL conical flask and stirred for 15 minutes at 20 °C using a magnetic stirrer. 1.8 mL of TMOS was then slowly added and the reaction was left to proceed at *ca.* 20 °C for 8 hours under stirring. The mixture was then aged overnight. The white solid was isolated by centrifugation for an hour at 8300 x g. The supernatant was removed and the silica was rinsed five times with deionised water. The white powder was dried in an oven at 45 °C for three days, and then it was calcinated to remove the surfactant at 550 °C for 12 hours. The experiment was repeated five times and the combined products were gently ground and mixed together.

6.2.2.3 Synthesis of glycidoxypropyl –mesoporous silica

2 g of the mesoporous silica (prepared in Section 6.2.2.2 and then dried in an oven at 120 °C overnight) and 150 mL dried toluene were transferred to a two-necked round-bottomed flask (250 mL). The mixture was sonicated for 15 min and stirred at 90 °C for an hour under N_2. 1 mL of GMOS (dissolved in 10 ml of dried toluene) was then added slowly through a dropping funnel. The reaction was left to proceed at *ca.* 90 °C for 7 hours under N_2 and then allowed to cool. The white solid was isolated by centrifugation, rinsed five times with dried toluene, and then dried under continuous vacuum for 5 hours.

6.2.2.4 Synthesis of C_{18}-glycidoxypropyl-mesoporous silica

2 g of glycidoxypropyl-mesoporous silica (prepared in Section 6.2.2.3) and 150 mL of dried toluene were transferred to a two-necked round-bottomed flask (250 mL). The mixture was sonicated for 15 min and 1 mL of $C_{18}SiCl_3$ (dissolved in 10 mL of dried toluene) was slowly added dropwise, using a dropping funnel. The reaction mixture was stirred at 90 °C under N_2 for 7 hours. The white solid was isolated by centrifugation for 15 min at 8400 x g and then rinsed five times with dried toluene and three times with ethanol and dried under continuous vacuum for 6 hours.

6.2.2.5 Opening the epoxide ring

100 mL of deionised water was transferred to a 250 mL round-bottomed flask and the pH was adjusted to 3 using conc. HCl. 1 g of C_{18}- glycidoxypropyl -mesoporous silica (prepared in Section 6.2.2.4) was added and the mixture was sonicated for 15 min. The reaction mixture was refluxed at 100 °C overnight. The white solid (HOC_{18}-nanoscavenger) was isolated by centrifugation for 30 min at 8400 x g, rinsed five times with deionised water and dried in an oven at 60 °C for 4 hours.

6.3 Characterization of the HOC_{18}-nanoscavenger

The synthesized HOC_{18}-nanoscavenger was characterized by a variety of methods.

6.3.1 Thermogravimetric analysis

Thermogravimetric analysis (TGA) was carried out using a Perkin-Elmer TGS-2 instrument connected to a Perkin-Elmer Datastation-3700 and a System 4 thermal analysis microprocessor. ~10 mg of the HOC_{18}-nanoscavenger was weighed in a Pt crucible and heated in air oven at the temperature range 30 °C to 750 °C, at a rate of 10 °C min^{-1}. TGA analyses of the unmodified mesoporous silica (starting material) and the glycidoxypropyl-mesoporous silica were also carried out under these conditions.

6.3.2 Fourier transform infrared (FTIR) spectroscopy

Infrared spectroscopy was carried out using a Perkin–Elmer System 2000 FTIR spectrometer equipped with an IR microscope. The reflectance IR spectra were measured over the range 400 cm^{-1} to 4600 cm^{-1} and 32 scans were carried out. No sample preparation is required for the analysis**.** ~10 mg of the HOC_{18}-nanoscavenger was placed on a gold-coated specimen plate inside the spectrometer sample compartment.

6.3.3 Scanning electron microscopy (SEM)

Scanning electron microscopy of the silica samples was carried out using a Philips XL-30 scanning electron microscope (FEI Company, Hillsboro, Oregon, USA) equipped with a Secondary Electron detector (SE). To prepare the sample for SEM studies, *ca.* 10 mg of the HOC_{18}-nanoscavenger was dispersed in 10 mL of methanol and the resulting suspension was sonicated for 2 min. A drop (~1−10 μL) of the suspension was applied on to a very clean, acetone-washed glass slide (1 cm^2), which was fixed to an aluminium SEM stud. The samples were kept in a desiccator to dry at room temperature. In order to prevent charging of the surface, to promote the emission of secondary

electrons so that the specimen conducts evenly, and to provide a homogeneous surface for analysis and imaging, the samples were then coated with a thin layer of gold (*ca.* 10 nm thick film) under high vacuum. A Hummer sputtering system (Anatech Ltd) was employed using the conditions summarized in Table 22. After gold coating, the sample was placed inside the SEM sample chamber and the SEM was operated under the high vacuum with an accelerating voltage of 30 kV.

Table 22: The parameters employed for gold coating

Parameters	Value
Gas employed	Argon 5 psi
Current	~ 15 mA
Time of coating	4 minutes

6.3.4 Surface area measurement

Measurements of the surface areas of the mesoporus silica before and after modification were obtained from nitrogen adsorption–desorption isotherms (77 K) using a Quantachrome NOVA 3000 automated gas sorption system with NOVA software (version 1.11). 0.1 g of the sample was accurately weighed to four decimal places and then transferred to a pre-weighed tube. The tube was re-weighed and then degassed overnight at 160 °C. The tube was left to cool to room temperature, weighed and then placed in the NOVA 3000 instrument. Surface area was estimated using the multipoint BET (Brunauer–Emmett–Teller)[260] equation (6.1), measuring the volume of nitrogen adsorbed by the sample.

$$\frac{1}{V\left[\left({P_\circ}/{P}\right)-1\right]} = \frac{1}{V_m C} + \frac{C-1}{V_m C}\left(\frac{P}{P_\circ}\right) \qquad (6.1)$$

where P and $P_\circ$ are the equilibrium and saturation pressure of adsorbates at the temperature of adsorption, V is the volume the adsorbed gas, V_m is the volume of the

monolayer adsorbed gas and C is the BET constant. The pore diameter was obtained from the pore size distribution curve using the Barrett–Joyner^{o}–Halenda (BJH) formula. The BJH method for calculating pore size distribution is based on a model of adsorbent as a collection of cylindrical pores. The method account for capillary condensation in the pores using the classical Kelvin equation[261] (6.2).

$$\ln \frac{P}{P_o} = \frac{2\gamma M_V}{r R_C T} \qquad (6.2)$$

Where P is the actual vapour pressure, P_o is the saturation vapour pressure, γ is the surface tension, M_V is the molar volume, R_C is the universal gas constant and r is the radius of the droplet.

6.3.5 Measurement of octadecyl groups on the silica surface

To develop the partitioning of butyltin species on to the HOC_{18}-nanoscavenger for the purposes of extraction and preconcentration, a fine balance has to be established between the amount of C_{18} and the amount of diol on the surface of the HOC_{18}-nanoscavenger. The loading of C_{18} on the surface of the modified nanoscavenger silica particles was assessed using a modification of the method described by Genieser *et al.*[262], which is based on the cleavage of the silicon–carbon bonds by fusion in potassium hydroxide. The cleavage products were *n*-octadecane and 1-octadecanol, and these compounds can be measured by gas chromatography. The sum of both of these products gives the amount of octadecyl loaded on the surface.

6.3.5.1 Materials and standards

n-Octadecane (99%), 1-octadecanol (99%), 1-hexadecanol (99%) and triethylene glycol dimethylether (90%) were purchased from Aldrich (UK). Potassium hydroxide and hexane were purchased from Fisher Scientific (Leicestershire, UK). The stock solutions were prepared by dissolving 10 mg of octadecane, 1-octadecanol and 1-hexadecanol

individually in 10 mL of *n*-hexane. 1-hexadecanol was used as an internal standard. A series of dilutions from the stock solutions were carried out to obtain mixtures of working standard solutions containing octadecane, 1-octadecanol and 1-hexadecanol (35, 200 and 200 µg /mL), (17.5, 100 and 100 µg /mL) and (8.75, 50 and 50 µg /mL), respectively.

6.3.5.2 Instrumentation

A Perkin-Elmer 8700 Chromatograph was used that was equipped with flame ionization detection (FID). Splitless injection of 1µL was performed, with a split delay of 1 min and a split ratio of 50:1. The separation was carried out using a non-polar quartz capillary column (Alltech Econo-cap SE-54, fused silica 30 m x 0.25 mm), coated with the non-polar stationary phase (5% phenyl–95% methylpolysiloxane), 0.25 µm film thickness) and N_2 carrier gas flow rate of *ca.* 1 mL min^{-1}. The operating conditions used for the gas chromatographic determination of the octadecyl group (C_{18}) on silica surface are summarized in Table 23.

Table 23 Operating conditions for the gas chromatographic measurement of the C_{18} groups

Carrier gas	Nitrogen (1 ml min^{-1})
Injector temperature	250 °C
Column oven programme	60 °C (3 min hold) to 200 °C at 25 °C/min to 250 °C at 5 °C/min , hold 5 min.
Detector temperature	300 °C

6.3.5.3 Cleavage procedure

Three 10 mg (dry weight) samples of the HOC_{18}-nanoscavenger were accurately weighed and transferred into small test tubes. 100 mg of KOH was added to each tube and the tubes were left in a desiccator (containing P_2O_5) overnight. 200 µL of triethylene glycol dimethyl ether was added to each tube and they were then heated in a sand bath at 216 °C for 2 hours. After 1 min of cooling, 250 µL of deionied water was added to each tube, followed by 100 µL of 1- hexadecanol (1 mg/ mL) as an internal standard and 140 µL of 37 % (w/v) HCl for neutralization. The tubes were shaken for 3 min and then 2 mL of *n*-hexane was added. After shaking the tubes for 1 min they were centrifuged for 5 min. The hexane phase was transferred to a 1 mL volumetric flask and made up to 1 mL with *n*-hexane. 1µL of the hexane solution was analysed by gas chromatography.

6.4 Results and discussion of the characterization of the HOC_{18}-nanoscavenger

6.4.1 Thermogravimetric analysis of the synthesized materials

Thermogravimetric analyses of the unmodified mesoporous silica (after removing the template), the glycidoxypropyl-mesoporous (intermediate product) and the HOC_{18}-nanoscavenger (final product) are shown in Figure 31. For unmodified mesoporous silica, three areas of weight loss were identified (Figure 31a). The first region (I) from 50 °C to around 150 °C (*ca.* 2.3% weight loss) is associated with removal of residual solvent and physically adsorbed water. The second weight loss of (*ca.* 1.6%) over the temperature region 200–460 °C (region II) is due to the removal of residual organic chemicals. At temperatures above 600 °C (third region III), a slight weight reduction (<1.3%) is observed. This corresponds to water losses due to condensation of the silanol groups to siloxane bonds[263]. All these small weight losses indicate that the template was removed successfully. TGA analysis of the glycidoxypropyl-mesoporous silica shows two main weight loss steps (Figure 31b), the first between 100 and 230 °C associated with residual solvent resulting in small mass loss (*ca.* 1.4%). The most important observation was the dramatic weight loss of 7% at about 230 °C resulting from highly exothermic oxidation of the glycidoxy group. The TGA curve of the HOC_{18}-nanoscavenger (Figure 31c) shows two main distinct weight loss steps, the first between 100 and 220 °C, associated with residual solvent. The second weight loss is the main organic oxidation between 270 and 550 °C resulting from a gradual oxidation of octadecyl groups, constituting about 27% weight loss, indicating the loading of the octadecyl groups on the surface of the silica. The remaining of the organic material was gradually burnt off between 550 °C and 650 °C. There was no significant weight loss after 650 °C.

