Journal of Chromatography, 580 (1992) 191–214
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam

CHROMBIO. 6346

Review

Chromatographic separation of marine organic pollutants

H. Hühnerfuss and R. Kallenborn

Institut für Organische Chemie der Universität Hamburg, Martin-Luther-King-Platz 6, W-2000 Hamburg 13 (Germany)

(First received December 23rd, 1991; revised manuscript received February 4th, 1992)

ABSTRACT

A review and discussion of the chromatographic separation of marine organic pollutants is given, including sampling and clean-up procedures, fractionation and enrichment of marine pollutants, capillary gas chromatography (cGC) and high-performance liquid chromatography applying both classical and chiral stationary phases. The potential of multi-dimensional cGC for the analysis of marine organic trace pollutants is discussed for polychlorinated biphenyls (PCBs). The chromatographic separation of coplanar PCBs and of the enantiomers of chiral pollutants provides a further insight into the toxic potential of these marine organic pollutants.

CONTENTS

Correspondence to: Dr. H. Hühnerfuss, Institut für Organische Chemie der Universität Hamburg, Martin-Luther-King-Platz 6, W-2000 Hamburg 13, Germany.

0378-4347/92/$15.00

LIST OF ABBREVIATIONS

AAS	Atomic absorption spectrometry
AHH	Aryl hydrocarbon hydroxylase
cGC	Capillary gas chromatography
DCPP	2-(2,4-Dichlorophenoxy)propionic acid
p,p'-DDE	*p,p'*-Bis(4-chlorophenyl)-2,2-dichloroethene
DDT	1,1-Bis(4-chlorophenyl)-2,2,2-trichloroethane
ECD	Electron-capture detection
EI	Electron impact ionization
EROD	7-Ethoxyresorufin-O-deethylase
FAAS	Flame atomic absorption spectrometry
FPD	Flame photometric detection
GC	Gas chromatography
GPC	Gel permeation chromatography
α-HCH	α-1,2,3,4,5,6-Hexachlorocyclohexane
γ-HCH	γ-1,2,3,4,5,6-Hexachlorocyclohexane
HPLC	High-performance liquid chromatography
HPTLC	High-performance thin-layer chromatography
ICP-AES	Inductively coupled plasma atomic emission spectrometry
ICP-MS	Inductively coupled plasma mass spectrometry
LC	Liquid chromatography
MID	Multiple-ion detection
MS	Mass spectrometry
NPD	Nitrogen–phosphorus detection
OLLE	On-line liquid–liquid extraction
PAHs	Polycyclic aromatic hydrocarbons
PAR	4-(2-Pyridylazo)resorcinol
PCB	Polychlorobiphenyl
β-PCCH	β-1,3,4,5,6-Pentachloro-1-cyclohexene
γ-PCCH	γ-1,3,4,5,6-Pentachloro-1-cyclohexene
SIM	Selected Ion Monitoring
TBT	Tributyltin
TLC	Thin-layer chromatography

1. INTRODUCTION

At present, about 60 000 organic substances are being used by mankind, and presumably some thousands of these substances are being transferred into the marine ecosystem. Therefore, it would appear to be hopeless to aim at a comprehensive analysis of all anthropogenic marine compounds. A more appropriate approach would include the main pollutants and their degradation products that may be assumed to contribute to the toxic potential for the most part. However, a reliable assessment of the toxic potential of marine pollutants in turn depends on appropriate analytical techniques. This review provides an insight into the most recent achievements in this field.

Investigations on "organic carbon in sea water" were performed as early as 1909 by Pütter [1]. However, as the concentrations of biogenic and anthropogenic marine substances are of the order of nanograms per litre or in the low picograms per litre range, many analytical problems had to be solved. In particular, the complex matrix of organic compounds gave rise to co-elution problems with several compounds. As a consequence, concentrations of PCBs and DDT

identified in the earlier studies sometimes were significantly higher than their concentrations in marine samples collected after 1985 [2–4]. These observations may reflect an actual decline in polychlorobiphenyl (PCB) and 2,2-bis(4-chlorophenyl)-1,1,1-trichloroethane (DDT) levels in the open ocean or an improvement in analytical methods, including confirmation of analyte identities by gas chromatography–mass spectrometry (GC–MS) [2]. In the earlier studies, PCBs and pesticides were determined by single-column, single-detector analysis [nearly exclusively GC with electron-capture detection (ECD)]; confirmation analysis of identified analytes was not performed. This basic matrix problem inspired many workers to improve clean-up and fractionation techniques. This review takes into account the vital importance of this first step in the analysis of marine compounds by giving a brief overview over some recent developements in this field.

Regarding the determination of marine organic compounds, two chromatographic methods have been dramatically improved in the last decade, *viz.*, high-performance liquid chromatography (HPLC) and capillary gas chromatography (cGC). As a consequence, separations of homologous compounds, isomers and enantiomers of chiral compounds have been achieved in problematic cases that had given rise to co-elution in earlier studies. Because of the dominance of these two analytical methods, this review focuses upon these two techniques.

A new field of marine analysis that has been advanced in recent months is the determination of chiral compounds at trace levels. This new experimental approach was possible because new chiral stationary phases have been developed, which include, in particular, cyclodextrin derivatives. Hence the fate of many prochiral or chiral pollutants can be monitored in the marine ecosystem with regard to enzymatic (*e.g.*, microbial) and non-enzymatic (*e.g.*, photochemical) degradation pathways. This new aspect, which is considered to have promising perspectives, will be discussed here in both the HPLC and the cGC sections.

2. SAMPLING AND CLEAN-UP PROCEDURES

The low concentrations of marine organic pollutants require sampling procedures that avoid contamination of the sample. Therefore, rigorous cleaning of all parts of the sampler, solvents, adsorbent material, and chemicals is necessary prior to application. Furthermore, possible contamination by penetration of the sampler through the surface layers of the sea, which may be highly enriched by pollutants, has to be excluded. The following samplers fulfil these basic requirements: the Blumer sampler [5], the DHI sampler [6] and a high-volume water sampler designed to pump water from a defined depth below the sea surface outside the wake of the survey vessel [2].

The air–sea interface is of particular interest, because sea slicks may significantly accumulate organic pollutants. For a review on specific microlayer samplers suitable for collecting the upper water layer of 50–400 μm thickness, the reader is referred to the survey by Hühnerfuss [7,8] and to a recent paper by Sauer *et al.* [2].

The subsequent processing of the water samples may include different steps that allow both the extraction and enrichment of the organic pollutants.

2.1. Extraction and enrichment of marine organic pollutants

In general, liquid–liquid or liquid–solid extraction techniques are applied. Recent publications dealing with this subject focused predominantly on hexachlorocyclohexane (HCH) isomers and PCBs. In addition, organometallics, polycyclic aromatic hydrocarbons (PAHs), organophosphates, chlorophenols, nitrogen-containing pollutants and other organochlorines have been investigated.

2.1.1. Liquid–liquid extraction

A typical procedure being used in connection with the DHI sampler is the following (further technical details of the sampler were given by Gaul and Ziebarth [6]). On board the ship, the water samples can be extracted within the sam-

pler, thus minimizing potential losses by adsorption effects. The water samples are extracted with 200 ml of hexane that has been purified by column fractional distillation. The extracts are stored in a refrigerator at about 253 K until the end of the cruise. Ashore the extracts are purified and analysed by known procedures [6,9], which include purification of the hexane solution by column chromatography over alumina and subsequent on-column GC analysis using a nickel-63 electron-capture detector. It should be noted that the alumina must be prepared carefully according to the procedure described by Ernst *et al.* [9], *i.e.*, first the alumina has to be kept for 3 h at 1123 K (lower temperatures give rise to modification of the alumina and thus to varying recovery rates, in particular of the standard ε-HCH!), and then 5% of water is added.

In addition, the samples can be fractionated further by column chromatography over Florisil, where the first fraction contains the PCB derivatives and the second fraction mostly consists of the HCH isomers [9].

The different procedures described in the literature are mainly distinguished by the application of different solvents, *e.g.*, cyclohexane or *n*-hexane [6,10], *n*-pentane [11], dichloromethane [2], tetrachloromethane [10], different ethers [12] or ethyl propionate [12]. In general, the solvent must be insoluble in water; however, the choice of the appropriate solvent depends on the polarity of the substances to be extracted.

2.1.2. Solid-phase extraction

During the extraction of environmental samples, both biogenic and anthropogenic compounds are extracted into the organic solvent, and this complex matrix may interfere seriously with the determination of the respective analytes. For example, lipid material and aliphatic and polyaromatic hydrocarbons are co-extracted with organochlorine compounds into a non-polar solvent such as *n*-hexane. As a consequence, chromatographic peaks may overlap, substances are deposited on the detector foil of the electron-capture detector and the electron density and the ECD response may be affected considerably. In summary, complex environmental matrices cannot be handled by simple liquid–liquid extraction, but additional subsequent clean-up procedures are required. Very elegant experimental approaches are based on liquid–solid extraction methods that include extraction, fractionation and clean-up of the sample.

Numerous techniques have been suggested for the removal of fats and other interfering substances from solvent extracts of environmental samples. Gel permeation chromatographic (GPC) separation [13] and adsorption chromatography involving alumina [6,9], silica gel [14] and Florisil [9] microcolumns have been most commonly used. Examples of clean-up with alumina and Florisil microcolumns have already been given in Section 2.1.1. With regard to silica gel column chromatography, Takada and Ishiwatari [14] used this technique for the determination of alkylbenzenes with normal C_{10}–C_{14} and branched C_{11}–C_{13} alkyl chains in sediments and suspended matter. This method was reported to be applicable to environmental samples containing nanogram amounts of alkylbenzenes per gram of sample (dry mass). The recoveries of alkylbenzenes were 81–94% and the reproducibility was good. An improved two-step clean-up procedure involving alumina–silica column chromatography and GPC of air particulate matter and river sediment extracts and a GPC clean-up procedure for marine biota samples were described for the determination of PAHs with two to five rings and selected polychlorinated biphenyl congeners, respectively [13]. Bio-Beads SX-3 and SX-12 (200–400 mesh) were used as packing materials. The recoveries obtained varied from 52 to 78% depending on the compound. In general, GPC should be considered as an alternative technique to remove the higher-molecular-mass co-extractive lipids which so often interfere in the GC analyses of organophosphorus and organochlorinated pesticides, PCBs and PAHs. The separation mechanisms in GPC involve adsorption, partition and size exclusion. The predominance of one mechanism over the others is largely determined by the choice of the mobile phase and the packing pore size [13] and, therefore, GPC is as-

sumed to be a very versatile technique.