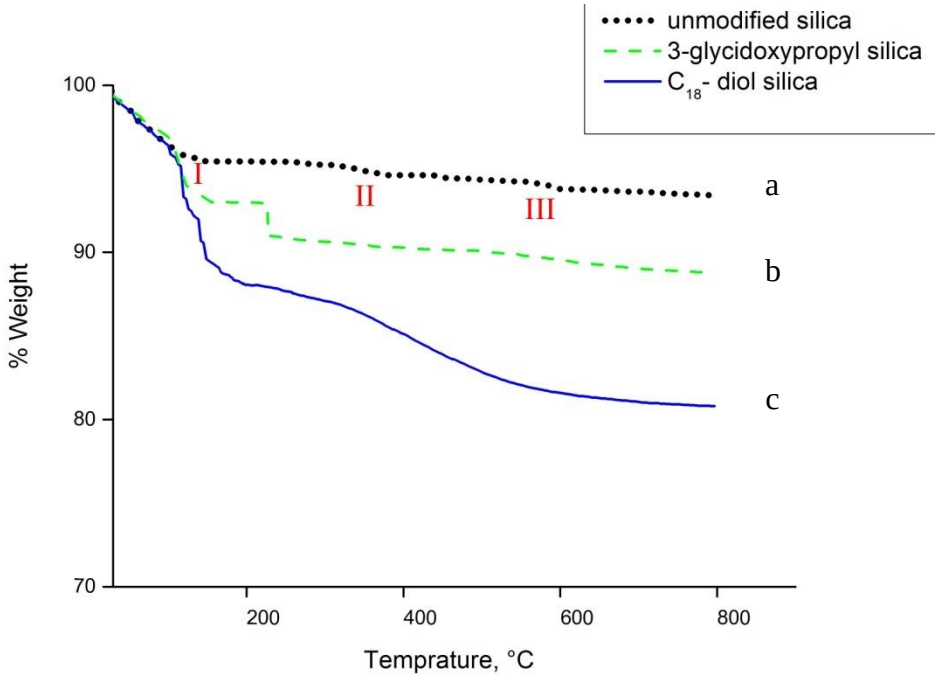


Fig. 31 Thermogravimetric curves of (a) unmodified mesoporous silica, (b) glycidoxypropyl-mesoporous silica and (c) HOC_{18}-nanoscavenger

6.4.2 Fourier transform infrared (FTIR) spectroscopy

Fourier transform infrared spectroscopy (FTIR) was carried out of the mesoporous silica before and after modification. Both spectra (Figure 32 a,b) show a broad band in the range 3000–3900 cm^{-1}, which is attributed to the hydroxyl groups from silanol, residual water and alcohol[264]. Hydroxyl groups will also have resulted from the hydrolysis of epoxy groups to their corresponding diols. The main absorption due to the silanol groups (around 3880 cm^{-1}) is hidden. The band at 1640 cm^{-1} is attributed to water and alcohol adsorbed on the silica surface[263]. Bands appearing between 1100 and 1060 cm^{-1} were associated with the Si–O–Si stretching[265]. The band at around 965 cm^{-1} was assigned to the Si–OH[264]. The most important bands of the spectrum of the HOC_{18}-nanoscavenger (Figure 32b) compared with that of the unmodified silica (Figure 32a) are bands for asymmetric and symmetric C–H stretching from $–CH_2$ and $–CH_3$ groups located at 2800–3000 cm^{-1}. Also, the band at 1468 cm^{-1} is related to C–H bending. The existence of CH_2 and CH_3 can confirm successful modification but cannot confirm whether the

modification on the the silica surface took place for both of the groups (octadecyl and glycidoxypropoyl) or for just one of them. However, the results obtained are closely in agreement with published data[266, 267].

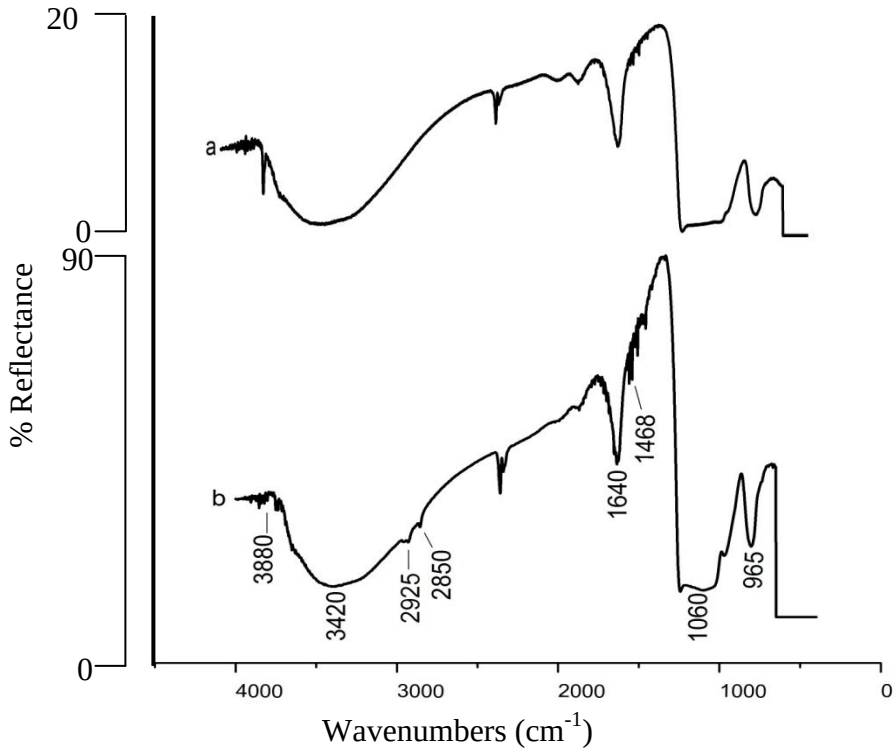


Fig. 32 Fourier transform infrared spectroscopy of (a)- mesoporous silica (before modification) and (b) the HOC_{18}-nanoscavenger

6.4.3 Scanning electron microscopy (SEM)

The surface morphology of the HOC_{18}-nanoscavenger was studied by SEM as described in Section 6.3.3. The SEM image shows the material to be spherically shaped with a mean diameter of 250 ± 80 nm based on the measurement of 150 individual particles using the SEM measurement tools (Figure 33).

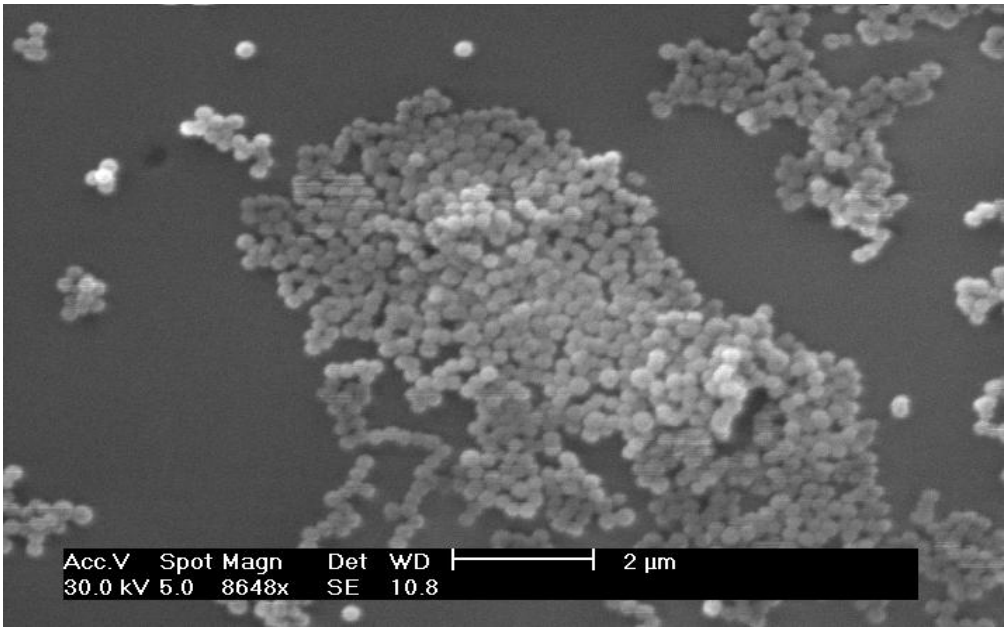


Fig. 33 SEM image of the HOC18-nanoscavenger

6.4.4 Nitrogen adsorption-desorption measurements

The nitrogen adsorption–desorption isotherms of the unmodified mesoporous silica (starting material) and the HOC_{18}- nanoscavenger (final product) show typical Type IV isotherms (IUPAC)[268] with a small hysteresis loop between the adsorption and desorption branches, which is associated with the presence of mesopores[269] (Figure 34). The BET surface area (using a multipoint method) of the mesoporous silica was very high (approximately 1610 m^2/g) before the modification and decreased after the modification with C_{18} and diol groups to 1200 m^2/g. This is due to the hydrophobic chain, which can block the pores and prevent the nitrogen molecules from entering[267]. The total pore volume, which was estimated from the amounts adsorbed at a relative pressure (P/P^0) of 0.99 was 0.76 $cm^3\ g^{-1}$. The BJH pore size of the HOC_{18}-nanoscavenger, calculated from the adsorption branch data, was 2.9 nm (Figure 35).

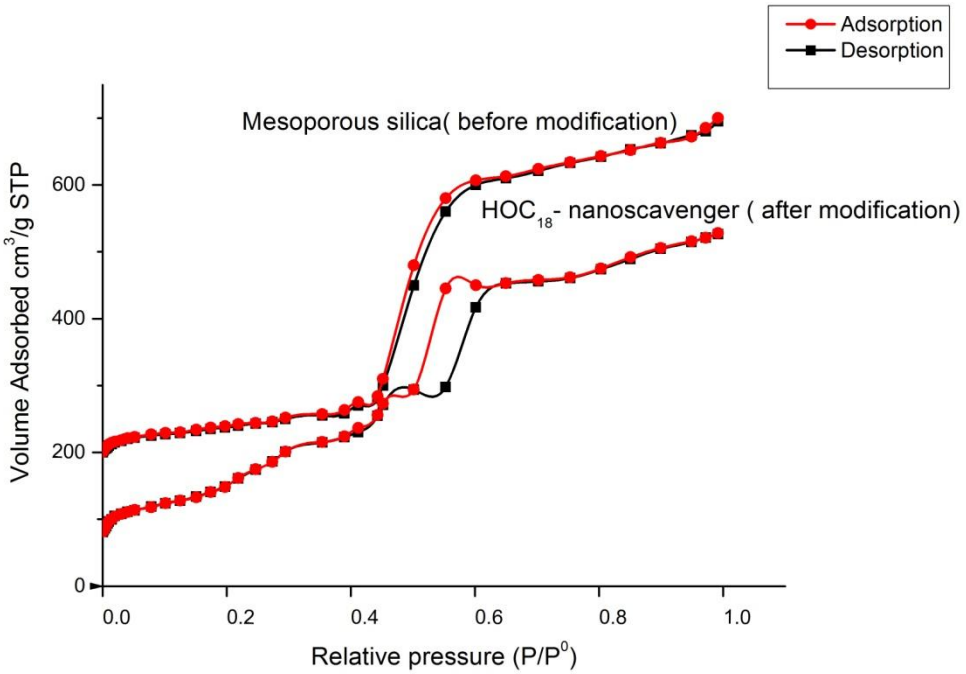


Fig. 34 Nitrogen adsorption–desorption isotherm of the unmodified mesoporous silica and the HOC_{18}- nanoscavenger

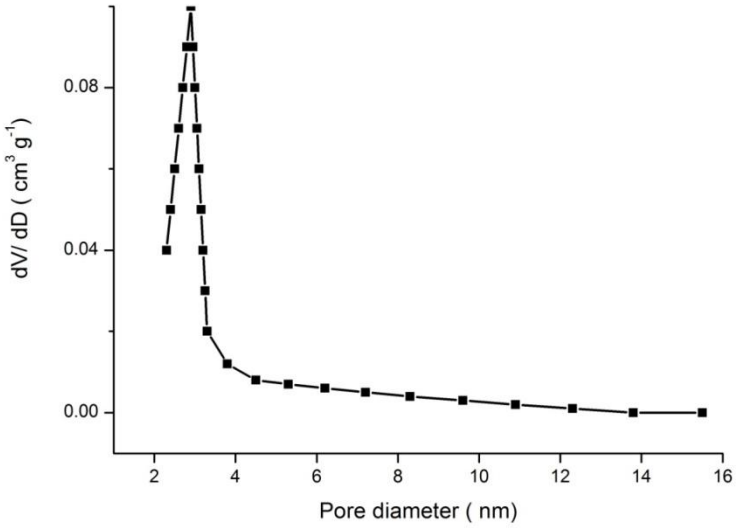


Fig. 35 BJH pore size distribution for the HOC_{18}- nanoscavenger

6.4.5 The octadecane content of HOC18-nanoscavenger

Measurement of the loading C_{18} on the surface of the HOC_{18}-nanoscavenger was carried out as described in Section 6.3.5. The cleavage products were mainly *n*-octadecane (96%) and small amounts of 1-octadecanol (4%). The released amounts of these compounds were analysed using GC-FPD and quantified using an internal standard method. The concentration of the released *n*-octadecane (C_{18}) on the surface of the silica was 0.67 mmol/g, while the concentration of the released 1-octadecanol was 0.03 mmol/g. The aqueous wettability and dispersion of the C_{18}-mesoporous silica was improved by conversion of the epoxy to a diol group (Figure 36a and b).

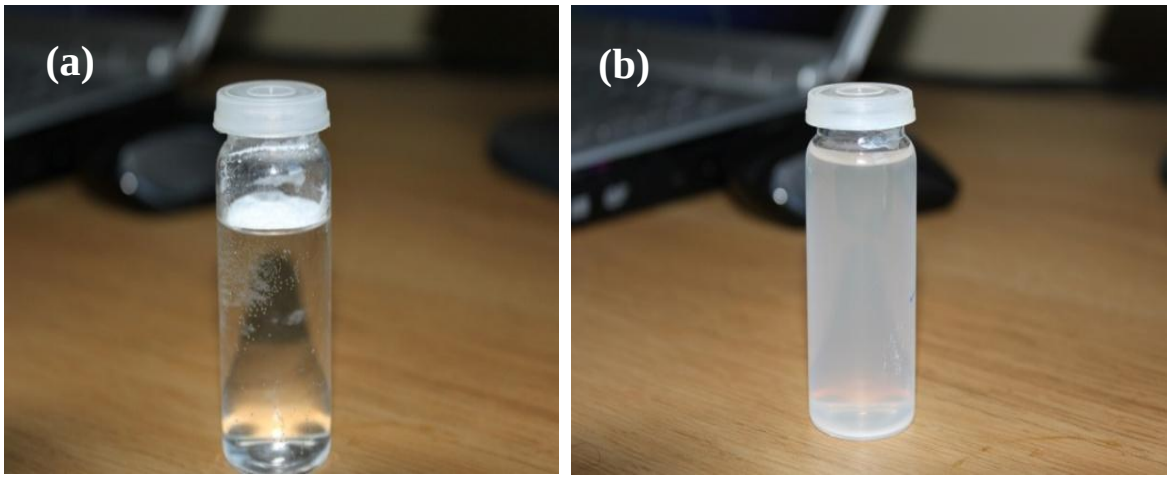


Fig. 36 Dispersion of the C_{18}-mesoporous silica in water (a) before opening the epoxy ring and (b) after opening the epoxy ring

6.5 Determination of butyltin compounds in water

The determination of TBT, DBT and MBT was carried out using the newly developed HOC_{18}-nanoscavenger for the SPDE extraction and preconcentration of the compounds from water. This was performed using two approaches: (a) butyltin compounds were collected on the surface of the HOC_{18}-nanoscavenger and then derivatized using a Grignard reagent (Grignard approach), (b) butyltin compounds were derivatized in aqueous solution using sodium tetraethylborate and the alkylated products were extracted using the HOC_{18}-nanoscavenger (sodium tetraethylborate approach).

6.5.1 HOC_{18}-nanoscavenger SPDE followed by Grignard derivatization

6.5.1.1 Introduction

Butylin compounds can be potentially collected from water by exploiting hydrophobic interactions between the solutes and the particles. Extraction is performed as the nanoscavenger particles move naturally through the samples and scavenge the analytes. Continuous physical movement is not therefore required. The analyte-loaded nanoscavenger can then be recovered from the sample by filtration. The collected butyltin species are derivatized using Grignard reagent and finally extracted back into *n*-hexane, before being quantified by gas chromatography with pulsed flame photometric detection.

6.5.1.2 Materials

All reagents were of analytical reagent grade unless otherwise stated. Chemicals that have been described in Section 3.2.1 were used. With the addition of sodium acetate (anhydrous, 99%) and glacial acetic acid (99.99%) were obtained from Fisher (UK). Sodium tetraethylborate ($NaBEt_4$, 97 %) was purchased from Acros Organics (UK) and was kept in a desiccator or glove box under a nitrogen atmosphere to prevent its degradation by atmospheric moisture. The 1 M pH 5 buffer solution (acetic acid/sodium

acetate) was prepared by mixing 21.4 g of sodium acetate with glacial acetic acid until a pH of 5 was obtained in 250 mL deionised water. Polyvinylidene fluoride membrane filters (47 mm diameter, pore sizes 0.22 µm) were supplied by Millipore (Billerica, USA).

6.5.1.3 Preparation of standards

Preparation of the standard solutions was carried out as described in Section 3.2.3, but the mixed working solutions were prepared dialy over the concentration range (0.5 to 2 µg Sn/ L).

6.5.1.4 Water sample collection

To assess the applicability of the analytical procedure, sixteen estuarine water samples were collected at high tide from different areas of Southampton Water during Jun 2010 (Figure 37). Samples were collected by submersing 2 L acid-prewashed, high-density polyethylene (HDPE) plastic bottles. The samples were acidified at pH 2 using conc. HCl to preserve the butyltin stability in the water[93]. The collected samples were immediately analysed without filtration to avoid losing the butyltins associated with suspended matter.

For the spiking experiment, an ´uncontaminated´ seawater sample was collected from a location expected to be relatively uncontaminated with tributyltin compounds. The sample was collected by Dickie B. from the Atlantic Ocean between Cape Verde Islands and the Canary Islands during February 2009. Its salinity was 33.8% and it was filtered using a glass fibre filter (47 mm pore size, MF300, Fisher).

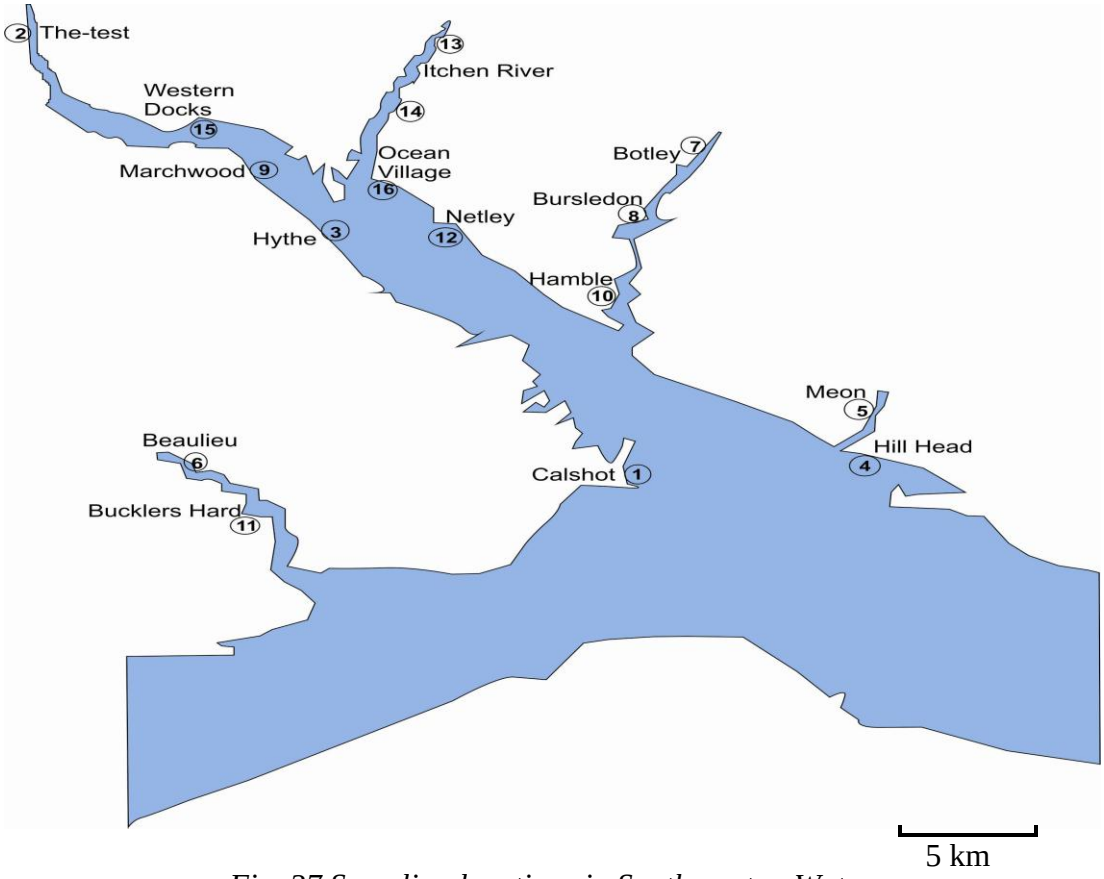


Fig. 37 Sampling locations in Southampton Water

6.5.1.5 Apparatus

The butyltin compounds were determined by gas chromatography PFPD system detection. The instrumental operating conditions have been described in Section 3.2.2.

6.5.1.6 Analytical procedure for determination of TBT, DBT and MBT

200 mg of HOC_{18}-nanoscavenger was sonicated in 10 mL of deionised water. The suspension was transferred to a 1000 mL volumetric flask, which was then filled to the mark with deionised water. 5 µL of a mixed standard solution of TBTCl, DBTCl, MBTCl and TPTCl (containing 0.5 µg Sn/mL as each compound) was added to the flask using a Hamilton microlitre syringe. The flask was left for 4 hours, the suspension was then vacuum filtered using a 0.22 µm Polyvinylidene fluoride (PVDF) membrane filter.

The filter paper was carefully transferred to a 20 mL glass vial and dried in a desiccator using activated silica gel desiccant for 6 hours. The dried filter paper was carefully cut into small pieces inside the PTFE glass vial. 2 mL of hexane were added into the glass vial and mixed well with the filter paper pieces. 1 mL of hexylmagnesium bromide (2 M in ether) was added and shaken violently. The reaction was left to proceed at *ca*. 20 ^{0}C for 2 hours. 2 mL of hydrochloric acid solution (5% v/v) was then added to quench the excess Grignard Reagent and the vial was shaken well. The hexane layer was transferred in a clean vial dried with anhydrous Na_2SO_4. The dried hexane layer was transferred to a 2 mL volumetric flask and the flask was made up to the mark using hexane. 5 µL of the solution was analysed by GC-PFPD.

6.5.1.7 Quantification

The identification of organotin compounds was based on their retention times and quantification was based on peak height. Figure 38 shows a typical chromatogram obtained by GC-PFPD to identify and quantify TBT, DBT and MBT from the spiked water samples. Quantitative analyses were performed using an internal standard method as described in Section 3.2.6. A 0.5 ng Sn/L solution of TPTCl was used as an internal standard to correct the concentration values of analytes for losses during manipulation, extraction inefficiency, incomplete conversion during derivatisation and evaporative losses. 0.5 ng Sn (as TPTCl)/L (IS) was added in with the butyltins standard solutions (0.5– 2 ng Sn/L) and 5 µL of the solutions was injected into the chromatograph daily. Linear calibration graphs were constructed by least-squares regression of the peak height ratio (analyte/IS) vs. concentration (Figure 39). Good linearity was found over the tested concentration range, with coefficients higher than 0.98 in all cases. A blank was generated by analysis of unspiked deionized water for TBT, DBT and MBT following the analytical procedure described in Section 6.5.1.6. Procedural blanks were performed for every set of experiments.

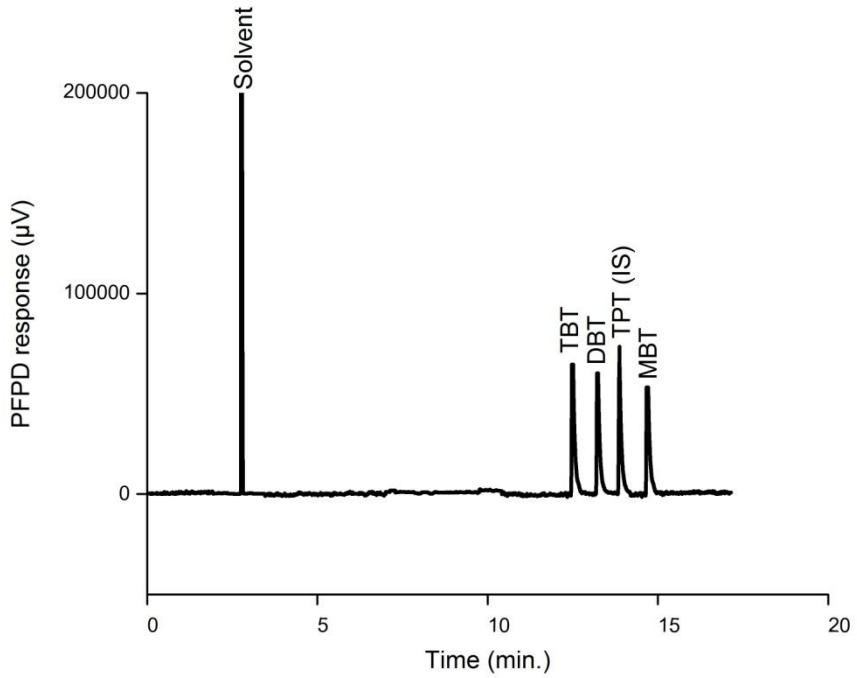


Fig. 38 Typical chromatogram of hexylated of butyltins derived from spiked deionised water samples. (Concentrations of all butyltins were 1.25 ng Sn / L)

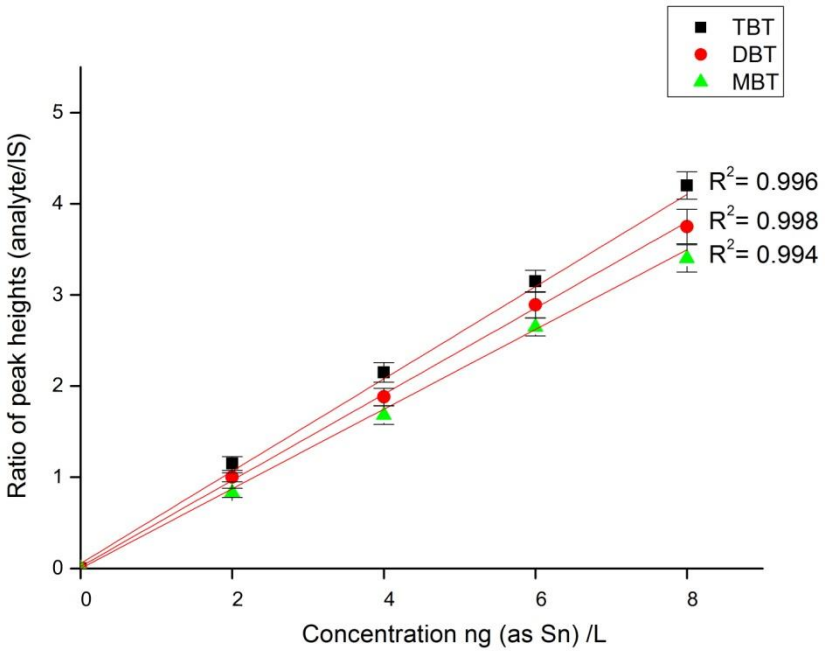


Fig. 39 Linearity of measurements established from the ratio of analyte and IS peak heights, covering the concentration range 0.5 to 2 ng Sn /L as TBT, DBT and MBT

6.5.1.8 Amount of dispersed HOC_{18}-nanoscavenger

The appropriate amount of the HOC_{18}-nanoscavenger required to achieve maximum extraction efficiency was examined. Six deionised water samples (1000 mL) were spiked with 5 µL of a mixed standard solution of TBTCl, DBTCl, MBTCl and TPTCl (containing 0.5 µg Sn/mL as each compound). 50, 100, 150, 200, 250 and 300 mg of the HOC_{18}-nanoscavenger were each dispersed in a butyltin solution. The dispersions were equilibrated, filtered, derivatized and analysed as described in Section 6.5.1.6.