In addition, reversed-phase columns of different polarity have been used for clean-up procedures in recent years [15–17]. Sperling *et al.* [15] used a combination of clean-up methods that proved to be very effective for the separation of various organohalides from commercial cod-liver oil. The method consisted of a liquid–liquid partitioning between dimethylformamide and hexane followed by a reversed-phase (octadecyl) partitioning. A systematic comparison between different reversed-phase columns (C_{18} phase, phenyl phase) and various high- and low-volatile halogenated hydrocarbons by Pfaffenberger [17] showed that C_{18} phases are superior in many instances to phenyl phases because of their more general applicability. However, C_{18} phases are not recommended for application to the analysis of high-volatile halocarbons, because the recovery may be as low as 10–20% [17]. Further, it should be noted that reversed-phase columns should not be used for trace analyses of marine organic pollutants without careful purification with the solvent prior to application. In general, glass columns are less problematic than plastic columns, which may cause serious interferences due to leaching of plasticizers and other contaminants.

Sometimes, lipophilic organic substances are dissolved in sea water in such minute concentrations that the chemical characterization and determination of single compounds is possbile only after a sufficient amount has been collected by concentration from relatively large volumes of water, typically of the order of 100–1000 l. In this case, sorption and enrichment on specific solids, *e.g.*, XAD-2 resins, may be a suitable experimental approach. However, prior to application of the resins careful purification according to the procedure described by Ehrhardt has to be performed [18,19].

Materials used for the above chromatographic clean-up methods suffer from disadvantages, such as lot-to-lot variation, relatively poor column efficiency, slow elution and high consumption of solvents. In comparison, HPLC columns packed with microparticles exhibit the advantages of high reproducibility, low consumption of solvents, high efficiency and higher sample loading capacity [20]. A rapid, efficient HPLC procedure was reported by Krahn *et al.* [21], who used a size-exclusion column to separate the analytes of interest from interfering compounds in the sample matrix. Analytical results were presented for organic contaminants from mussel and oyster tissues and from sediment extracts.

2.1.3. On-line liquid–liquid extraction (OLLE)

Fogelqvist *et al.* [22] developed a mechanized system for extractive sample work-up for GC coupled on-line to an on-column injector. Extraction of the water sample is performed in a liquid–liquid segment flow in a glass coil internally coated with a hydrophobic layer. After extraction the phases are separated with the aid of a hydrophobic membrane supported by a screen coated with Teflon. The organic phase is fed to a loop injector. A schematic diagram of the OLLE system is shown in Fig. 1.

One of the important advantages of the system is that it is closed from the atmosphere. Further, it was shown to provide rapid and precise work-up of sea-water samples for the determination of high-volatile halocarbons, *e.g.*, chloroform, bromodichloromethane, dibromochloromethane, bromoform, trichloroethylene, tetrachloroethylene, tetrachloromethane and 1,1,1-trichloroethane. Compared with manual extraction, smaller volumes of sample and organic phase are needed. Reliability was tested by Fogelqvist *et al.* [22] during a three-week cruise when 350 sea-water samples were processed without failure. Concentrations down to the picograms per litre level in water can be determined, using injection volumes up to 130 μl. Further, an intercalibration experiment was performed utilizing both the classical liquid–liquid extraction method and the OLLE method. It turned out that the relative standard deviations in the manual technique were about 20% and with the latter system about 10%. The mean values of the two determinations were found not to be statistically different. For further details of this method, which is assumed to be the most sensitive technique for high-volatile halo-

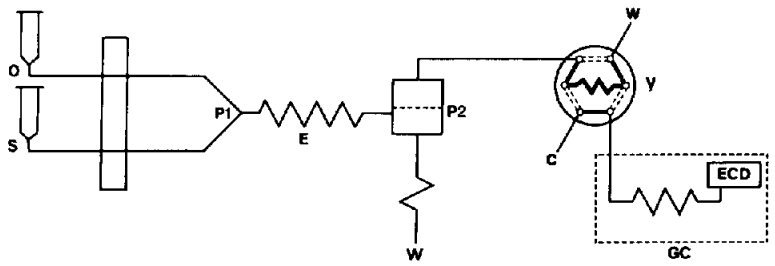

Fig. 1. Schematic diagram of the OLLE system according to ref. 97. O = 50-ml glass syringe with extraction solvent, flow-rate 138 μl/min; S = 50-ml glass syringe with water sample, flow-rate 1430 μl/min; P1 = phase segmentor; E = polymer-coated glass extraction coil (5 m × 0.7 mm I.D.); P2 = phase separator with Teflon membrane, 0.2-μm pore size; W = waste; V = rotary valve with variable loop (load position); C = carrier gas inlet, hydrogen 5 ml/min; GC = gas chromatograph, oven temperature 318 K isothermal, 9 m × 0.3 mm I.D. silanized retention gap and 22 m × 0.5 mm I.D. separation column with 0.5-μm immobilized SE-54; ECD = electron-capture detector 548 K, scavenger gas nitrogen at 30 ml/min.

carbons, the paper by Fogelqvist *et al.* [22] should be consulted.

2.1.4. Extraction of marine sediments and biota

At first glance, concentrations of marine organic pollutants often appear to be minute and negligible. As a consequence, many workers try to infer the toxical potential of specific parts of the world's oceans not by analysing the water itself, but by determining the level of pollutants in sediments and marine biota, which are known to concentrate pollutants by adsorption and/or bioaccumulation and biomagnification effects. This holds, in particular, for higher members of the ecological food web, and hence the analytical problem caused by detection limits can be avoided, although the organic matrices may be more complex and additional clean-up procedures have to be applied.

It is not the intention of this paper to give a comprehensive review of this subject. Only some of the most recent papers that have appeared in generally accessible journals will be cited, and reports and "grey literature" will be neglected. For older literature the reader should refer to the references given in the papers cited hereafter.

With regard to the analysis of sediments and marine biota that have been used as "indicator animals" the most recent papers are summarized in Table 1.

Determination of the substances in question in the respective marine compartments and tissues summarized above needs highly sophisticated methods. In general, a complete analysis of pollutants in, *e.g.*, animal tissues consists of the following five steps: (a) homogenization and drying; (b) extraction of all lipophilic substances by means of organic solvents; (c) separation of the substances in question from the excess of natural lipids ("clean-up"); (d) isolation of single compounds from the complex mixture, usually by cGC and sometimes by HPLC; and (e) identification.

It should be noted that with regard to the first step (homogenization, drying) a controversial debate exists in the literature, because two different

TABLE I

RECENT LITERATURE DEALING WITH THE EXTRACTION OF MARINE SEDIMENTS AND BIOTA

Matrix	References
Sediments	13, 14, 16, 21, 23 (interlaboratory study), 24–31, 125
Mussels	16, 21 32–36, 130, 136
Fish	13, 15, 32, 33, 36–45, 126, 130, 131, 137
Sea birds	46, 125
Common Eider ducks	47, 124, 129, 135, 138
Penguin blubber	20, 48, 136
Harbour seals	38, 39, 48–50, 124, 136
Hermit crabs	51, 52
Marine mammals	37, 40, 53, 127, 128
Plankton, algae	54, 55, 131
Benthic organisms	126, 131

approaches are being used by environmental laboratories, drying by means of freeze-drying and drying by pulverizing with the help of anhydrous sodium sulphate. The present authors performed comparative experiments with these two methods, and observed that freeze-drying bears the potential danger that losses of substances due to evaporation cannot be avoided in all instances and that extreme caution has to be applied when using this method. The second method, pulverizing with the help of anhydrous sodium sulphate, appeared to be less problematic regarding selective losses of substances.

The extraction and clean-up procedures summarized in the preceding section can be used in an analogous manner for sediments and animal tissues. As an example, Fig. 2 shows cGC–ECD traces for extracts of sediments (top) and oyster tissue (bottom) after the HPLC treatment described by Petrick *et al.* [20] for PCB congeners and *p,p'*-bis(4-chlorophenyl)-2,2-dichloroethene (*p,p'*-DDE). Additional examples for HPLC and cGC analyses of marine samples will be discussed in later sections.

3. HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

The unequivocal advantage of HPLC is its applicability both to the clean-up of the sample and to the determination of organic pollutants. In the last decade, the development and improvement of detectors, *e.g.*, fluorescence, inductively coupled plasma atomic emission spectrometric and other specific detectors, gave rise to an increase in sensitivity and to a decrease in detection limits. In addition, many investigators were able to use HPLC at concentrations down to the picograms per litre level in water, *e.g.*, by HPLC–GC–ECD combination, *i.e.*, they replaced the HPLC sensor by the ECD sensor of the gas chromatograph for the analysis of chlorinated compounds.

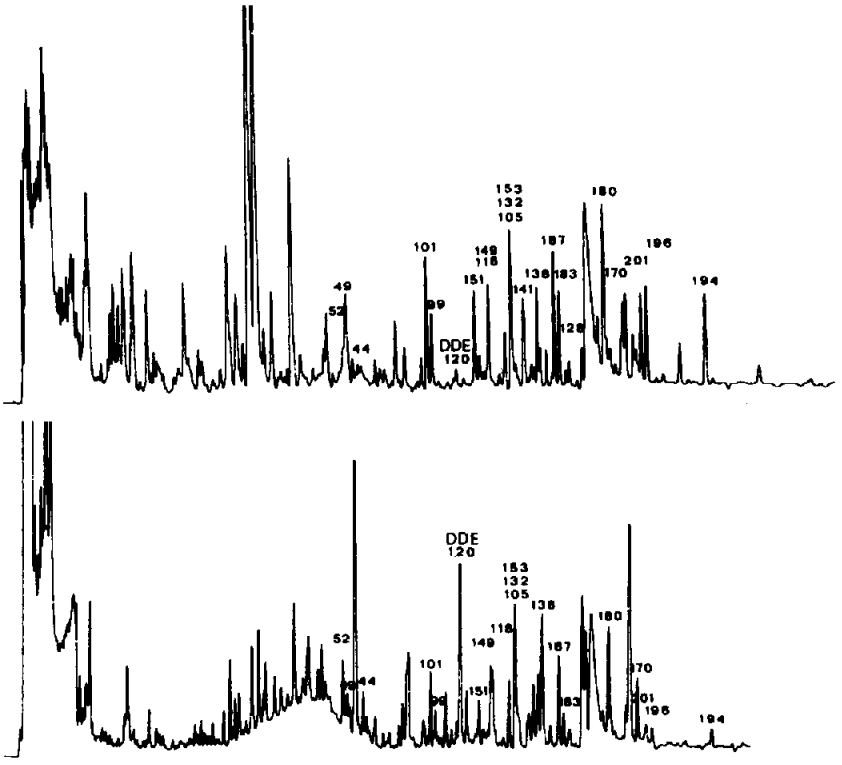

Fig. 2. cGC–ECD of (top) sediment and (bottom) oyster tissue originating from the Mediterranean, after the HPLC treatment described by Petrick *et al.* [20] for PCB congeners and *p,p'*-DDE. PCB numbers according to ref. 118; chromatograms from ref. 20.