6.5.1.9 Rate of butyltin chlorides uptake by HOC_{18}-nanoscavenger

The time required to achieve quantitative collection of butyitin chlorides from aqueous solution by nanoscavenger dispersion was assessed. Ten deionised water samples (1000 ml) were spiked with 5 µL of a mixed standard solution of TBTCl, DBTCl, MBTCl and TPTCl (containing 0.5 µg Sn/mL as each compound). 200 mg of HOC_{18}-nanoscavenger was dispersed in each solution. The dispersions were then left for different equilibrium times (30–300 min) prior to vacuum-filtration through 0.22 µm Polyvinylidene fluoride (PVDF) membrane filters. Each filter paper was transferred to a 20 mL glass vial and carefully cut into small pieces inside the glass vial. 2 mL of hexane was added to the vial and mixed well. After derivatization the hexane layer was transferred to a clean vial and analysed as described in Section 6.5.1.6.

6.5.1.10 Drying time effect

As the determination of butyltin compounds was carried out in aqueous solution, complete drying of the sample is necessary before Grignard derivatization. Different drying times were evaluated. Four deionised water samples (1000 mL) were spiked with 5 µL of 0.5 µg Sn/mL mixed butyltin standard solution. 200 mg of HOC_{18}-nanoscavenger was dispersed in each solution. The suspensions were left for 4 hours and then vacuum filtered. The filter papers were carefully transferred to 20 mL glass vials and allowed to dry for different drying times (4, 6, 8 and 10 h) in a desiccator using

activated silica gel desiccant. At the end of each drying period the hexane extracts were derivatized, isolated and analysed as described in Section 6.5.1.6.

6.5.1.11 Effect of hexylation reaction time

In order to assess the time required to obtain quantitative derivatization of the butyltin chlorides, six deioninsed water samples (1000 mL) were spiked with 5 µL of 0.5 µg Sn/mL mixed butyltin standard solutions and then extracted (Section 6.5.1.6). 1 mL of Grignard reagent was then added to each butyltin extract and the reaction was allowed to proceed for different reaction times (20, 50, 80, 120, 150 and 180 min.). At the end of each derivatization, 2 mL of aqueous sulphuric acid 5% (v/v) was added to destroy the excess Grignard reagent. After isolation, the hexane layer was analysed for butyltins according to the procedure described in Section 6.5.1.6.

6.5.1.12 Acidification effect

The effect of acidification of water samples on the butyltin extraction efficiency was investigated. This is necessary as many water samples need acidification to preserve the butyltin stability in water over a long time period to prevent the microbial activity responsible for butyltin degradation[92]. Therefore, it is important to investigate the suitability of HOC_{18}-nanoscavenger dispersion extraction for analysis of acidified water samples. Six deionised water samples (1000 mL) were spiked with 5 µL of 0.5 µg Sn/mL mixed butyltin standard solution. Three of the six samples were acidified at pH 2 using conc. HCl before analysing as described in Section 6.5.1.6. The rest of the samples were analysed following the same procedure, but without acidification.

6.5.1.13 Effect of salinity

The effect of salinity on the extraction efficiency was examined. This is essential as seawater samples have high salinity, which could affect the extraction efficiency. Three 1 L aliquots of deionised water and three 1 L aliquots of uncontaminated seawater were

spiked with 5 µL of a 0.5 µg Sn/mL mixed butyltin standard solution. All subsamples were analysed according to the procedure described in Section 6.5.1.6. Blank deionised water and seawater samples were also analysed and no butyltins were detected.

6.5.1.14 Extraction recovery

The extraction efficiency of the new procedure described in Section 6.5.1.6 was investigated using spiked deionised water and spiked seawater samples. An experiment was carried out in two stages. Stage 1 involved the analysis of the unspiked ´uncontaminated´ seawater and deionised water samples for TBT, DBT and MBT, as described in Section 6.6.1.6. In Stage 2, six ´uncontaminated' seawater samples (1000 mL) and six deionised water samples (1000 mL) were spiked with 5 µL of the 0.5 µg Sn/mL mixed butyltin standard solution (corresponding to 2.5 ng Sn/L as TBT, DBT and MBT). The spiked samples were analysed following the procedure as described in Section 6.5.1.6 with the results being calculated using the internal standard method.

6.5.2 Results and discussion of the HOC_{18}-nanoscavenger SPDE/Grignard derivatization

6.5.2.1 Amount of the extractant

The impact of changing the amount of nanoscavenger dispersed in the aqueous solution was assessed (Section 6.5.1.8). Maximum recoveries for all studied butyltin compounds were achieved with 200 mg of the HOC_{18}-nanoscavenger; no further improvement was observed above that quantity (Figure 40). This is a partition phenomenon with mass of the analyte extracted depending on the partition coefficient.

$$K = \frac{C_{aq}}{C_s} \rightarrow K = \frac{m_{TBT,aq}/V_{aq}}{m_{TBT,s}/m_s} \quad (6.3)$$

where, K is the partition coefficient $C_{aq}$and C_s are the concentration of the analyte in aqueous solution and solid phase, respectively, $m_{TBT,aq}$ and $m_{TBT,s}$ are the mass of TBT in aqueous solution and solid phase, respectively and V_{aq} and m_s are the volume of water and the mass of solid phase, respectively. This partition only works if there is a large excess of solid phase compared with the mass of solid required to hold the analyte. This was achieved by dispersion of 200 mg of the HCO_{18}-nanoscavenger. Larger quantities of the particles became aggregated and this affected their ability to collect butyltin from water.

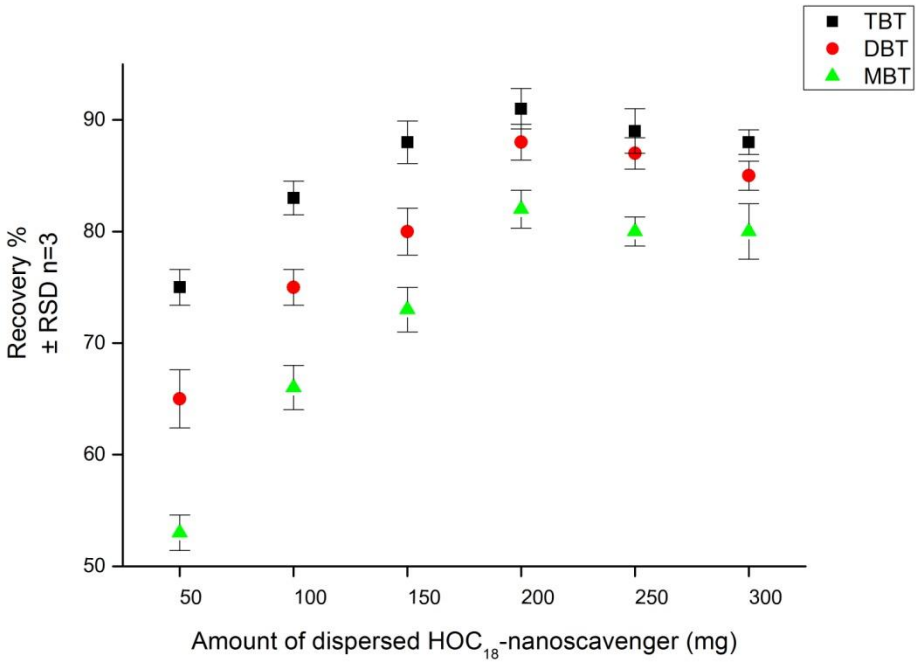


Fig. 40 Recoveries of butyltin compounds achieved with 200 mg HOC_{18}-nanoscavenger dispersed in 1 L deionised water containing 2.5 ng Sn/ L as TBTCl, DBTCl and MBTCl

6.5.2.2 Equilibrium time

The HOC_{18}-nanoscavenger was dispersed in spiked sample solutions for time periods ranging from 30 to 300 min (Section 6.5.1.9). Maximum recovery was achieved within 240 min and no significant improvement was observed after that time (Figure 41). This can be explained based on equilibrium principles. The time is required to allow the system to reach equilibrium. Movement of particles is slow and most particle/butyltin collisions may not result in binding anyway. Only after 4 hours is the equilibrium established.

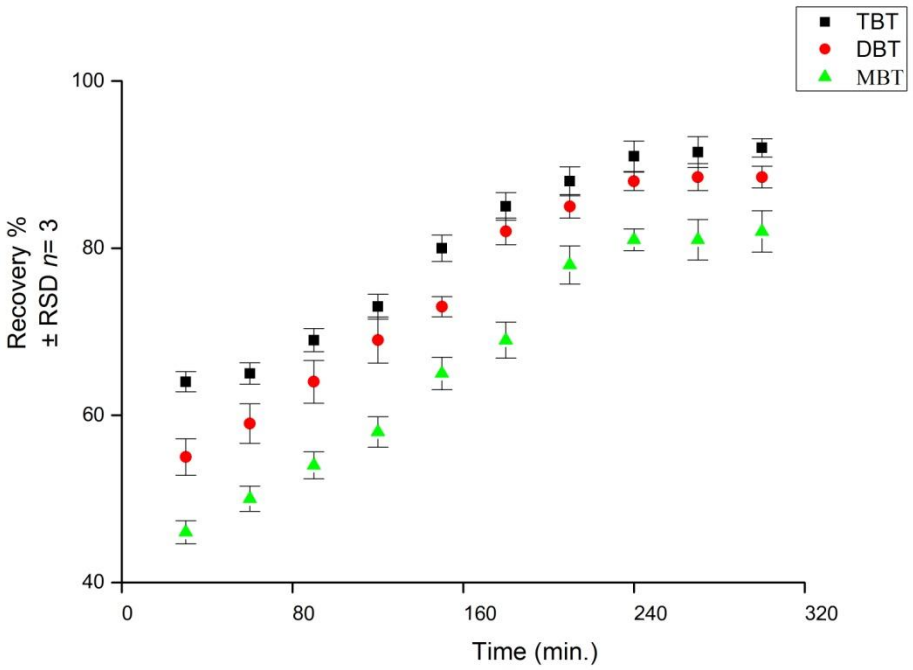


Fig. 41 Effect of time on butyltin chloride uptake by HOC_{18}- nanoscavenger (deionised water containing 2.5 ng S / L as TBTCl, DBTCl and MBTCl)

6.5.2.3 Effect of the drying time

Maximum recovery was achieved by drying the filter paper for 6 hours with no improvement being observed with longer drying (Table 24).

Table 24 Impact of differ drying times. (Spiked water samples containing 2.5 ng Sn/ L as each compound of TBT, DBT and MBT)

Time (min)	Recovery % ± RSD % (mean of 3 values)		
	TBT	DBT	MBT
240	84 ± 3	82.7 ± 4.1	77 ± 2
360	91 ± 2	88 ± 3	82.1 ± 2.3
480	91.5 ± 1.4	86.1± 3.2	80 ± 2
600	85.1 ± 2.2	85 ± 2	79.3 ± 1.7

6.5.2.4 Derivatization reaction time

Recoveries of butyltin compounds were gradually increased by increasing the reaction time up to 120 min, but no significant improvement was obtained after that time (Figure 42). Therefore, the derivatization reaction time was set at 120 min for subsequent work. The reasons for this are: some butyltin chloride may be localized within the pores of the nanoscavenger and some of the nanoscavenger particles are held inside the pores of the filter paper. Therefore, the Grignard reagent needs time to penetrate the pores to react with all the butyltin chloride. In addition, some of the Grignard may destroyed by localized wet regions in the silica.