The analysis of natural marine substances in sea-water, *e.g.*, amino acids [56,57], amines [58], ammonium ions [58,59], photosynthetic pigments [60,61] and carotinoids [62], is well described in many publications and reviews. The same holds for biogenic pollutants such as toxins [63]. In this review, emphasis is placed on the HPLC analysis of man-made organic pollutants, *i.e.*, organometallics, PAHs, pesticides and halogenated pollutants.

3.1. Classical stationary phases

3.1.1. Organometallics

There has been a growing realization that the form in which a trace metal occurs, so-called speciation, is of vital importance in a number of fields, *e.g.*, toxicology and environmental monitoring. A prime example is the marine antifouling agent tributyltin (TBT), which is known to affect seed oysters and dog whelks at environmental concentrations below 100 ng/l. Inorganic tin species do not act in this manner. This has led to increased interest in methods for trace metal speciation. A promising analytical approach to such speciation is to couple the capability of chromatography for species separation with the selectivity and sensitivity of atomic spectrometry for detection. If two species co-elute and only one contains the metal of interest, the use of metal-specific detection means that only the metal-containing species is detected. Hence complete chromatographic separation is not required.

A simple yet effective HPLC–flame atomic absorption spectrometry (FAAS) coupling utilizing pulse nebulization and a slotted tube atom trap was described by Ebdon *et al.* [64] for the speciation of tin in natural waters. A detection limit of 200 ng for tin was obtained and the separation of tin(II), tin(IV) and tributyltin (TBT) within 8 min was possible. Thus, large preconcentration factors are required in order to measure the levels in environmental samples.

Another procedure for the determination of TBT ions in estuarine waters by HPLC with fluorimetric detection using morin in a micellar solution was described by Ebdon and Alonso [65]. TBT was quantitatively retained from 100–500 ml samples on a 4-cm-long octadecyl silica (ODS) column. After washing off the salts with 20 ml of distilled water, the ODS column was backflushed with methanol–water (80:20) containing 0.15 *M* ammonium acetate on to a 25-cm-long Partisil SCX analytical column. The detection limit of this method is 16 ng of TBT (as tin).

Branch *et al.* [66] investigated the coupling of HPLC with inductively coupled plasma mass spectrometry (ICP-MS) utilizing two different types of plasma torch, and assessed the implications for the analysis of tin species. The principal practical difficulty with the use of ICP-MS is the requirement to introduce a mobile phase containing a high concentration of organic solvent [66]. In spite of these difficulties, Branch *et al.* [66] reported an improvement of several orders of magnitude over coupling of HPLC with flame atomic absorption spectrometry (FAAS), *i.e.*, a limit of detection of 0.025 μg/ml TBT was achieved, which is equivalent to 1.6 ng of tin injected.

Recently, Tolosa *et al.* [67] compared four different methods for the determination of organotin derivatives in sea-water, marine sediments and mussels, *viz.*, cGC coupled with flame photometric detection (FPD), with ECD and with MS was compared with liquid chromatography (LC) with MS detection. The coupling of cGC with single- or dual-flame FPD using a 600-nm interference filter exhibited the best performance for the trace level determination of alkyl- and aryltin compounds as methyl derivatives in aquatic matrices [67,132]. Limits of detection in the picograms to nanograms per litre and per gram for water and for sediments and biota, respectively, were obtained. Characteristic cGC–FPD traces for methyl derivatives of organotin compounds are shown in Fig. 3 for extracts from water, sediments and biota.

On the other hand, the cGC–electron-impact ionization (EI) MS in the SIM mode can be concurrently used as a confirmatory technique for cGC–FPD assignments. The most common multiple-ion detection (MID) mode has not exhibited adequate sensitivity for the analysis of real environmental samples.

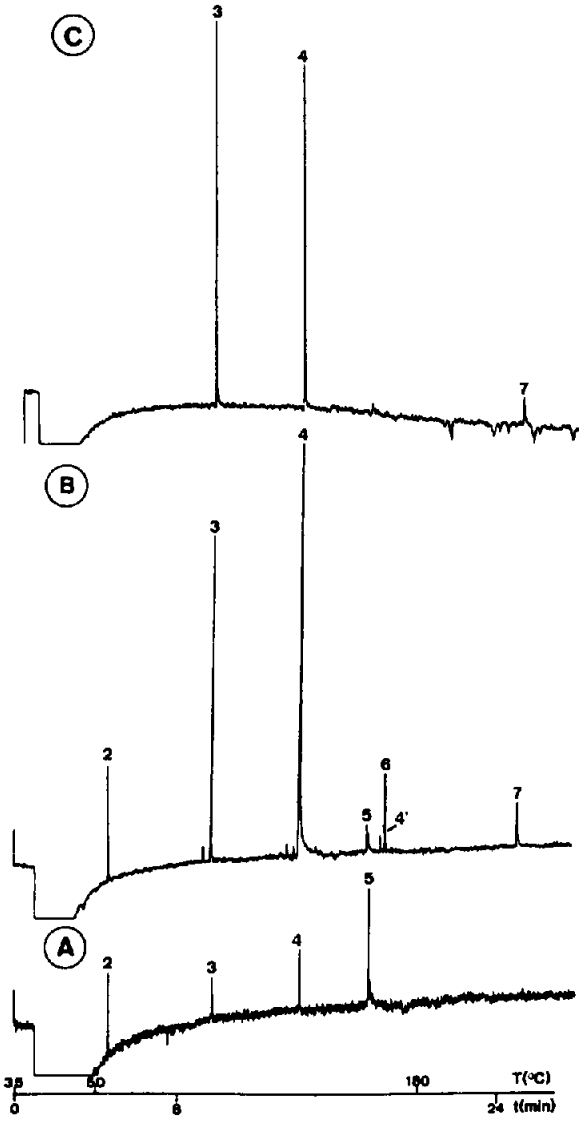

Fig. 3. Characteristic cGC–FPD traces of methyl derivatives of organotin compounds in (A) water, (B) sediment and (C) biota (from ref. 67). Compounds: 2 = *n*-butyltin; 3 = di-*n*-butyltin; 4 = tri-*n*-butyltin; 5 = tetra-*n*-butyltin; 6 = diphenyltin; 7 = triphenyltin; 4' = hydroxy derivative of tri-*n*-butyltin.

In 1982 and 1985, two comprehensive reviews on the application of HPLC to organometallic and metal coordination compounds were published by Willeford and Veening [68] and Nickless [69], respectively; the latter also included other chromatographic techniques. However, since then, much additional experimental evidence has been reported, shedding more light on the speciation problem of metals in sea-water.

Mackey [70] performed an investigation on naturally occurring complexes of Cu, Zn, Fe, Mg, Ni, Cr, Mn and Cd using HPLC with atomic fluorescence detection. The metal–organic complexes were isolated from coastal sea-water on to octadecyl-bonded silica (Sep-Pak cartridges). Organic complexes of Cu, Zn, Fe, Mg, Ni and Mn were found to be present but no complexes of Cr or Cd were detected. The complexes covered a wide range of polarities with no specific complexes being predominant. Typical values of the amounts of trace metals isolated by this technique corresponded to concentrations in the original sea-water of >65 ng/l (Cu), >27 ng/l (Fe) and >41 ng/l (Zn). According to the experience of the present authors with polar organic pollutants, it has to be assumed that a significant fraction of the metal organics present was too polar to be completely retained by the Sep-Pak cartridges.

In order to overcome this basic problem, Mackey and Higgings [71] compared the potential of different reversed-phase chromatographic supports such as Sep-Pak C_{18} cartridges, Sep-Pak cartridges packed with Styragel and XAD-2 resin and subsequent elution with methanol, acetonitrile or tetrahydrofuran. Analysis of the extracts by HPLC with atomic fluorescence detection of trace metals showed that methanol extracts remain unchanged for up to three months in polypropylene at temperatures between 293 and 253 K and in glass at 253 K. Further, high-intensity lamps improved the sensitivity of the atomic fluorescence detector by 1–2 orders of magnitude, and Zn, Mg, Cu, Ni and Fe could be determined simultaneously with detection limits ranging from 0.05 μg/l (Zn) to 7 μg/l (Fe). It should be noted that all the chromatographic columns investigated (C_{18} Resolve, C_{18} Nova-Pak, PRP-1 and Protein-Pak 125) contained impurity sites that could interfere with the analysis of organometallic compounds at the ultra-trace level. As this observation is in full accordance with the experience of the present authors with C_{18} phases

available in Europe, it is strongly recommended that attention be paid to potential interferences caused by impurities in the phases. Mackey and Higgins [71] claimed that interference effects could be reduced by the addition of modifying agents containing alkali metal salts at a concentration of 50 m*M*. The most suitable salt is potassium trifluoromethanesulphonate.

The speciation of trace amounts of organometallic compounds such as metallothionein, a low-molecular-mass sulphur-containing and metal-binding protein, is of special interest, because it has been characterized as the primary protein responsible for trace metal detoxification of both invertebrates and vertebrates. Mazzucotelli *et al.* [72] used a high-performance liquid chromatograph equipped with an off-column spectrophotometric reactor for the determination of such organometallic compounds. The metallothioneins were determined by absorbance measurements at 520 nm after an off-column reaction of the metal-containing eluates with a buffered 4-(2-pyridylazo)resorcinol (PAR) solution. The proposed HPLC PAR method appears to be more sensitive and selective and faster than the classical procedure based on macro-column separation with subsequent fraction collection and atomic absorption spectrometric (AAS) determination. From a biological point of view, the importance of the results is that it had never previously been demonstrated that zinc *per se* can stimulate the neosynthesis of metallothioneins in marine organisms. In addition, it has been demonstrated that Cd/Zn-binding proteins isolated from mussel tissues belong to the class of metallothioneins. Zinc in turn can be displaced by exposure of the animals to metals such as Cu, Cd and Hg, which have a much stronger affinity for –SH residues [72].

The applicability of this experimental approach was extended by the addition of further detectors such as ultraviolet diode-array and electrochemical detectors [73].

Recently, Mazzucotelli *et al.* [74] investigated the potential of the combination of HPLC and inductively coupled plasma atomic emission spectrometric (ICP-AES) detection for the determination of trace amounts of metalloproteins in marine mussel samples. It turned out that some problems may occur; however, after having overcome these obstacles, the method is mainly an improvement on most previous methods in that microsamples are analysed more rapidly, allowing a high sample throughput per day.

Lower sensitivities and high sample throughput were also the aim of Nagaosa *et al.* [75], who investigated the separation and simultaneous determination of Al, Fe and Mn in natural water samples by using HPLC with spectrophotometric and electrochemical detection, and of Ohashi *et al.* [76], who determined simultaneously Mo, V, Ga, Cu, Fe and In as 8-quinolinolate complexes by HPLC. The versatility of ICP-MS was demonstrated by McLaren *et al.* [77]. The simultaneous determination of seven trace elements was achieved. The authors claimed that ICP-MS exhibits a detection power far superior to ICP-AES, and a much larger number of trace elements can be directly determined in concentrates prepared from sea-water samples. Further, ICP-MS complements other sensitive techniques such as graphite furnace AAS in permitting the certification of new elements for which there were previously an insufficient number of independent methods of determination. The use of ICP-MS in combination with HPLC offers a powerful new approach to the determination of trace element speciation which has been applied to, *e.g.*, arsenic speciation in fish tissue and tin speciation in a harbour sediment.

3.1.2. Polycyclic aromatic hydrocarbons

The analysis of PAHs in the environment is of considerable importance owing to increasing concern about the mutagenicity and carcinogenicity of many of these compounds. The main difficulty associated with a PAH determination is the extreme complexity of environmental matrices. Even after extensive fractionation, the PAH fraction may contain several hundred compounds. In order to determine selected compounds in such mixtures, methods that offer combinations of good chromatographic resolving power and detector selectivity are required. As a result of a

need to establish reliable values for a set of PAHs in different marine compartments, several groups have investigated the applicability of various combinations.

Theobald [78] investigated petroleum hydrocarbons in sea-water using HPLC with fluorescence detection. Bo-Xing and Yu-Zhi [79] described a method for the concentration and determination of several PAHs in water by flotation enrichment and subsequent HPLC. Another approach was chosen by Núnez and Centrich [80], who first extracted the samples with light petroleum–diethyl ether (85:15) and concentrated the samples before analysis by HPLC with fluorescence detection. In this instance, the recoveries of individual PAHs from spiked water samples were 0.16–0.27 ng/ml. Detection limits in the picogram range were obtained for each compound. The determination of PAHs by HPLC with simultaneous MS and ultraviolet diode-array detection in sediment material was reported by Quilliam and Sim [81]. EI-MS with the moving-belt interface provides high sensitivity and selectivity, in addition to structural information such as molecular mass, functional groups and elemental composition. The diode-array detector helps to differentiate isomeric structures and confirm compound identity.

A straightforward HPLC analysis was possible for samples obtained in the Gulf of Naples because of their high contamination, which in turn was due to the concentration of industries in that area including an important steel plant and municipal developments [82]. The levels of sixteen PAHs were investigated in five shellfish and in fourteen fish species along the coast of the Gulf of Naples. The average total amount of PAHs in shellfish was 217 μg/kg wet mass, with a range 185–295 μg/kg, and in fish species 94–1930 μg/kg wet mass.

Galgani *et al.* [130] investigated PAHs in fish from northwest France, in order to gain an insight into the correlations between 7-ethoxyresorufin-O-deethylase (EROD) activity and the content of various pollutants including PCBs, HCHs and PAHs.

Rapid, semi-quantitative screening of sediments for aromatic compounds using sonic extraction and HPLC–fluorescence analysis was reported by Krahn *et al.* [83]. The concentrations of aromatic compounds determined by the screening method were highly correlated with the sum of the aromatic hydrocarbons determined by a standard analytical method which used HPLC clean-up and GC–MS determination.

Van de Nesse *et al.* [84] used on-line coupling of LC with thin-layer chromatography (TLC) for the identification of PAHs in marine sediment by fluorescence excitation and emission spectrometry. Eleven PAHs were thus identified. High-performance thin-layer chromatography (HPTLC) obviously combines the advantages of TLC and HPLC.

Hofstraat and co-workers [85,125] and Mastenbroek *et al.* [86] compared a standard HPLC technique with a new experimental approach, high-resolution Shpol'skii spectroscopy, for the determination of PAHs in harbour and marine sediments. The technique affords comparable low detection limits to a standard procedure based on HPLC with fluorimetric detection, but offers much greater selectivity. As the Shpol'skii effect is a matrix effect, the selectivity of the method can be significantly increased by using selective excitation, even with a conventional broad-band light source. The results obtained with Shpol'skii spectroscopy were in good agreement with those given by a standard method employing HPLC with fluorescence detection. A typical Shpol'skii fluorescence spectrum obtained with 304 nm excitation is shown in Fig. 4. The spectrum was used for the determination of PAHs in a harbour sediment sample [85].

In particular, the low-temperature Shpol'skii technique provides high-resolution fluorescence spectra of PAHs that can serve as fingerprints. Thus, information concerning peak purity was obtained and the number of components identified was roughly doubled [86].

3.1.3. Pesticides and halogenated pollutants

GC still appears to be the major technique used for marine organic pesticides and halogenated pollutants owing to its high separation pow-

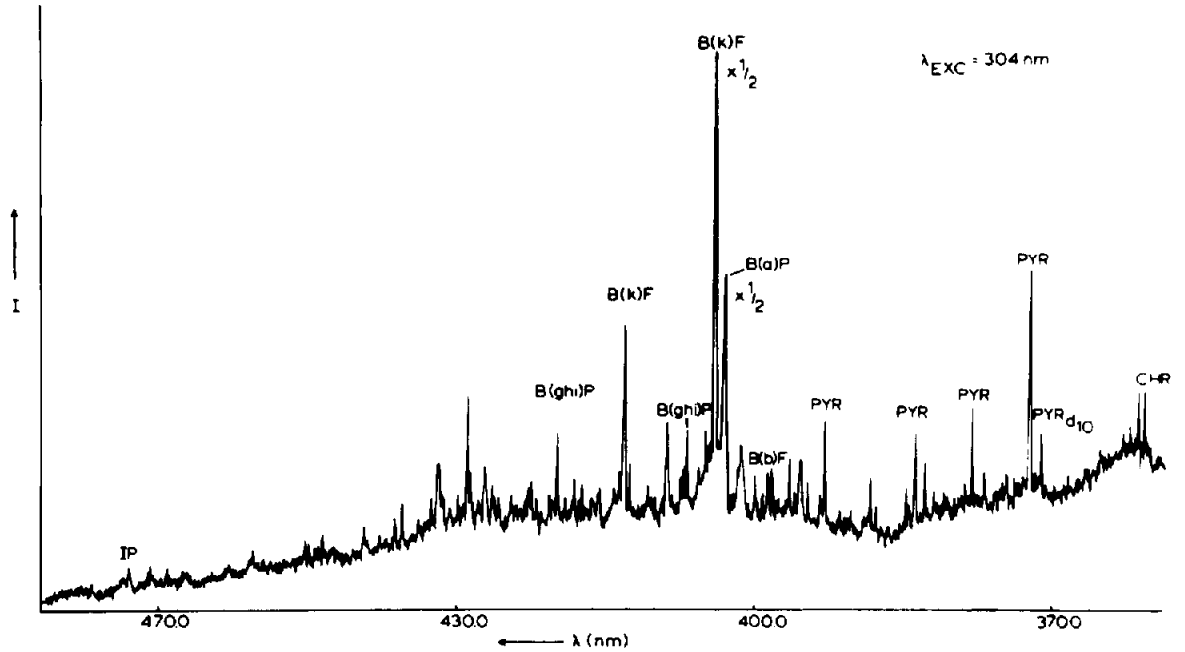

Fig. 4. Shpol'skii fluorescence spectrum of a harbour seal sample, excited at 304 nm (from ref. 85). The main peaks were assigned to chrysene (CHR), pyrene (PYR), benzo[*b*]fluoranthene (B(b)F), benzo[*a*]pyrene (B(a)P), benzo[*k*]fluoranthene (B(k)F), benzo[*ghi*]perylene (B(ghi)P), indeno[1,2,3-*cd*]pyrene (IP) and pyrene-d_{10} ($PYR_{d_{10}}$).

er (capillary column) and the availability of selective and sensitive detectors. However, the application of HPLC is growing, especially for the determination of pesticides that cannot be subjected directly to cGC owing to poor volatility, polarity and/or thermal instability of the compounds. Special reversed-phase columns have been introduced for the analysis of such groups of pesticides, *e.g.*, carbamates, phenylurea herbicides, hydroxytriazines and nitrophenols. Schlett [87] gave a survey of pesticides that cannot be determined by cGC or only after a cumbersome and time-consuming derivatization, but which can be determined directly by HPLC. This list of 69 substances includes many environmental pollutants that are assumed to have a severe impact on marine ecosystems. However, only a limited number of substances can be measured in a single run. One has to focus on the 20–30 compounds that are relevant in the water catchment area. The chromatographic separation is performed in a gradient mode (for details see ref. 87). A good separation is possible, as shown in Fig. 5 for two different wavelengths, 245 and 230 nm. Substances that interfere can be separated by changing either the pH of the mobile phase or the gradient programme.

The factor currently limiting the application of HPLC to marine water samples is the low concentration of the pesticides and organohalide compounds generally encountered in the world's oceans. In order to overcome this basic disadvantage limiting the applicability of HPLC to pesticides and halogenated pollutants, many investigators prefer a HPLC GC combination. Thus the unequivocal advantages of HPLC for the clean-up procedure and for fractionation and the sensitivity of ECD or nitrogen–phosphorus detection (NPD) in GC can be used. Examples of this experimental approach are given in Section 4.