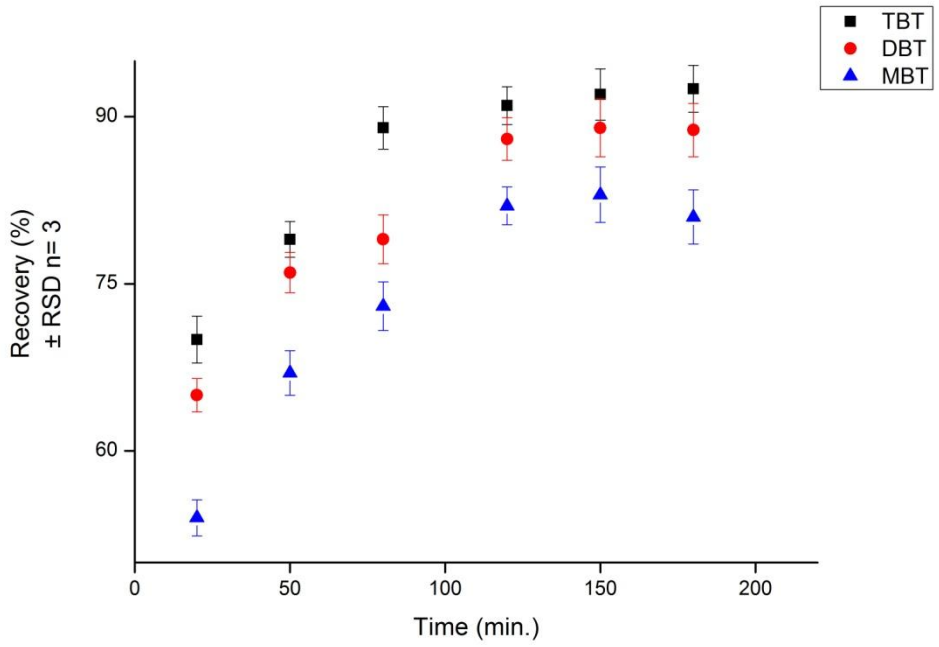


Fig. 42 Recoveries of butyltin compounds achieved with different derivatization times

6.5.2.5 Acidification effect

Generally, acidification in real water samples may increase the solubility of butyltin compounds in water[270] and therefore decrease their affinity for hydrophobic sites on the HOC_{18}-nanoscavenger particles. However, acidification might not have the same effect on spiked samples, as butyltin compounds are introduced in their chloride forms, which are hydrophobic. In our experiment, as expected, acidification of the spiked deionised water did not affect the extraction yield of the butyltin compounds (confirmed by *t*-test at 95% confidence) indicating that the HOC_{18}-nanoscavenger dispersion method is suitable for analysis of acidified or non-acidified water samples.

6.5.2.6 Effect of salinity

The effect of salinity on the extraction efficiency of the HOC_{18}-nanoscavenger dispersion extraction was investigated (experiment in Section 6.5.1.13). With seawater samples (Figure 43) the extraction of all butyltin compounds was higher than with deionised water samples (confirmed by *t*-test at 95% confidence). This is because the presence of the salt increases the ionic strength of the aqueous solution and affects the solubility of organic solutes. This can be explained by the engagement of water molecules around the ionic salt (salting-out effect). This reduces the local concentration of water available to dissolve the butyltin compounds. Also, the chloride ions present in seawater associate with the hydrated cations of butyltin compounds forming covalent butyltin chlorides. This result indicates that the HOC_{18}-nanoscavenger dispersion extraction method was as suitable for the analysis of saline water samples as it is with low ionic strength water samples.

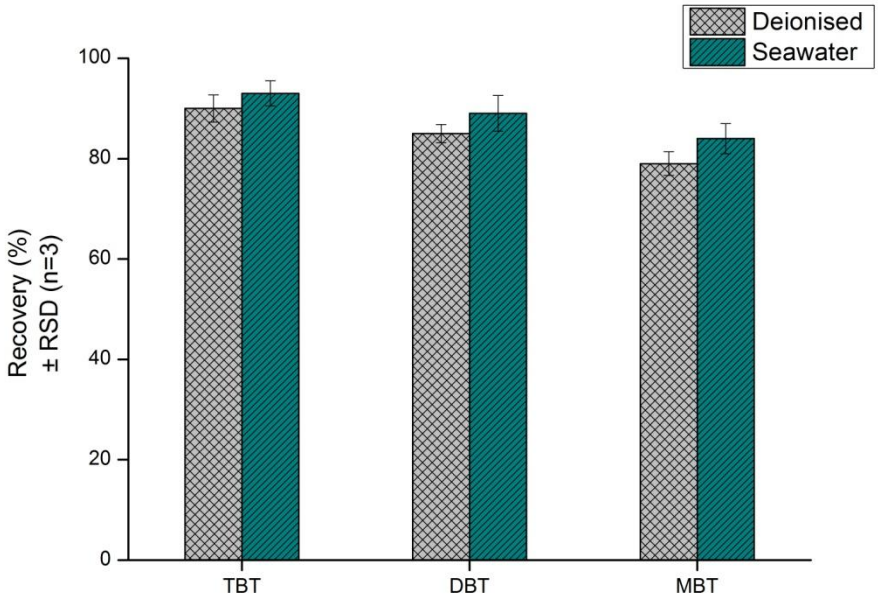


Figure 43 Effect of salinity on the recovery of butyltin compounds from spiked water samples containing 2.5 ng Sn/ L as each compound of TBT, DBT and MBT

6.5.2.7 Extraction efficiency

After applying all the changes to the analytical procedure, the effectiveness of the nanoscavenger dispersion extraction/Grignard derivatization method was assessed. Analysis of the unspiked 'uncontaminated' seawater and unspiked deionised water showed them to contain less than the detection limits (1.5 ng Sn (as TBT)/L, 1 ng Sn (as DBT)/L and 2 ng Sn (as MBT)/L. On spiking with butyltin compounds to a level of 2.5 ng Sn/L, recoveries of 94% ± 2%, 90% ± 4% and 86% ± 3% in seawater and 91% ± 3% , 88% ± 2% and 82% ± 3% in deionised water were obtained for TBT, DBT and MBT respectively (experiment described in Section 6.5.1.14).

6.5.2.8 Detection limit

The limit of detection was derived from the lowest injected butyltin concentration that yielded a signal of three times the noise from a blank. A blank was measured four times

per day over 4 days, yielding an average noise of 300 ± 120 µV. However, the concentration detection limit depends on the sample size, the concentration of the extract and the amount injected into the GC. In the present study, 1000 mL of water was extracted, and the extract was concentrated to 2 mL, then 5 µL were analysed. The detection limits obtained for the three different species were: for TBT 1.5 ng Sn/ L, DBT 1 ng Sn/ L and MBT 2 ng Sn/ L. These detection limits are comparable with other similar analytical techniques[90] and just adequate for the UK environmental quality standard (EQS), set at 2 ng/ L[68].

6.5.2.9 Repeatability and reproducibility

Repeatability was assessed as the variability of the measurements obtained from the analysis of six aliquots of ´uncontaminated' seawater repeatedly, following the procedure described in Section 6.5.1.6. Reproducibility was assessed as the variability of the measurements obtained by the analysis of six aliquots of ´uncontaminated' seawater on three different days. In both cases a procedural blank was also analysed. The repeatability and reproducibility values are expressed as relative standard deviation (RSD %). For repeatability, values better than 7% were obtained for all compounds. For reproducibility, values better than 13% were obtained for all compounds.

6.5.2.10 Application to Southampton Water samples

The applicability of the developed method, described in Section 6.5.1.6, to real water samples was studied. Sixteen estuarine real water samples were collected from different areas of Southampton Water. Southampton Water was selected to assess the impact of TBT antifouling paint in coastal waters, since both commercial vessels and pleasure yachts are in regular use in this area. TBT, DBT and MBT concentrations found in these areas are presented in Table 25. TBT concentrations ranged from 1.6 to 8.1 ng Sn/L with an average concentration of 3.9 ± 1.1 ng Sn/ L, the DBT from 1.2 to 5.2 ng Sn/ L with an average concentration of 2.4 ± 0.6 ng Sn/ L and the MBT ranged from 3 to 3.6 ng Sn/ L with an average concentration of 3.3 ± 0.5 ng Sn/ L. Generally, concentrations of

TBT in the samples were below or near the UK environmental quality standard (EQS), set at 2 ng/L, with all samples collected from estuarine areas frequented by pleasure craft alone. However, higher concentrations of TBT compared with the UK EQS were observed mainly in areas frequented by large commercial vessels. The highest concentrations of butyltin compounds were found in the Western Dock region. The average concentrations of triplicate TBT analysis in this area was 8.1 ± 1.2 ng Sn/L (four times higher than the UK EQS). Other high TBT concentrations were observed at the Marchwood Port, with 5.3 ± 1.0 ng Sn/L. This port has been used intensively by UK naval and supply vessels. Also, high TBT concentrations (4.9 ± 0.9 ng Sn/L, more than two times of the UK EQS) were observed at the Hamble marina. This is understandable as many commercial fishing vessels (> 25 m), which may have had made legal use of TBT, before the ban, are moored in this region. Also, the marina has a heritage of refitting and maintaining racing yachts, therefore high TBT would be expected in that region. TBT was determined at 5.5 ± 1.1 ng Sn/L in the sample obtained from Hythe marina. This is surprising, since only small boats (< 25 m) are moored in this marina and it was only opened in 1988[68], after the ban in TBT on small vessels was applied in the UK[9]. This may suggest a small amount of illegal TBT antifouling paints was used in this area and that there is a legacy deposit in this area. Similar observations about this marina have been reported previously[68, 271]. Two samples were collected from different locations on the estuary of the Itchen River (Sampling point 14 and 15). TBT was found only at sampling point 15 (collected about 0.5 km from the Cobden Bridge on the Itchen River estuary). This is to be expected, as some small boatyards are located in this area. High concentrations of DBT and MBT were found in samples collected from Western Docks and Hamble Port. This is because the boat activity is high and large commercial vessels are frequent in this area, and the water circulation in both marinas is restricted. Therefore, conversion of TBT to DBT and MBT by sunlight or by microbial activity is more likely. The results indicate that TBT is still found in significant levels in some marinas of Southampton Water. These results can be compared with those obtained in 1998 for the same area[68] and the TBT concentrations do not appear to have fallen substantially during this period. Sources of TBT in Southampton Water especially at the

marina sites include the historical use of antifouling paint and the effect of tidal transport of re-suspended sediments. Regarding the ecotoxicological effects of TBT, and taking into consideration the UK environmental quality target of 2 ng/L, the contents found in Southampton Water could be adversely affecting the ecosystem and living organisms present in the environment.

Table 25 TBT, DBT, and MBT concentration (ng Sn/L) in the Southampton Water

Sampling Points[a]	Location	Position	Salinity ($^{o}/_{00}$)	Butyltin concentration (ng Sn/L ± S.D.,*n*=3[b])		
				TBT	DBT	MBT
1	Calshot	50° 48.26′N,01° 18.55′W	33.3	4.5 ± 1.0	< DL[c]	< DL
2	River Test	50° 55.43′N,01° 29.13′W	0.20	< DL	< DL	< DL
3	Hythe Marina	50° 52.46′N,01° 23.89′W	29.7	5.5 ± 1.1	1.5 ± 0.4	< DL
4	Hill Head	50° 48.85′N,01° 13.72′W	30.6	1.6 ± 0.6	< DL	< DL
5	River Meon	50° 59.14′N,01° 07.18′W	0.20	< DL	< DL	< DL
6	Beaulieu	50° 49.87′N,01° 27.69′W	0.21	< DL	< DL	< DL
7	Botley	50°49.14′N ,01°18.31′W	0.30	< DL	< DL	< DL
8	Bursledon	50° 53.11′N,01° 17.46′W	26.6	< DL	< DL	< DL
9	Marshwood	50° 54.07′N,01° 27.36′W	26.6	5.3 ± 1.0	2.1± 0.7	< DL
10	Hamble Marina	50°51.05′N, 01°18.39′W	30.0	4.9 ± 0.9	3.1± 0.9	3.0 ± 0.5
11	Bucklers Hard	50°48.10′N,01° 25.38′W	29.7	2.1 ± 0.5	< DL	< DL
12	Netley	50° 53.55′N,01° 23.28′W	31.0	2.3 ± 0.8	< DL	< DL
13	Itchen River at Park side	55° 56.04′N,01° 22.28′W	0.20	< DL	< DL	< DL
14	Itchen River at Cobden Bridge	50° 55.523′N,01° 22.43′W	3.00	2.4 ± 0.7	1.5 ± 0.4	< DL
15	Western Docks	50°54.03′N, 01° 24.53′W	27.5	8.1 ± 1.2	5.2 ± 1.0	3.6 ± 0.7
16	Royal Victoria	50° 51.57′N, 01° 20.43′W	26.8	1.9 ± 0.9	< DL	< DL

[a] Three subsamples.

[b] Sampling points are shown on the map Fig. 37.