In summary, there is a great need for a chlorine-selective detector in LC that will allow trace analyses for organic halogenated pollutants. Var-

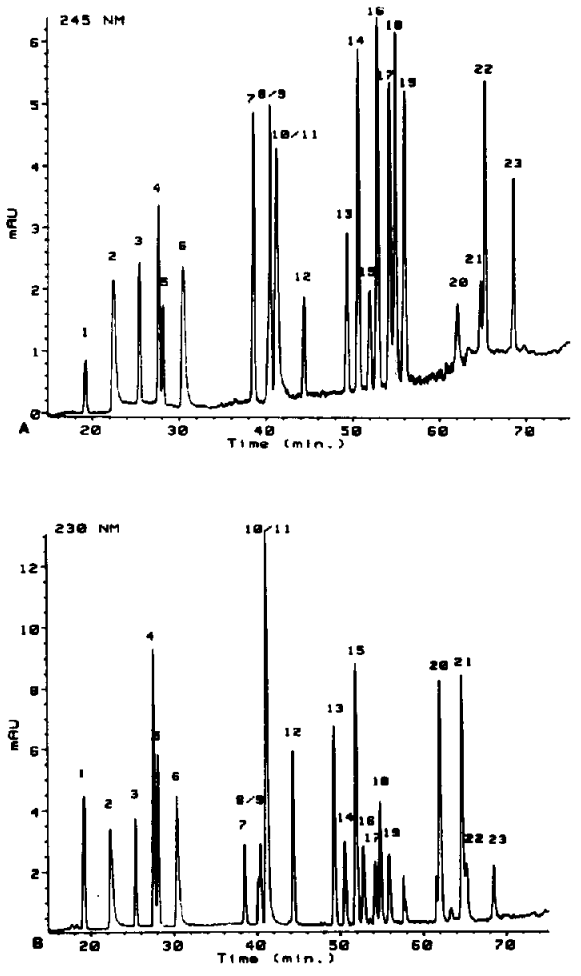

Fig. 5. Chromatographic separation of pesticides by HPLC and detection at two different wavelengths: (A) 245 nm and (B) 230 nm (from ref. 87). Peaks: 1 = desisopropylatrazine; 2 = hydroxysimazine; 3 = metamitron; 4 = chloridazone; 5 = desethylatrazine; 6 = hydroxyatrazine; 7 = hexazinone; 8/9 = metoxuron/bromacil; 10/11 = simazine/hydroxypropazine; 12 = desethylterbutylazine; 13 = methabenzthiazuron; 14 = chlortoluron; 15 = atrazine; 16 = monolinuron; 17 = diuron; 18 = isoproturon; 19 = metobromuron; 20 = propazine; 21 = terbutylazine; 22 = linuron; 23 = chloroxuron.

ious attempts have been made to modify existing halogen-sensitive GC detectors for use with LC. A very promising approach was suggested by Folestad *et al.* [88], who developed a novel design of a chlorine-selective flame-based detector adapted to microcolumn reversed-phase LC. The total column effluent (20–70 μl/min) is introduced via a thermospray interface into a heated-oven system. Chlorinated compounds are converted, after pyrolysis in a stream of hydrogen, into indium(I) chloride, which is subsequently excited in a cool hydrogen diffusion flame to emit at 360 nm. The detection limit is 9 pg/s for 1,1,2-trichloroethane in water. A linear response from 5 to 70 ng for 1,1,2-trichloroethane in methanol–water (15:85) was observed. Non-volatile compounds such as chlorinated uracils, guanine and guanosine could be detected selectively. Hence this detector is suitable for work with columns of 1 mm I.D. For applications where higher concentrations of organic solvents (> 50%) and where higher sensitivity are necessary, more studies are needed.

3.2. Chiral stationary phases

As recently suggested by Faller *et al.* [89], the chromatographic separation of the enantiomers of marine pollutants allows a discrimination between enzymatic and non-enzymatic processes in marine and terrestrial ecosystems. Hühnerfuss and co-workers [89,90] developed a GC method applicable to environmental samples exhibiting low concentrations of non-polar organic pollutants (see Section 4.2), and subsequently [91] aimed at analysing polar chiral compounds including phenoxycarboxylic acids. In the latter instance, the application of HPLC with chiral stationary phases turned out to be a very promising method. Ludwig *et al.* [91] investigated the microbial degradation of 2-(2,4-dichlorophenoxy)-propionic acid (DCPP) by marine microorganisms with the help of HPLC using the chiral phase Chiral-α_1-AGP (100 mm × 4 mm I.D.). They reported the notable result that the microorganisms which were obtained from the North Sea area German Bight degraded DCPP enantioselectively, *i.e.*, the *R*-enantiomer was exclusively decomposed to 2,4-dichlorophenol whereas the *S*-enantiomer remained unaffected. Typical HPLC traces illustrating this phenomenon are shown in Fig. 6 after a degradation period of 21 days for a sterile control and for a mixed culture of marine microorganisms.

Basically, this experimental approach is ex-

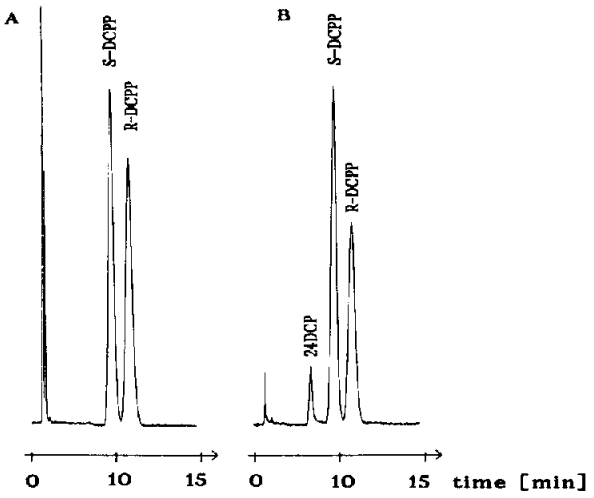

Fig. 6. HPLC of the *S*- and *R*-enantiomers of 2-(2,4-dichlorophenoxy)propionic acid (DCPP) and of 2,4-dichlorophenol (24DCP). (A) of a sterile control and (B) after degradation by marine microorganisms (21 days) (from ref. 91). α_1-AGP column (100 mm × 4 mm I.D.); eluent, water–2-propanol (96:4) containing 10 mmol of phosphate buffer (pH 4.85); flow-rate, 0.9 ml/min; UV detection at 240 nm.

pected to be applicable to many environmental problems, in particular to investigations on the fate of organic chiral and/or prochiral compounds and their degradation products. However, the detection limits discussed in Section 3.1.3 also hold for this experimental approach.

Another interesting variation of the chromatographic separation of chiral compounds was reported by Einarsson *et al.* [92] who synthesized a new chiral reagent, (+)-1-(9-fluorenyl)ethyl chloroformate. This reagent can be used for the chiral derivatization of primary and secondary amino acids, and also other compounds with reactive amino groups, thus forming stable, fluorescent and easily separable products. The derivatives thus obtained have favourable chromatographic properties, as demonstrated, *e.g.*, by a reversed-phase separation of the D- and L-forms of seventeen primary amino acids in a single run [92]. However, the potential for the analysis of marine pollutants with reactive amino groups must be further investigated.

3.3. Possibilities and limitations of HPLC

As already outlined above, the low concentrations of many environmental pollutants, in particular of halogenated hydrocarbons, currently set limits on more general applicability. However, the unequivocal advantage of HPLC regarding its potential for clean-up and fractionation of organic substances gave rise to an HPLC–GC combination that is frequently used in environmental trace analysis. In summary, there is a great need for a chlorine-selective detector in LC that allows trace analyses for organic halogenated pollutants. With regard to the analysis of organometallics and PAHs in marine environmental samples, in particular in sediments and marine biota, HPLC is widely used.

Very promising perspectives are the application of chiral phases, examples of which are discussed in Section 3.2, and miniaturization of columns. The latter experimental approach, which has attracted much attention in recent years, may give rise to considerably shorter retention times and to various other improvements [88,93], *e.g.*, the development of mass-sensitive detectors is possible, because the flow-rates remain sufficiently large in spite of miniaturization. This implies that an increase in signal is expected with an increase in the mobile phase flow-rate. A linear relationship can be obtained typically in the range 20–60 μl/min. An increase in signal is accompanied in turn by a decrease in noise, as higher flow-rates yield a more stable flame with less flickering [88].

4. CAPILLARY GAS CHROMATOGRAPHY

Because of the volatility of most marine organic pollutants or their derivatives, GC has been and still remains a major force in organic separation and analysis, although more recently even its predominant position has and is being challenged by modern LC. However, as already outlined in Section 2, the organic matrix of environmental samples, be it samples of sea-water, sediments or marine biota, mostly is so complex that prior to GC analysis a thorough clean-up

and fractionation procedure has to be performed.

Because of the large number of publications available in this field, it is not possible to comment on all of them. However, it is the intention to try to discuss the current status of the GC analysis of marine pollutants including its problems, and to show some perspectives of this chromatographic application.

Substances discussed here include high-volatile organic compounds, in particular volatile anthropogenic halocarbons, and low-volatile organic compounds as summarized in Table 2.

4.1. Classical stationary phases

4.1.1. High-volatile organic compounds

The major problem connected with the analysis of high-volatile organic compounds is the preconcentration of these marine pollutants without substantial losses. Extraction of, *e.g.*, 100 ml of water with 5 ml of hexane or pentane gives rise to an enrichment factor of 20. However, often considerably larger enrichment factors are required in order to achieve appropriate concentration levels. Further concentration of the extract by a gentle stream of nitrogen, as accomplished in solutions with low-volatile hydrocarbons, in general leads to considerable losses of the volatile compounds. In addition, when dealing with sea-water, special problems are encountered, as a number of volatile halocarbons produced by marine organisms can be present in the sample and interfere with the determination of anthropogenic halocarbons [94–97].

Common preconcentration methods applied to sea-water samples usually include liquid–liquid extraction [95], purge-and-trap [98,99] and OLLE [22]. In addition, sampling of marine air is performed by using solid-phase tubes, *e.g.*, Dekabon tubing [98] or Tenax TA [99,100].

Sample work-up according to liquid–liquid extraction and by purge-and-trap is mostly very time consuming and bears the risk of contamination. So far, OLLE (for details, see Section 2.1.3) appears to be superior to these two preconcentration methods. Further, intercalibration between the two classical methods and OLLE showed [22] that the relative standard deviations in the manual techniques were about 20% and in the OLLE set-up about 10%. An improvement in the relative standard deviation from 20 to 10% means a considerably enhanced applicability of the method, in fact a reduction of the detection limit by more than a factor of 2. Fogelqvist *et al.* [22] claimed that the better repeatability of the mechanized OLLE system compared with manual methods is a result of the modified extraction technique and the closing of the system.

The aims of the analysis of high-volatile marine organic pollutants are currently devoted mainly to the discrimination between biogenic and anthropogenic halogenated hydrocarbons [95–97] and to studies of distribution patterns of halogenated hydrocarbons in coastal areas [101] and of fluorinated hydrocarbons in deeper ocean layers [98,102–106] as tracer substances for the study of water mixing and transport processes. Further, the study of the sources and distribution of halocarbons in marine air is a subject of great interest in order to gain further insight into global transport processes of airborne pollutants [98–100].

TABLE 2

RECENT LITERATURE DEALING WITH LOW-VOLATILE MARINE ORGANIC POLLUTANTS

For publications on organometallics and PAHs, see Sections 3.1.1 and 3.1.2, respectively.

Compound	References
HCH isomers	2, 6, 20, 47, 89, 90, 107, 108, 127, 130, 131, 134, 137, 138
PCBs	2, 4, 13, 15, 16, 20, 21, 23, 32, 33, 35–40, 43, 46, 48–50, 53, 108–114, 124, 127, 128, 130, 131, 134, 136
Organophosphates	108, 115, 116
Chlorophenols	116, 117
Nitrogen-containing pollutants	12
Other organochlorines	2, 15, 16, 20, 21, 24, 34, 39, 43, 49, 127, 130, 131, 134
PAHs	133
Organotin derivatives	67, 132

4.1.2. Low-volatile organic compounds

Recent investigations on low-volatile marine organic pollutants were focused predominantly on HCH isomers and PCBs. In addition, organophosphates, chlorophenols, nitrogen-containing pollutants and other organochlorines have been analysed. The most recent publications dealing with these low-volatile organic pollutants are summarized in Table 2. For publications on organometallics and PAHs, see Sections 3.1.1 and 3.1.2, respectively.

As α-HCH, γ-HCH and the PCBs are ubiquitous environmental contaminants, most investigations have been focused on the distribution and fate of these pollutants in the world's oceans. The latter aspect includes their persistence and toxic implications to marine biota, the microbiological and photochemical degradation pathways, the biotransformation in marine ecological food webs and seasonal differences in the distribution in marine biota.

Special emphasis has been placed on the investigation of highly toxic coplanar PCBs; of the 209 theoretically possible isomers and congeners, 20 members may attain coplanarity due to non-*ortho*-chlorine substitution in the biphenyl rings. Earlier studies suggested that the toxic nature of technical PCB mixtures may be associated with the presence of trace levels of particular coplanar PCBs having four or more chlorine atoms at both *para* and *meta* positions such as 3,3′,4,4′-tetrachlorobiphenyl (PCB77, according to the nomenclature introduced by Ballschmiter and Zell [118]), 3,3′,4,4′,5-pentachlorobiphenyl (PCB126) and 3,3′,4,4′,5,5′-hexachlorobiphenyl (PCB169). They are isostereomers of highly toxic 2,3,7,8-tetrachlorodibenzo-*p*-dioxin and 2,3,4,7,8-pentachlorodibenzofuran and are known to elicit common toxic and biological responses including body weight loss, thymic atrophy, immunotoxicity, high binding affinity to hepatic cytosolic receptor protein and high induction potency of 3-methylcholanthrene-type hepatic microsomal enzymes (for secondary literature on the above toxic effects, see the review of Tanabe et al. [37]).

Although there is much evidence on the extreme toxic potential of these coplanar PCBs, very limited information is available on their environmental distribution [4,37,119]. The reason for this lack of information appears to be the difficulty in separating the peaks of coplanar PCBs from interfering peaks of other PCB congeners. Special techniques, *e.g.*, multi-dimensional GC with ECD [4] or variations of the usual technique [120] (prolongation of the column, multi-step temperature programming) are necessary in order to obtain a sufficient separation of the co-eluting PCB congeners in question. This aspect will be further pursued in the Section 4.1.3.

Tanabe *et al.* [37] detected three toxic coplanar PCBs in all specimens analysed. The residue values ranged from 11 pg/g to 42 ng/g for 3,3′,4,4′-tetrachlorobiphenyl (PCB77), 5 pg/g to 4.0 ng/g for 3,3′,4,4′,5-pentachlorobiphenyl (PCB126) and 1 pg/g to 3.6 ng/g for 3,3′,4,4′,5,5′-hexachlorobiphenyl (PCB169), on a wet mass basis. The highest concentrations, about 40 ng/g PCB77, 4 ng/g PCB126 and 4 ng/g PCB169, were found in a killer whale caught off the Pacific coast of Japan but reared in an aquarium for two years before its death. Significant concentrations were also detected in other marine vertebrates such as fish and finless porpoise from coastal regions and Dall's porpoise, Baird's beaked whale and Pacific white-sided dolphin living in the open ocean. When considering the difference in accumulation patterns between males and females in those specimens where both sexes were available in Tanabe *et al.*'s study [37], lower concentrations of coplanar PCBs and total PCBs were found in females, possibly because of transplacental and lactational transfer.

When comparing the residue levels of coplanar PCBs and total concentrations of other PCB congeners, the coplanar PCBs were found to be 3–5 orders of magnitude lower than total PCBs [37]. In all instances biological specimens with high concentrations of PCBs appear to have high residue values of coplanar PCBs.

When inferring the relative toxic contribution of coplanar PCBs in the various compartments of the marine ecosystem from the results of Tanabe *et al.* [37], it is necessary to take into consideration their toxic potencies and residual concen-

tration. However, the toxic effects of trace levels of coplanar PCBs, polychlorinated dibenzo-*p*-dioxins and polychlorinated dibenzofurans on a long-term basis on marine biota are neither fully understood nor clearly demonstrated. The most useful approach to elucidating the environmental toxic significance of these chemicals may be by comparing their biological response to induce the microsomal enzymes. The aryl hydrocarbon hydroxylase (AHH) and the EROD induction potencies of coplanar PCBs and some representative dioxin and dibenzofuran derivatives have been determined in rat hepatoma cell lines, and the direct quantitative correlation between *in vitro* dose–response biological effect (microsomal enzyme induction) and *in vivo* toxic effects (body weight loss and thymic atrophy) of these chemicals have also been demonstrated in rats (for secondary literature on these toxic aspects, see Tanabe *et al.* [37]). Some light has been shed on the toxic potential of coplanar PCBs by normalizing the residual concentrations of PCB congeners based on their individual induction capabilities relative to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. Interestingly, one of the toxic coplanar PCBs, 3,3′,4,4′,5-pentachlorobiphenyl (PCB126)), revealed the highest value of tetrachlorodibenzodioxin equivalent (T_4CDD equivalent) among all the toxic chemicals examined, including the most toxic 2,3,7,8-tetrachlorodibenzo-*p*-dioxin itself (see Fig. 7).

In summary, the environmental data so far available for coplanar PCBs show that more intensive research on this subject is necessary in order to gain a more comprehensive insight into the toxic potential of coplanar PCB congeners in the marine ecosystem.

Another problem in marine chemistry is related to the chromatographic separation of the enantiomers of chiral marine pollutants and their degradation products [47,89,90,107,138]. This aspect and its toxic implications are discussed in Section 4.2.

The toxic potential of organophosphates and nitrogen-containing marine pollutants is well recognized [12,115]. In particular, these substances are acetylcholinesterase inhibitors in mammals. However, only a few data are avaible on the distribution of these pollutants in the marine ecosystem [12,108,116]. According to a *Chemical Abstracts* literature search, some results on chloro- and nitrophenols [121] and on *sym*-triazine herbicides [122] in sea-water have been published in a Russian journal; however, this was not available to the reviewers and therefore cannot be discussed here.

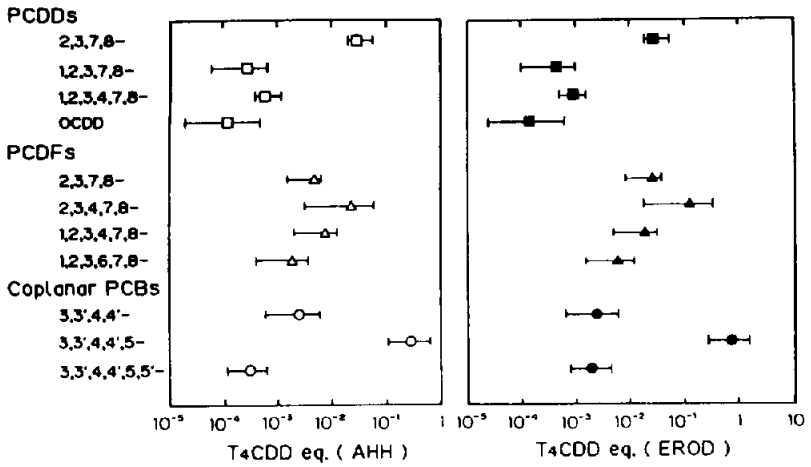

Fig. 7. T_4 equivalent of polychlorodibenzodioxins (PCDDs), polychlorodibenzofurans (PCDFs) and coplanar polychlorobiphenyls (PCBs) with regard to the induction potencies for the enzymes AHH and EROD (from ref. 37).

4.1.3. Multi-dimensional gas chromatography

A multi-dimensional GC technique was suggested by Duinker *et al.* [4] for the effective separation of PCB congeners that cannot be separated on a single SE-54 or other column by conventional methods. Two capillary columns are arranged in series, such that the second column receives only small, preselected fractions eluting from the first column. The technique offers complete separation and increased sensitivity. The possibilities of this experimental approach were demonstrated for toxic coplanar PCB congeners, PCB 37, PCB77, PCB81, PCB118, PCB123, PCB114, PCB105, PCB126, PCB167, PCB156, PCB157, PCB169 and PCB189, which were determined accurately for the first time in Clophen (see Fig. 8) and Aroclor commercial mixtures and a seal blubber extract. The relative concentrations differed considerably between the blubber extract and the commercial mixture. This technique supplies very accurate data, but special two-oven gas chromatographs and skilled personnel are necessary for performing these time-consuming analyses.

On the other hand, Vetter *et al.* [120] claimed that at least some of the coplanar PCB congeners, *e.g.*, a separation of the three co-eluting PCBs PCB153, PCB132 and PCB105, can be achieved by a considerably simpler technique, *i.e.*, by using a 50-m SE-54 column and a multi-step temperature programme. They proposed the application of an efficient SE-54 column as standard column to determine PCB153 without interference from PCB105 and PCB132. OV-1701 is suitable as a stationary phase in the second column, and an OV-225 column can be an interesting addition to apolar phases, as the elution sequence on this phase is very different from that on an apolar phase. Vetter *et al.* [120] obtained the best resolution of PCB153, PCB132 and PCB105 on this phase. However, a simultaneous separation of several coplanar PCBs can presumably only be achieved by multi-dimensional techniques.