[c] Values are below the detection limit of the developed method (1.5 ng Sn/ L, 1 ng Sn/ L and 3 ng Sn/ L for TBT, DBT and MBT respectively).

6.5.3 Ethylation followed by HOC18-nanoscavenger SPDE

6.5.3.1 Introduction

Another approach to the determination of tributytin compounds in water samples was ethylation of the TBT species using sodium tetraethylborate ($NaBEt_4$) and collection of the ethylated butyltin using the HOC_{18}-nanoscavenger. The ethylated TBT-loaded nanoscavenger can be recovered from the sample by filtration, then extracted back into *n*-hexane before being quantified by gas chromatography with pulsed flame photometric detection. The conventional ethylation of TBT using $NaBEt_4$ has been described elsewhere[90, 272, 273]. The $NaBEt_4$ ethylates TBT in an aqueous solution buffered at pH 5. Therefore, the use of tetraethylborate as a derivatizing agent for TBT in water samples seems to be convenient as the direct derivatization of the analyte in aqueous solution is possible, resulting in a reduction in the number of analytical steps.

6.5.3.2 Calibration curve

The standard solution of tri(*n*-butyl)ethyltin (*n*-Bu_3EtSn) in hexane was obtained from the Quality Assurance of Information for Marine Environmental Monitoring in Europe (QUASIMEME) project office. This contained 10.2 µg Sn/L. From this solution a stock solution containing 500 µg Sn / L as *n*-Bu_3EtSn in *n*-hexane was prepared. This was kept in the dark, refrigerated at 4 ºC. A series of standard solutions (0.5 to 2 µg Sn / L) was prepared daily in *n*-hexane, by diluting from the stock solution.

6.5.3.3 Method

970 mL of deionised water was transferred to a 1 L volumetric flask. 5 µl of 1 µg Sn (as TBTCl /mL solution was added to the flask using a Hamilton microlitre syringe (corresponding to 5 ng Sn /L of TBT). The pH of the solution was adjusted to 5 by adding 20 mL of the 1 M pH 5 buffer solution (acetic acid/sodium acetate). 50 mg of sodium tetraethylborate was weighed in the glove box and directly added to the solution. The solution was shaken violently for 2 min. The reaction was then left to proceed at *ca.* 20 ^{0}C for 20 min. 200 mg of HOC_{18}-nanoscavenger was sonicated in 10 mL of deionised water and the suspension was transferred to the TBT solution and left for 2 hours. The suspension was then vacuum filtered using a 0.22 µm Polyvinylidene fluoride (PVDF) polymer membrane filter. The filter paper was carefully transferred to a 20 mL glass vial and dried in a desiccator using activated silica gel desiccant for 30 min. The dried filter paper was carefully cut into small pieces inside the PTFE glass vial. 2 mL of hexane was added into the glass vial and mixed well with the filter paper pieces. The hexane was removed from the filter paper pieces and dried with activated Na_2SO_4. The dried hexane was transferred to a 2 mL volumetric flask, which was then filled to the mark with hexane. 5 µL of the solution was analysed by gas chromatography.

6.5.3.4 Quantification

The identification of tri(*n*-butyl)ethyltin was based on its retention time and quantification was based on peak height (Figure 44). Quantitative analyses were performed using an internal standard method (described in Section 6.6.1.7). 0.5 ng Sn (as TPTCl) /L (IS) was added in with the tri(*n*-butyl)ethyltin standard solutions (0.5 — 2 ng Sn /L) and 5 µL of the solutions was injected into the GC-PFPD daily. Linear calibration graphs were constructed by least-squares regression of the peak height ratio (analyte/IS) vs. concentration (Figure 45). Good linearity was found over the tested concentration range, with a regression coefficient of 0.998. A blank was generated by analysis of unspiked deionized water for TBT following the analytical procedure described in Section 6.6.4.3. Procedural blanks were performed for every set of experiments.

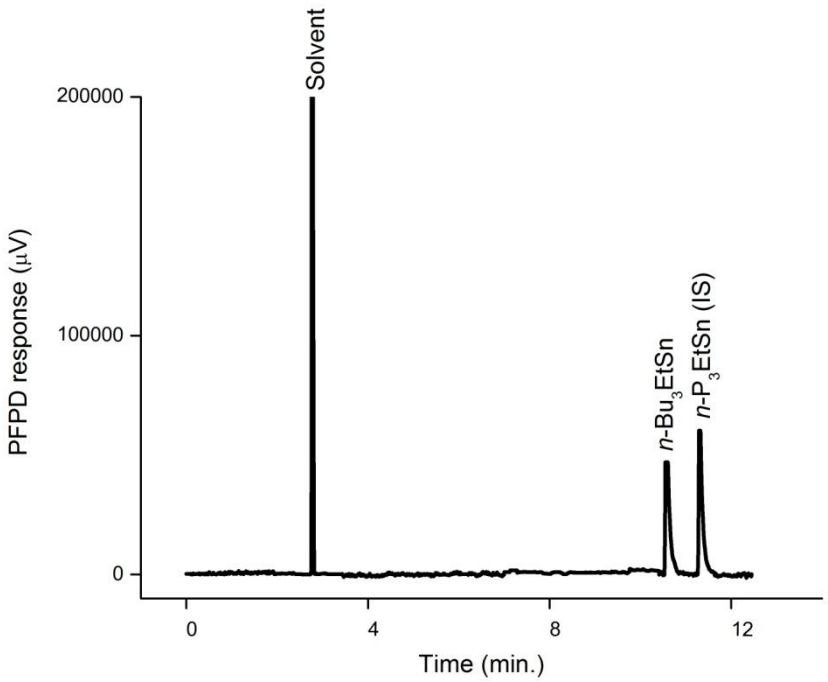


Fig. 44 Typical chromatogram obtained from the ethylation of TBT derived from a spiked deionized water sample (concentration 1.25 µg Sn (as TBT) / L)

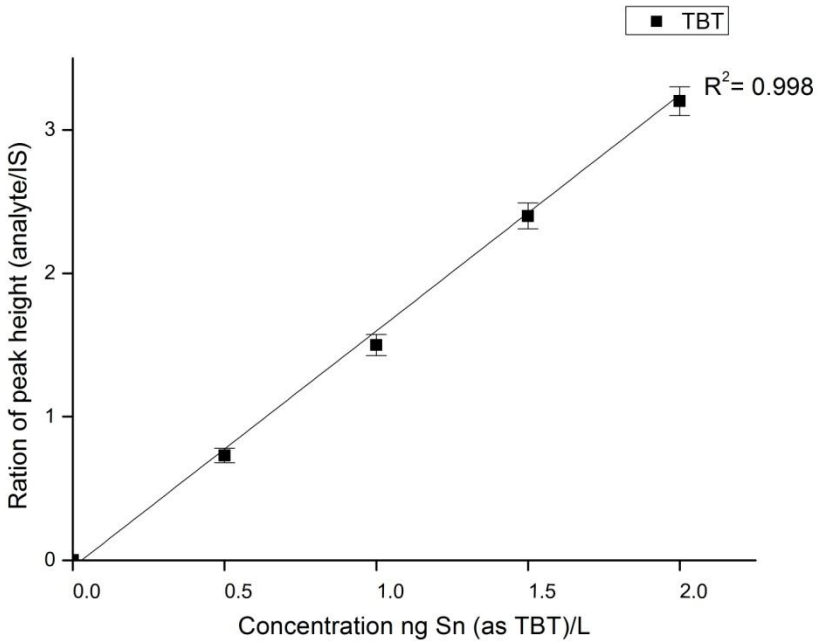


Fig. 45 Linearity of measurements established from the ratio of analyte and IS peak heights, covering the concentration range 0.5 to 2 ng Sn (as TBT) /L

6.5.3.5 Amount of sodium tetraethyl borate reagent

The amount of sodium tetraethyl borate required to achieve maximum derivatization reaction was assessed. Six deionised water samples (1000 mL) were spiked with 5 µL of a 1 µg Sn (as TBTCl)/mL solution. The pH of the solutions was adjusted to 5 using acetic acid/sodium acetate buffer solution. Different amounts of sodium tetraethylborate (20, 30, 40, 50, 60 and 70 mg) were added to the solutions. The solutions were shaken violently for 2 min and the reaction was then left to proceed at *ca*. 20 ^{0}C for 20 min. After the derivatization reaction, 200 mg of HOC_{18}-nanoscavenger was dispersed in each solution and then left to equilibrate for 2 h. The solutions were then vacuum filtered, extracted with hexane and analysed as described in Section 6.5.3.3.

6.5.3.6 Rate of n-Bu_3EtSn uptake by HOC_{18}-nanoscavenger

The rate of *n*-Bu_3EtSn extraction by the HOC_{18}-nanoscavenger was investigated. Six deionised water samples (1000 mL) were spiked with 5 µL of a 1 µg Sn/mL standard solution of *n*-Bu_3EtSn. 200 mg of HOC_{18}-nanoscavenger were dispersed in each solution. The solutions were then left for different equilibriation times (30, 60, 90,120, 150 and 180 min). At each the end of the equilibration time, the solutions were vacuum filtered, extracted with hexane and analysed as described in Section 6.5.3.3.

6.5.3.7 Extraction recovery

To evaluate the recovery of TBT obtained for the sodium tetraethylborate approach described in Section 6.5.3.3, four ´uncontaminated' seawater samples (1000 ml) and four deionised water samples were spiked with 5 µL of a mixed standard solution of TBTCl and TPTCl (containing 1 µg Sn/mL as each compound). This was corresponding to 5 ng Sn/L of TBT). The spiked samples were analysed following the procedure described in Section 6.5.3.3. Blank solutions of the seawater and deionised water samples were also analysed and no TBT was detected. The results were calculated using the internal standard method.

6.5.4 Results and discussion of the ethylation/ HOC_{18}-nanoscavenger method

6.5.4.1 Amount of derivatizing reagent

Varying the quantity of sodium tetraethylborate (20–70 mg) showed lower yields when 20 mg of the reagent was employed, but no significant improvement was obtained using more than 50 mg, confirmed by t-student test at 95% confidence, (Figure 46). 50 mg was therefore used for subsequent work.

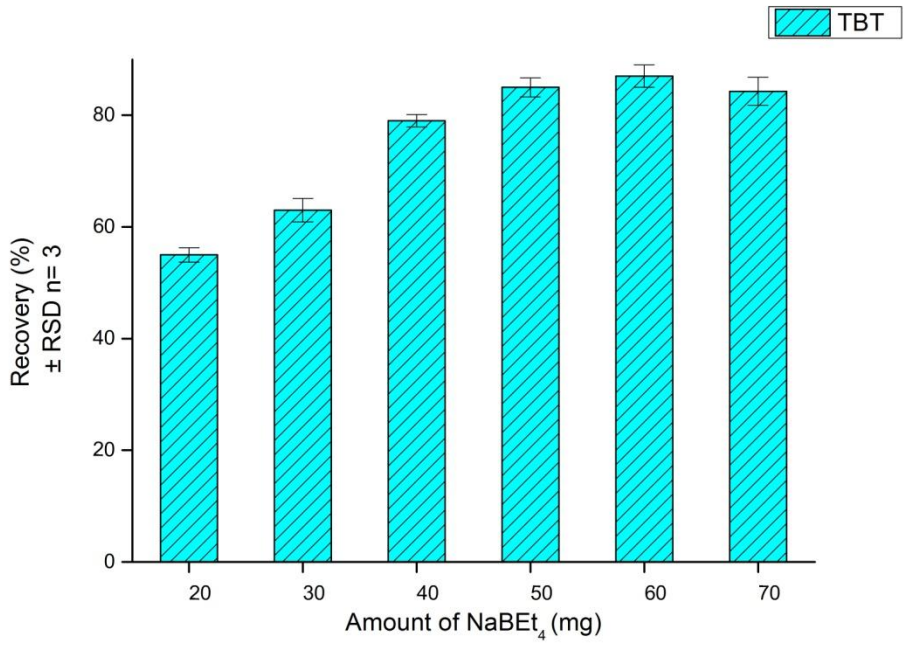


Fig. 46 Recoveries of TBT using different quantities of $NaBEt_4$ (mean values and RSD (n = 3) are represented). (Spiked deionised water containing 5 ng Sn /L as TBTCl)

6.5.4.2 Rate of n-Bu_3EtSn uptake by octadecyl-diol modified silica

n-Bu_3EtSn was extracted from water using HOC_{18}-nanoscavenger over a range of equilibration times (30 to 180 min) (experiment in Section 6.5.3.6). Maximum recovery was achieved within 120 min. and no significant improvement was observed after that time (Figure 47).