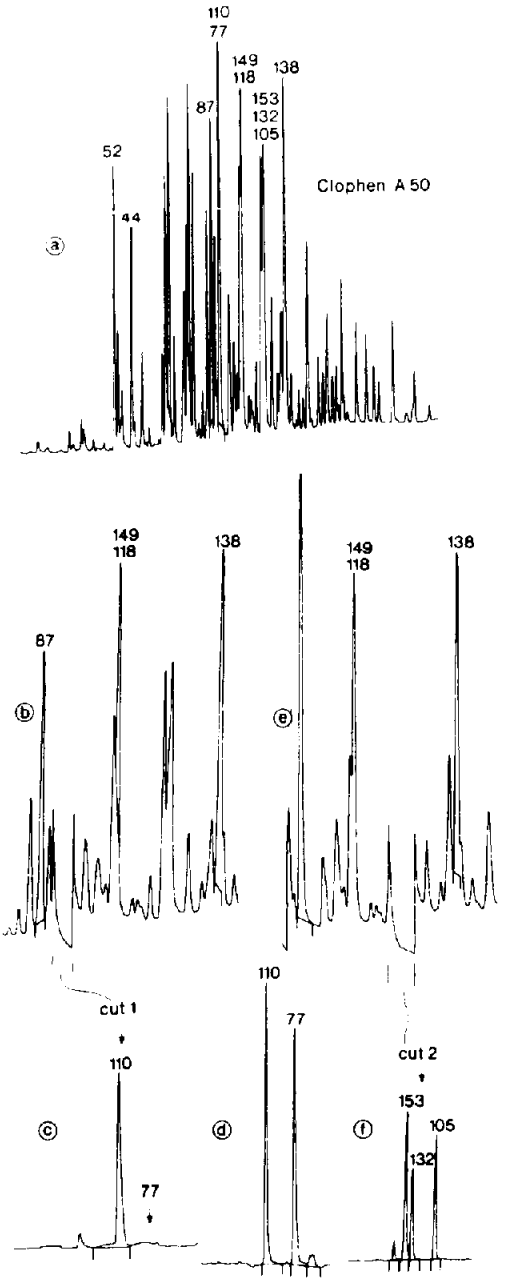

Fig. 8. Multi-dimensional eGC (from ref. 4). (a) Chromatogram of Clophen A50 on the first column (SE-54) as detected with the monitor ECD. (b,e) Details of the chromatograms recorded with the monitor ECD. The peaks labelled 110, 77, and 153, 132, 105, eluting from the first column, have been cut consecutively from this eluate to pass through the second column. (c, f) Chromatograms recorded with ECD reflecting the compositions of the peak cuts in (b) and (e). The position where congener 77 would have eluted if present is indicated by an arrow. (d) Signal of congeners 110 and 77 on the main electron-capture detector after cutting the corresponding peak from the first column after injection of a synthetic mixture of these congeners. Numbering of PCB congeners according to ref. 118.

4.2. Chiral stationary phases

4.2.1. Theory and experimental approach

GC measurements with classical stationary phases supply an insight into the distribution of organic pollutants and their degradation products in the marine ecosystem. However, a decision as to whether or not enzymatic or non-enzymatic processes were responsible for the degradation of the respective pollutants cannot be inferred from concentration patterns.

Recently, Faller *et al.* [89] suggested a new experimental approach that allows a discrimination between these two processes in marine and terrestrial ecosystems: the application of heptakis(3-O-butyryl-2,6-di-O-pentyl)-β-cyclodextrin as a chiral stationary phase for the first time offers the possibility of determining the enantiomeric excess of chiral pollutants even at low concentrations as encountered in a North Sea water sample by means of cGC. The method is expected to be generally applicable to many environmental problems in which chiral biogenic and anthropogenic substances and chiral degradation products are involved. Regarding the discrimination between enzymatic and non-enzymatic processes, it can be safely assumed that chiral substances will be decomposed by non-enzymatic processes non-enantioselectively, whereas enzymatic processes will give rise to enantioselective degradation. In the latter instance, an enantiomeric excess of the educt and/or the degradation product can be expected, which in turn can be determined by the new GC method using cyclodextrin phases.

4.2.2. Application for studying the fate of organic marine pollutants

The feasibility of this method was demonstrated by Faller *et al.* [89] by analysing the enantiomeric ratio of α-HCH in a water sample taken in the North Sea Skagerrak area south of the Norwegian coast. Of the eight HCH isomers, only α-HCH is chiral, exhibiting two enantiomers, whereas the insecticide γ-HCH (lindane) is optically inactive. Technically produced α-HCH shows an enantiomeric ratio of 1:1. The same enantiomeric ratio would have to be expected in the marine environment if the decomposition and/or isomerization of α- and γ-HCH were dominated by non-enzymatic processes, because (a) the HCH isomers are exclusively man-made, so the enantiomeric ratio at the sources basically should be identical with that of technical α-HCH, and (b) non-enzymatic processes are not assumed to lead to an enantiomeric excess of chiral compounds. If microbiological processes dominate the decomposition of HCH isomers, however, be it in sea-water or in terrestrial ecosystems, an enantiomeric excess can be expected, because the decomposition by microorganisms in general proceeds enantioselectively.

In the case of the North Sea water sample taken in the Skagerrak area, Faller *et al.* [89] determined a ratio (+)-α-HCH/(−)-α-HCH = 0.88, which implies that microbiological processes are significantly influencing the degradation of the HCH isomers. The same conclusion was drawn by Faller *et al.* [90] from a more extensive data set obtained from water samples of all characteristic North Sea areas.

Ludwig and co-workers [107,123] performed systematic laboratory investigations on the decomposition of α- and γ-HCH by marine microorganisms and by photochemical processes, determining the enantiomeric excesses by GC with chiral stationary phases. They drew the following conclusions. (a) The prochiral γ-HCH is decomposed by marine microorganisms to γ-1,3,4,5,6-pentachloro-1-cyclohexane (γ-PCCH) or isomerized to α-HCH non-enantioselectively. However, the further degradation of the chiral γ-PCCH is effected enantioselectively. α-HCH and β-PCCH are decomposed by marine microorganisms enantioselectively. These results imply that in the case of HCH isomers, a prochiral substrate (γ-HCH) is not sufficient for an enantioselective microbial degradation or isomerization, and a chiral substrate (α-HCH, β-PCCH, γ-PCCH) is required in order to obtain enantioselective decomposition. (b) Measurements of the air–water transport of γ- and β-PCCH showed that more than 99% of the γ-PCCH that had been formed by degradation of γ-HCH was transported from the water into the

air, whereas with β-PCCH this process can be neglected. (c) Photochemical degradation of α-HCH and of β-PCCH in sea-water by artificial sunlight turned out to be non-enantioselective. However, enantiomeric excesses of β-PCCH that may have been formed by enzymatic processes may be modified by photochemical processes provided that enzymatic processes become less important, *e.g.*, owing to seasonal variations of microbial activity. (d) Determination of the enantiomeric excess of α-HCH, γ-PCCH and β-PCCH in seven water samples from the North Sea and fourteen water samples from the Baltic Sea gave the following average values: North Sea, (+)-α-HCH/(−)-α-HCH = 0.87; Baltic Sea, (+)-α-HCH/(−)-α-HCH = 0.85, γ_1-PCCH/γ_2-PCCH = 1.15 and β_1-PCCH/β_2-PCCH = 0.97. These values can be explained by assuming significant contributions of both enzymatic (enantioselective) and non-enzymatic (non-enantioselective) processes.

4.2.3. Application for studying processes in the ecological food web

Kallenborn *et al.* [47,135] investigated the (+)-α-HCH/(−)-α-HCH enantiomeric ratio in liver, kidney and muscle tissue of the Eider duck (*Somateria mollissima* (L.)). The Eider duck was chosen because it largely favours mussels (*Mytilus edulis* L.) in its diet. Mussels, in turn, are capable of strongly enriching pollutants and thus serve as indicator organisms to provide an insight into the state of an aquatic environment. In Fig. 9 the gas chromatograms of the α-HCH enantiomers are shown for water, mussel and liver of the Eider duck. Whereas the mussel largely reflects the characteristics of the adjacent water area, *i.e.*, an enantiomeric excess of the (−)-α-HCH enantiomer, in the liver of the Eider duck the (+)-α-HCH enantiomer is dominant.

Basically, in all three extracts of liver, kidney and muscle (+)-α-HCH was clearly enriched; almost enantiomerically pure (+)-α-HCH was present in liver extracts. In contrast, the (+)-α-HCH/(−)-α-HCH enantiomeric ratio was about 7.0 in muscle samples and about 1.6 in kidney extracts; the values for these organs were slightly

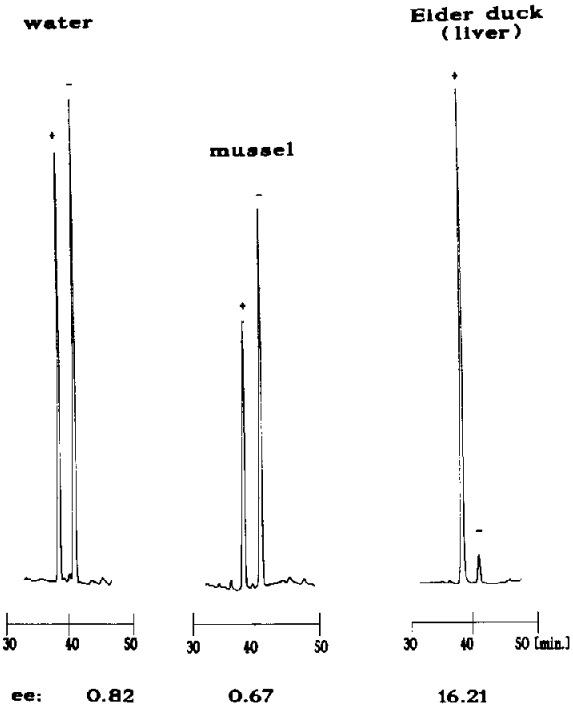

Fig. 9. Enantiomer separation of α-HCH extracted from a Baltic Sea water sample, from a mussel (*Mytilus edulis* L.) and from the liver of a common Eider duck (*Somateria mollissima* (L.)) using a fused-silica capillary column coated with 50% heptakis(2,3,6-tri-O-*n*-pentyl)-β-cyclodextrin and 50% OV-1701. Column temperature programme: initial 323 K, increased at 10K/min to 388 K; carrier gas, helium (45 kPa); on-column injection; ee = enantiomeric excess.

larger or smaller for different Eider ducks [47].

The authors could not give an exact explanation for the appearance of different enantiomeric ratios in the organs of the Eider ducks. However, they assumed that the reason lies in the different physiological functions of the organs. For muscle and kidney, whose main functions are locomotion and excretion, respectively, the content of extractable lipids was about 2%; these organs can, therefore, store lipophilic pollutants. Liver, which contains about 2.5% of extractable lipids, serves as a detoxification organ and is therefore not capable of storing toxic compounds but can metabolize them to substances that the body can tolerate or excrete. As the (+)-α-HCH found in the liver is almost enantiomerically pure, (−)-α-HCH is presumably more readily degraded en-

zymatically than the (+)-enantiomer in the liver.

This is only a first example of ongoing investigations. However, this chromatographic approach appears to have encouraging perspectives.

4.2.4. Possibilities and limitations of cGC using chiral stationary phases

The first chiral cyclodextrin phases used for trace analysis in the marine environment consisted exclusively of β-cyclodextrin derivatives, which tolerated a maximum oven temperature of 423 K, when working with environmental samples that exhibited such low concentrations that application of ECD was required. This gave retention times of more than 3 h. At considerably higher temperatures, *e.g.*, 443 K, bleeding of the column would have given rise to a considerably noisier baseline. These problems were not encountered, however, when working with concentrations such that flame-ionization detection could be applied.