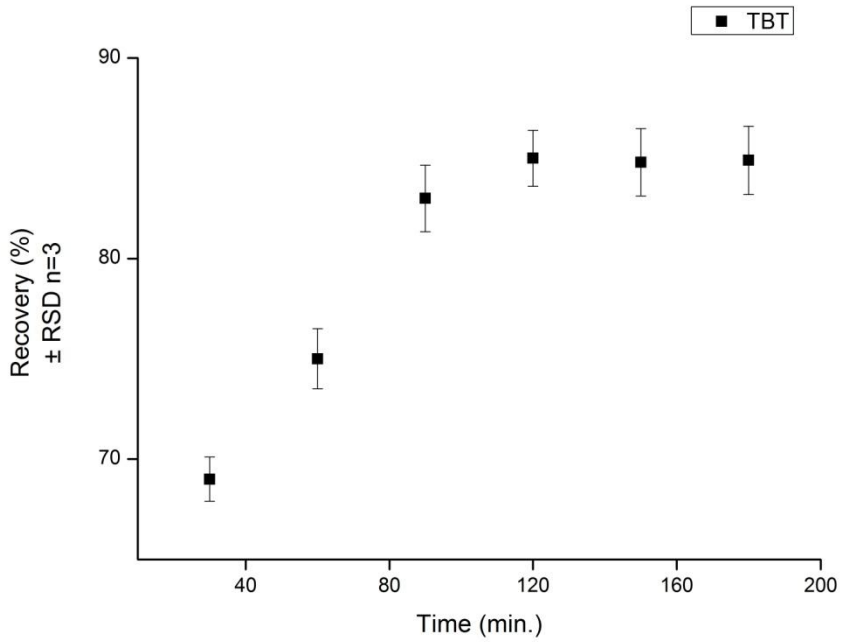


Fig. 47 Effect of time on TBTCl uptake by $HOCC_{18}$- nanoscavenger (spiked deionised water at a level of 5 ng Sn / L as TBTCl)

6.5.4.3 Extraction efficiency

Analysis of the unspiked 'uncontaminated' seawater and deionised water showed the samples to contain less than the detection limit (3 ng Sn (as TBT)/L). On spiking to a level of 5 ng Sn (as TBTCl) /L ,recoveries of 88% ± 1% in seawater and 85% ± 2% in deionised water were found for TBT (experiment described in Section 6.5.3.7).

6.5.4.4 Detection limit

The detection limit of the method was calculated as the concentration which gives three times the standard deviation of the peak heights for six replicates of the blank. The detection limit obtained for TBT was 3 ng Sn/ L, when l000 mL of water sample was extracted, the volume of the final extract was 2 mL and 5 µL was analysed.

6.5.4.5 Repeatability and reproducibility

Repeatability of the method was assessed by analysing six aliquots (1000 mL) of ´uncontaminated' seawater repeatedly, following the procedure described in Section 6.5.2.3. Reproducibility was assessed by analysis of six aliquots of ´uncontaminated' seawater on three different days. In both cases a procedural blank was also analysed. The repeatability and reproducibility values are expressed as relative standard deviation (RSD%). Values of 3% and 8% were obtained for repeatability and reproducibility, respectively.

6.5.4.6 Comparison of the two analytical approaches

The two analytical approaches, the Grignard approach and the sodium tetraethylborate approach, were compared for: (a) extraction recovery, (b) detection limit, (c) precision and (d) the duration of analytical steps. The Grignard approach yields a higher TBT extraction recovery from seawater and deionised water than is obtained by the sodium tetraethylborate approach. This is due to the higher reactivity of the Grignard reagent. Also, the product of the Grignard reaction with TBT (MHTBT) has a higher affinity for the hydrophobic sites on the HOC_{18}-nanoscavenger than the n-Bu_3EtSn, as the affinity of the organic compounds will increase as the alkyl chain attached to the tin atoms is increased. A lower TBT detection limit (1.5 ng Sn/L) was obtained with the Grignard approach than with the sodium tetraethylborate approach (3 ng Sn/L). The precision was assessed by examining repeatability and reproducibility of the detector response using the two approaches. The sodium tetraethylborate approach shows itself to be more

precise than the Grignard method. This is because, with Grignard reagent, additional analytical steps are needed, owing to the necessity for a drying environment and destruction of the excess Grignard. This can lead to an increase in the complexity of analytical procedure and variability in the results. Grignard reagents offer the possibility of changing the alkyl chain length for the derivatization to meet specific separation requirements. However, the Grignard method is time consuming, and requires strict anhydrous conditions and non-protic solvents, which necessitates solvent exchange when polar solvents are used as extracting agents. The sodium tetraethylborate approach has the advantage of simplicity and a shorter duration of analytical steps in comparison with the Grignard approach, as the derivatization step is carried out directly in aqueous media leading to a consequent reduction in the number of analytical steps.

6.6 Conclusions

An analytical procedure has been developed for the determination of butyltin compounds in water samples. This procedure involved the use of HOC_{18}-nanoscavenger as a replacement for organic solvents in the extraction and pre-concentration of butyltin compounds from water samples based on the solid phase dispersion extraction technique (NSDE). The nanoscavenger was prepared from mesoporous silica templated using C_{12}TMABr. The surface of the silica was sequentially modified with glycidoxypropyl groups and then with octadecyl groups. Increasing the wettability of the particles was achieved by converting the epoxide ring to a diol groups. The morphology of the particles was found to be spherical with a size in the range 200-290 nm. These particles can be suspended within the sample solution for several hours, during which time they can selectively scavenge dissolved species from solution. This specific particle size makes the dispersion, filtration and centrifugation process straightforward. Two approaches were developed for the determination of butyltin compounds from aqueous solution based on the use of NSDE; 1) - butyltin compounds were collected from the water on the surface of the HOC_{18}-nanoscavenger and the butyltins loaded-nanoscavenger were derivatized using Grignard reagent then analysed using GC-PFPD. 2) - In aqueous solution, buffered at pH 5, TBT was ethylated using sodium tetraethylborate ($NaBEt_4$) and the ethylated product was collected from the aqueous solution using the HOC_{18}-nanoscavenger. The amount of HOC_{18}-nanoscavenger and the time required to establish partition equilibrium of butyltin between an aqueous sample and the HOC_{18}-nanoscavenger were found to be the most important parameters affecting the extraction recoveries. The two approaches were shown to be suitable for trace analysis of acidified and non-acidified water samples and spiked deionised and seawater samples. Quantitative recovery of the butyltin compounds was achieved using the nanoscavengers solid phase dispersion. The TBT detection limits achieved in the both approaches were below or near the UK environmental quality standard (EQS) value for TBT in waters, set at 2 ng Sn /L[68]. The benefits, therefore, of using these methods is that satisfactory detection limit is achieved without long extraction time or time-consuming

concentration steps, so that many samples can be quickly analysed. The performance of the two analytical approaches (Grignard approach and $NaEt_4B$ approach) was compared. Lower detection limit and higher extraction yield of TBT was achieved with Grignard approach than with $NaEt_4B$ approach , but the $NaEt_4B$ approach was more precise than the Grignard approach. There are many advantages of the use of nanoscavenger as extractant. Large numbers of samples can be quickly treated, and this can be carried out at the sampling location, stabilizing the analyte during transportation and pre-analysis storage. The use of small quantities of organic solvents makes nanoscavengers a potentially environmentally friendly alternative extraction technique. The SPDE technique based on the HOC_{18}-nanoscavenger can therefore be a good replacement for conventional solvent extraction.

7 Final conclusions and future work

The main aim of this study has been to evaluate current methods for the measurement of tributyltin (TBT) and its breakdown products in environmental samples and to develop new methods for their determination. The project has been in two main parts: the first being focussed on the measurement of TBT, DBT and MBT in sediment, the second focussing on the analysis of water. Difficulties can arise in the analysis of sediments due to the presence of paint particles containing high concentrations of TBT. Paint can be deposited in sediment during boat maintenance (Chapter 4). An additional problem arises from the presence of high concentrations of sulphur compounds in the sediment which can interfere with butyltin quantification (Chapter 5).

The second part of this thesis has covered the development of new analytical methods for the determination of TBT, DBT and MBT in water (Chapter 6). A novel nanoscavenger (chemically modified mesoporous silica) was synthesized and used to replace of organic solvent in the extraction of butyltin compounds from water, based on a dispersion extraction (NSDE) technique. Initial work was directed towards the synthesis and characterization of a nanoscavenger with selective physico-chemical properties (particle size, shape, surface area and surface chemistries). Once this had been achieved, a new analytical procedure was developed for the determination of butyltins in water, based on the dispersion of the doubly functionalized nanoscavenger.

7.1 The measurement of butyltins in sediments

An analytical procedure has been developed based on acidification of the sample with acetic acid followed by hexane/tropolone extraction followed by Grignard derivatization to give tetraalkylated butyltins. The instrumental analysis was performed by gas chromatography coupled with pulsed flame photometric detection (GC-PFPD). Instrumental repeatability of the butyltin compound measurements (TBT and DBT) were 5% ± 2.1% and 6% ± 1.9% respectively, which was within the range of reported

values in the literature[90, 183]. Quantitative recoveries of the target analytes (93, 96 and 87% for TBT, DBT and MBT respectively) from the spiked sediment were obtained using the developed method. The detection limits for the analysis of 0.5 g dry weight of sediment and a final extract volume of 25 mL were: 5µg Sn (as TBT)/ kg, 7µg Sn (as DBT)/ kg and 11µg Sn (as MBT)/ kg . This limit is below the UK environmental quality target (EQT) value for TBT in sediments, set at 1-2 mg Sn/ kg[201]. Satisfactory detection limits have been achieved without extended extraction times or lengthly concentration steps; many samples can therefore be quickly analysed. The overall accuracy of the method was confirmed using the sediment certified reference material PACS-2.

The main difficulties encountered during the use of this method arose from the analysis of sediment samples with high TBT-paint particle content or having a high concentration of sulphur. TBT-containing paint particles needed harsh extraction conditions to open up the paint structure and release the TBT. The sulphur-rich sediments required a clean-up step to remove the sulphur compounds that could interfere with butyltin measurement[157]. In the determination of butyltin compounds in environmental samples there is still a large uncertainty in the reported results, making it difficult to compare data obtained by different groups and in different regions.

During the determination of low levels of butyltins all analytical steps (sample collection, extraction, derivatization and detection) are possible sources of errors[91]. An important task is therefore to assess the most reliable quantification method. Two different quantification methods were investigated in this study (external and internal standard methods) and the precisions and linearities of the two quantification methods were compared. Precision was assessed by investigating the repeatabilities and reproducibilities of the two methods. Better linearity and precision were observed with the internal standard method. This is expected because during the analytical steps, if a known quantity of standard is added to the unknown prior to any manipulations, the ratio of standard to analyte remains constant because the same fraction of each is lost in any step. Additionally, by having the analyte and the standard analysed in the same run, the run to run variability is eliminated giving more precise results. Other advantages of

using internal standards are the saving of time and money by having fewer instrumental runs. The internal standard method is therefore recommended for the most accurate determination of butyltin compounds in environmental samples.

7.1.1 Can TBT levels be predicted by the measurement of total Sn in sediment?

TBT analyses are lengthier and more difficult to perform than most total element analyses. The co-variation of the TBT concentration in sediment with total tin or other elements could therefore be very useful in the prediction of the presence of TBT in a particular area. This was investigated using sediments collected from a small boatyard in Southampton, UK; the idea being to use simple measurements of total tin to predict the distribution of organotin contamination. Total tin was measured in the sediments using XRF. The correlation between total tin levels and the concentration of TBT showed four different groups of sediment samples having unrelated levels of total tin and TBT contamination. Due to different sources of tin contamination in this area, XRF analysis of Sn alone does not appear to accurately predict the areas where highly TBT-contaminated sediment is found. Further work is necessary to explore relationships with other elements such as, Cu, Zn, Pb, Ni and Cr, which are easily measured using XRF, and to use multivariate statistical analysis to determine whether a correlation of TBT with total Sn and with other elements can be found. This work can be performed by generating a huge pool of TBT concentration data and total element measurements in different sediment samples and by carrying out multivariate statistical analysis of these data. This statistical modelling would be used to predict the presence of TBT in coastal sediment.

7.1.2 Extracting TBT from flakes of paint

During the determination of TBT in sediment, the major consideration is no longer the determination itself, but the extraction of TBT from particles of paint deposited into

sediment during boat refurbishment. In order to solve this problem, naturally-aged paint samples was isolated from the sediment and analysed for TBT. Characterization of the paint samples showed significant concentrations of Cu, Zn, Sn, Ti and Pb, all of which have been commonly used in paint manufacture. Pretreatment procedures were developed based on the treatment of the paint with dichloromethane and ultrasonicating followed by hexane/tropolone extraction. DCM was chosen as it is known to be a swelling solvent, causing the polymer structure to open out and TBT to be released. The DCM pretreatment reduced the number of sequential extractions required to maximize the extraction of available TBT from the paint from five to three and increased the total quantity of TBT extracted from the paint by 50 %. This may be attributed to the ability of DCM to penetrate the paint flakes and extract more TBT. One of the most important difficulties rising during this procedure is the need for a solvent exchange step. The exchange of DCM for hexane was necessary in order to avoid the Grignard reacting with the solvent. Losses of TBT were however observed during the exchange of DCM with hexane, which were believed to contribute to the increased variability of the results. Therefore, improved control of TBT losses is required during the evaporation step.

7.1.3 Identification and removal of sulphur interferences

During the determination of butyltin compounds in the Southampton sediment samples, sulphur interferences were observed. Whilst elemental sulphur is present naturally in marine sediment, alkylsulphides were also produced during the derivatization of the sediment extract[217]. Overlapping of some butyltin compound peaks and the sulphur compounds were observed. Re-optimization of the column oven temperature program resulted in a good separation of the interfering peaks and the problem of co-elution was completely eliminated and the optimization of the PFPD gate setting improved the detector selectivity to tin over that of sulphur. Additionally, the use of activated copper and a C_{18} solid phase extraction together removed completely the sulphur interferences, improving the quantification of the butyltin compounds. The main developed method was validated using the sediment certified reference material PACS-2. The advantages

of the developed clean-up procedure over alternatives are the effective removal of elemental sulphur and alkylsulphides interferences without butyltin losses during the clean-up step. The approach is rapid and simple.

7.1.4 The distribution of butyltin compounds in Southampton coastal sediments

TBT, DBT and MBT were measured in Southampton coastal sediments. TBT and DBT were found in all sediment samples collected from a boatyard on the River Itchen in Southampton. Generally, the high TBT levels were associated with sediments near the slipway area, where boat maintenance (cleaning and painting) had been carried out. Paint debris can remain for long periods of time, leaching out slowly into the water column. The average levels found were twice the UK environmental quality standard for TBT in sediments (1-2 mg Sn/ kg[201]). The direct input of TBT from antifouling paints into the water gives rise to contamination of water and sediment near the slipway area. Far away from the slipway area (about 0.5 km upstream from the boatyard) high concentrations of TBT were still found. The effects of tidal transport of resuspended sediments is likely to be the main source of the upstream TBT contamination[94]. TBT was still found in this area at high concentrations, even though it has been banned from use on small vessels since 1987. These levels appear to present a potential risk for a varity of marine organisms in this area. Therefore, continued research into the elucidation of the pathways and persistence of this pollutant in the natural environment is required. Also, appropriate remedial treatment is needed in this area to reduce the sources of butyltins.

7.2 Determination of butyltins in water using NSDE

Sub-micron sized chemically modified silicas have been shown to be suitable replacements for organic solvents in the extraction and preconcentration of butyltin organic compounds from aqueous solution; the approach having been demonstrated by

the extraction and preconcentration of tributyltin compounds from water using the doubly functionalised mesoporous silica (HOC_{18}-nanoscavenger) by the solid phase dispersion extraction.

The size of the nanoscavenger particles was deliberately chosen to be around *ca.* 250 nm. Particle size was a key point in the synthesis of this material. Very small particles (less than 100 nm) are difficult to recover from a suspension in water and small particles can be lost during filtration or centrifugation. Large particles (>500 nm) on the other hand, rapidly sediment under gravity and therefore mechanical suspension of the scavenger in the samples is required. 250 nm is an optimal size, offering good equilibrium times between the nanoscavenger and the analyte, resulting in high recoveries[244]. In our study, mesoporous silica was prepared from TMOS using a C_{12}TMABr template. This resulted in material with a surface area of around 1610 m^2 g^{-1} and uniform spherical particles *ca.* 250 nm in diameter. The dual functionalised nanoscavenger was prepared from this silica by the attachment of diol and C_{18} alkane groups. In general terms, the C_{18} groups are responsible for collecting tributyltin compounds from water based on hydrophobic interactions and the diol groups improve the dispersion of the nanoscavenger in the water.

A new analytical procedure has been developed for the determination of TBT in water, based on this dual functionality nanoscavenger. The analytical procedures were carried out in two approaches: in the first approach tributyltin compounds were collected from the water on the surface of the nanoscavenger by hydrophobic interactions. After recovery of the particles by filtration, the collected tributyltin compounds were derivatized using a Grignard reagent and then extracted back into hexane before analysis. In the second approach, the butyltin chlorides were ethylated in aqueous solution using sodium tetraethylborate and the ethylated products were collected on the surface of the nanoscavenger and then extracted back into hexane before analysis. The amount of HOC_{18}-nanoscavenger, the equilibrium time and the duration of the derivatization reactions were found to be the most important parameters affecting the extraction recoveries. In both approaches, the detection limits were satisfactory (1.5 and

3 ng Sn (as TBT)/ L for the Grignard and $NaEt_4B$ approaches respectively). There were both below or near the UK EQS of 2 ng Sn (as TBT)/ L. This detection limit was regarded as satisfactory, as a concentration step could be later incorporated if necessary to improve the procedural detection limit. Quantitative recoveries of TBT were obtained using the two approaches (94% and 88% for the Grignard and $NaEt_4B$ approaches respectively). The % RSDs achieved with the two approaches were approximately 7% for TBT spiked samples. The good recoveries, appropriate detection limits and satisfactory repeatabilities for butyltins make the solid phase dispersion techniques novel and promising approaches to the extraction of organic materials from water.

7.2.1 The advantages of nanoscavenger dispersion extraction

Nanoscavenger solid phase dispersion extraction is an effective means of determining butyltin compounds in water samples and the approach has several advantages over other extraction techniques, such as liquid-liquid extraction, solid phase extraction cartridges or solid phase extraction disks. Sample handling steps are minimized since the nanoscavenger can dispersed directly into the water sample without any additional treatment. Large numbers of samples can be quickly and simultaneously treated, even at the sampling site. Performing the dispersion in the field is very useful especially when unstable analytes are involved. When samples have to be transported from the field to the laboratory, losses of analyte can occur, for example by degradation or volatilization. Solid phase dispersion, when applied in the field, can reduce or eliminate such losses. Furthermore, carrying out this process in the field limits contamination of the sample during transportation, storage or in the laboratory environment. One of the most important physical advantages of this procedure is less human or mechanical effort is needed as no mechanical agitation is required. Even with the large number of samples and large volume samples, the particles move naturally through the sample by Brownian motion and sedimentation collecting the analytes. This method can be used with both Grignard or sodium tetraethylborate derivatization approaches. In addition, the technique is environmentally friendly as smaller volumes of organic solvents are used

than with the other extraction methods and only small quantities of nanoscavenger (50-200 mg) need to be dispersed. All of these advantages combine to make nanoscavenger solid phase dispersion extraction a powerful tool in the analysis of butyltin compounds in water samples and very useful in monitoring programs assessing butyltin contamination in natural water systems.

7.2.2 Distribution of tributyltins in Southampton Water

Butyltin compounds have been shown to still be present in the marine environments in several other countries. It was therefore considered to be important to investigate the situation in Southampton Water. Southampton Water samples were analysed for TBT, DBT and MBT using the nanoscavenger solid phase dispersion method. Generally, the levels of TBT were very much dependent upon the types of activity at each location. Samples collected from the Western Docks, the Marshwood Port, Hamble Marina, Hythe Marina and Calshot contained TBT at concentrations between 4 and 8 ng Sn (as TBT)/L. At other locations, subject to normal yachting activity, TBT concentrations were below the UK Environmental Quality Standard, of 2 ng Sn/L[68] . Although the use of TBT has been banned in the UK, the need for regular monitoring of TBT concentrations in UK coastal area is still required as significant concentrations of this compound are still to be found around some marinas. These contaminated materials can be transferred to different areas through the tidal movement. Suitable remediation methods may therefore have to be employed to remove or destroy TBT sources in Southampton Water.

7.3 Future work

The growing interest in butyltin measurements of environmental samples is reflected in the increasing number of papers published on the subject since 1980[1]. This increase has been generated by significant improvements in analytical methods and instrumentation. On the other hand the legislative restrictions on butyltin use in many western countries and the monitoring of these compounds in various ecosystems have contributed to increased interest in butyltin analysis. The legacy of TBT pollution of the environment may remain for a considerable period of time and many aspects related to the investigation of butyltin compounds in environmental samples still need to be further developed. This can be for a number of reasons: (1) high levels can still be found around some marinas and docks even though legislation is in place. (2) These concentrations are high enough to cause harmful effects toward a wide range of organisms. (3) In various countries no legal regulations exist on the use of butyltin compounds. (4) Limited research has been carried out into butyltin pollution in many developing countries[274]. The following sections describe a number of research areas that still require attention in this field of study.

7.3.1 Determination of TBT in paint

Removal of TBT-antifouling paint by scraping, blasting or hosing usually takes place on the hard standings of marinas and harbors and the generated paint particles are readily transported into the marine environment where they become interspersed with sediments, creating TBT hot spots[275]. During the determination of TBT in sediment a major consideration is the ability of the analytical method to extract TBT from within the centre of flakes of paint. Most research on TBT pollution has focused on extracting TBT from particles of sediment and biotic samples with no specific research having focussed on the determination of TBT in the aged paint deposited into sediment. TBT present as TBT-containing paint particles increases the TBT persistence in sediment. Extracting TBT from the paint particles is difficult as TBT is strongly attached to the

polymer and is inside the paint structure[28]. Therefore, improvements are necessary to the extraction methods used in the determination of TBT in paint particles. Opening out the paint particles by pre-treatment with DCM before the main extraction step improved the extraction of TBT , but has still not proven the extraction to be quantitative. To do so will require the development of paint reference materials of known TBT content. Further work could include improving the TBT losses during the removal of DCM and improving the extraction of TBT from paints using harsher extraction conditions. For example, the use of microwave assisted extraction, ultrasonic assisted extraction or accelerated solvent extraction. A balance must be achieved between the effectiveness of the extraction and the instability of the TBT and such studies must be based on aged paint samples containing a known TBT content.

7.3.2 Extraction of butyltin compounds using nanoscavengers

The HOC_{18}-nanoscavenger has successfully extracted butyltin compounds from water samples. The extracted butyltins has been derivatized using two different derivatizing agents (Grignard and sodium tetraethylborate) prior to GC determination. However, the detection method and extraction efficiency of sodium tetraethylborate approach still needs further development. A new procedure based on the use of sodium tetraethylborate with the HOC_{18}-nanoscavenger could be developed. In this procedure, the derivatization and extraction of TBT will be carried out in aqueous solution simultaneously. This approach seems to be convenient as sodium tetraethylborate can work in aqueous media. By dispersing the nanoscavenger and adding the sodium tetraethylborate, at the same time, to water samples spiked with the target analytes, the extraction and derivatization steps can be carried out simultaneously. Such an approach will reduce the sample handling and manipulation steps, thus reducing possible losses of analytes and saving analysis time.

7.3.3 Extraction of TBT and its determination as total tin

A simpler technique could be developed to extract and determine TBT based on the HOC_{18}-nanoscavenger dispersion extraction. This is based on the extraction of TBT from water using the HOC_{18}-nanoscavenger followed by its redispersion in water and then direct aspiration of the dispersion into ICP-MS or ICP-OES instrumentation to measure total tin content. This approach assumes the selective extraction of alkyltins from the water, with the nanoscavenger rejecting the inorganic species; total tin measurement of the nanoscavenger would give a good estimate of organotin compounds. This procedure would simplify sample handling and manipulation. Also, in this procedure the derivatization step is not required and this eliminates a potential source of uncertainty in the final results and can reduce analysis time significantly.

7.3.4 TBT in humans

No known adverse human health effects of the TBT have been documented yet[276] , but TBT enters the human body through the consumption of the TBT-contaminated shellfish and fish.[277] Cooking is not effective in removing butyltin compounds from foods.[278, 279] The estimated daily intake of TBT (from cooked food) based on a survey conducted in 1992 in Japan, was 6.7 μg/person[279]. The daily intake estimates for total butyltins in contaminated fish from the Asia-Pacific regions were, for comparison, in the range 0.04-2.1 μg/person.[280] These observations show that humans are exposed to butyltin compounds via their food intake. Very little work has focused on the determination of TBT in human tissues and blood. For example Kannan *et. al.* [281] found an average concentration of TBT in human blood of 4.59 ± 3.37 ng/ mL. Investigations of experimental toxicity, dietary intake, and the potential human health effects of TBT are clearly still necessary.

7.3.5 Other applications of nanoscavengers

Many different types of modified mesoporous silicas can be prepared making this field of research applicable to a wide range of other applications. Potential applications for these functionalized materials are in the fields of controlled drug delivery, catalysis and separation. By modifying silica particles with organic or inorganic groups the affinity of the particles can be tailored to a specific application. A wide range of organic materials can be extracted on the surface of nanoscavengers by hydrophobic interaction. This is can be achieved by modifying of the silica surface with different organic groups. Indeed, large particle size modified silica has been widely and successfully used for the pre-concentration of drugs and pesticides. It is possible to selectively extract target analytes or to improve the extraction of the analytes by modification of the silica surface with specifically selected organic or inorganic groups such as chelating agents or ion exchange groups[282] having high affinities toward the target analytes. Nanoscavengers have for example, been successfully used to extract and pre-concentrate a wide range of metal ions by chelation[244]. The modification of mesoporous silica with different groups could help in the removal of interferences from samples. For example, the removal of alkylsulphides from sediment extract during the determination of butyltin compounds could potentially be achieved by producing a new nanoscavenger modified with organic groups having a high affinity towards the alkylsulphide compounds. Such procedure could offer a very simple, rapid and effective clean-up procedure.

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