In the meantime, the chiral cyclodextrin phases applicable to environmental trace analysis have been improved considerably: capillary columns coated with 50% heptakis(2,3,6-tri-O-*n*-pentyl)-β-cyclodextrin and 50% OV-1701 give significantly better resolution and shorter retention times [107,123,138].

In particular, application of these latter chiral phases allows the determination of the enantiomeric ratios of many chiral pollutants and their chiral degradation products in environmental samples with good reproducibility. As ECD can be used, measurements can be performed at trace concentrations, which in turn supplies a new experimental approach for the discrimination between enzymatic (enantioselective; for exceptions, see Hühnerfuss and co-workers [107,123]) and non-enzymatic (non-enantioselective) processes both in marine waters [89,90,107,123] and in the marine food web [47].

5. CONCLUSIONS

This review has taken into account the vital importance of clean-up and fractionation techniques by giving a brief overview of liquid–liquid and solid-phase extraction methods. Liquid–liquid extraction is often applied to sea-water samples, while the more complex matrix of marine biota in general requires additional clean-up procedures including GPC separation and other adsorption chromatographic techniques. In particular, reversed-phase columns of different polarity have been used for clean-up procedures in recent years. Rapid and precise work-up of sea-water samples for the determination of high-volatile halocarbons can be achieved by means of OLLE.

The unequivocal advantage of HPLC is its applicability both to the clean-up of the sample and to the determination of organic pollutants, although the low concentrations of many environmental pollutants limits more general applicability. Therefore, much effort has been spent on the development and improvement of detectors, *e.g.*, fluorescence ICP-AES and other specific detectors that gave increases in sensitivity and decreases in detection limits. However there is still a great need for a chlorine-selective detector in LC that will allow trace analyses for organic halogenated pollutants. In order to overcome this problem, the HPLC–GC–ECD combination has been used, *i.e.*, the HPLC sensor is replaced with the ECD of the gas chromatograph. Hence pollutants can be determined at the picograms per litre level in sea-water. In this review, emphasis has been placed on HPLC analyses of organometallics, PAHs, pesticides and halogenated pollutants. Further, the recent advances achieved by the application of chiral stationary phases have been reported. The chromatographic separation of the enantiomers of chiral marine pollutants allows a discrimination between enzymatic and non-enzymatic processes in the marine ecosystem. Another experimental approach that has attracted much attention in recent years is the miniaturization of columns, which may give rise to considerably shorter retention times.

cGC still remains a major force in the separation of organic marine pollutants, although the high sensitivity of this method in general requires thorough clean-up and fractionation procedures

prior to its application. Recent investigations on marine organic pollutants were focused predominantly on HCH isomers and PCBs. In addition, organophosphates, chlorophenols, nitrogen-containing pollutants and other organochlorines have been analysed. Some recent papers include high-volatile organic pollutants. New promising techniques include multi-dimensional GC and the application of chiral stationary phases in cGC. The former method allows the separation of coplanar PCBs and, as a consequence, the toxic potential of the PCB fraction of environmental samples can be established much more reliably. cGC measurements with chiral stationary phases have allowed the discrimination between enzymatic and non-enzymatic degradation processes of marine pollutants at trace levels. The possibilities and limitations of this method have been reviewed.

6. ACKNOWLEDGEMENT

This work was supported by the Ministry of Science and Technology of the Federal Republic of Germany (BMFT-Project MFU 0620 "Prozesse im Schadstoffkreislauf Meer–Atmosphäre: Ökosystem Deutsche Bucht").

REFERENCES

1 A. Pütter, *Die Ernährung der Wassertiere und der Stoffhaushalt der Gewässer*, Fischer, Jena, 1909.
2 T. C. Sauer, Jr., G. S. Durrell, J. S. Brown, D. Redford and P. D. Boehm, *Mar. Chem.*, 27 (1989) 235.
3 G. R. Harvey and W. G. Steinhauer, *Science*, 180 (1973) 643.
4 J. C. Duinker, D. E. Schulz and G. Petrick, *Anal. Chem.*, 60 (1988) 478.
5 R. C. Clark, M. Blumer and S. O. Raymond, *Deep-Sea Res.*, 14 (1967) 125.
6 H. Gaul and U. Ziebarth, *Dtsch. Hydrogr. Z.*, 36 (1983) 191.
7 H. Hühnerfuss, *Meerestech. Mar. Technol.*, 12 (1981) 137.
8 H. Hühnerfuss, *Meerestech. Mar. Technol.*, 12 (1981) 170; 13 (1982) 24.
9 W. Ernst, R. F. Schaefer, H. Goerke and G. Eder, *Fresenius' Z. Anal. Chem.*, 272 (1974) 358.
10 P. G. Desideri, L. Lepri, D. Heimler, S. Giannessi and L. Checchini, *J. Chromatogr.*, 284 (1984) 167.
11 K. Abrahamsson and S. Klick, *J. Chromatogr.*, 513 (1990) 39.
12 K. Bester, *Diploma-Thesis*, University of Hamburg, Hamburg, 1991.
13 P. Fernández, C. Porte, D. Barceló, J. M. Bayona and J. Albaigés, *J. Chromatogr.*, 456 (1988) 155.
14 H. Takada and R. Ishiwatari, *J. Chromatogr.*, 346 (1985) 281.
15 K.-R. Sperling, D. Janssen and T. Wulf, *Fresenius' Z. Anal. Chem.*, 320 (1985) 1.
16 R. J. Ozretich and W. P. Schroeder, *Anal. Chem.*, 58 (1986) 2041.
17 B. Pfaffenberger, *Diploma-Thesis*, University of Hamburg, Hamburg, 1989.
18 M. Ehrhardt, *Int. Counc. Explor. Sea Tech. Environ. Sci.*, 4 (1987) 1.
19 M. Ehrhardt, *Mar. Chem.*, 26 (1989) 363.
20 G. Petrick, D. E. Schulz and J. C. Duinker, *J. Chromatogr.*, 435 (1988) 241.
21 M. M. Krahn, L. K. Moore, R. G. Bogar, C. A. Wigren, S.-L. Chan and D. W. Brown, *J. Chromatogr.*, 437 (1988) 161.
22 E. Fogelqvist, M. Krysell and L. G. Danielsson, *Anal. Chem.*, 58 (1986) 1516.
23 A. L. Alford-Stevens, W. L. Budde and T. A. Bellar, *Anal. Chem.*, 57 (1985) 2452.
24 J. Lohse, *Mar. Pollut. Bull.*, 19 (1988) 366.
25 R. Kniekmeyer and H. Steinhart, *Dtsch. Hydrogr. Z.*, 41 (1988) 1.
26 R. Kniekmeyer and H. Steinhart, *Dtsch. Hydrogr. Z.*, 42 (1989) 43.
27 M. M. Schantz, B. A. Benner, Jr., S. N. Chesler, B. J. Koster, K. E. Hehn, S. F. Stone, W. R. Kelly, R. Zeisler and S. A. Wise, *Fresenius' J. Anal. Chem.*, 338 (1990) 501.
28 M. Remberger, A. Hynning and A. H. Neilson, *J. Chromatogr.*, 508 (1990) 159.
29 M. Guiliano, P. Doumenq, A. Jawad and G. Mille, *Appl. Spectrosc.*, 43 (1989) 571.
30 I. Watanabe, T. Kashimoto, M. Kawano and R. Tatsukawa, *Chemosphere*, 16 (1987) 849.
31 T. Tsuda, H. Nakanishi, S. Aoki and J. Takebayashi, *J. Chromatogr.*, 387 (1987) 361.
32 V. Weigelt, *Chemosphere*, 15 (1986) 289.
33 C. Porte, D. Barceló and J. Albaigés, *J. Chromatogr.*, 442 (1988) 386.
34 I. Bauer, S. Weigelt and W. Ernst, *Chemosphere*, 19 (1989) 1701.
35 J. McDowell Capuzzo, J. W. Farrington, P. Rantamaki, C. H. Clifford, B. A. Lancaster, D. F. Leavitt and X. Jia, *Mar. Environ. Res.*, 28 (1989) 259.
36 W. M. Draper and S. Koszdin, *J. Agric. Food Chem.*, 39 (1991) 1457.
37 S. Tanabe, N. Kannan, A. Subramanian, S. Watanabe and R. Tatsukawa, *Environ. Pollut.*, 47 (1987) 147.
38 J. P. Boon and F. Eijgenraam, *Mar. Environ. Res.*, 24 (1988) 3.
39 D. C. G. Muir, R. J. Norstrom and M. Simon, *Environ. Sci. Technol.*, 22 (1988) 1071.

40 D. W. Kuehl, B. C. Butterworth, J. Libal and P. Marquis, *Chemosphere*, 22 (1991) 849.
41 X. Yan, P. J. Barlow and C. Craven, *Food Chem.*, 40 (1991) 93.
42 R. Knickmeyer and H. Steinhart, *Mar. Pollut. Bull.*, 20 (1989) 433.
43 R. Knickmeyer and H. Steinhart, *Chemosphere*, 19 (1989) 1309.
44 R. Knickmeyer and H. Steinhart, *Chemosphere*, 20 (1990) 109.
45 R. Knickmeyer and H. Steinhart, *Mar. Pollut. Bull.*, 21 (1990) 187.
46 W. A. Heidmann and M. Beyerbach, *Chemosphere*, 18 (1989) 2303.
47 R. Kallenborn, H. Hühnerfuss and W. A. König, *Angew. Chem.*, 103 (1991) 328; *Angew. Chem., Int. Ed. Engl.*, 30 (1991) 320.
48 J. C. Duinker, D. E. Schulz and G. Petrick, *Mar. Pollut. Bull.*, 19 (1988) 19.
49 M. Oehme, *Sci. Total Environ.*, 106 (1991) 43.
50 B. Luckas, W. Vetter, P. Fischer, G. Heidemann and J. Plötz, *Chemosphere*, 21 (1990) 13.
51 R. Knickmeyer and H. Steinhart, *Neth. J. Sea Res.*, 22 (1988) 237.
52 R. Knickmeyer and H. Steinhart, *Sarsia*, 73 (1988) 291.
53 J. C. Duinker, A. H. Knap, K. C. Binkley, G. H. van Dam, A. Darrel-Rew and M. T. J. Hillebrand, *Mar. Pollut. Bull.*, 19 (1988) 74.
54 K. A. Newman and P. M. Gschwend, *Limnol. Oceanogr.*, 32 (1987) 702.
55 R. Knickmeyer and H. Steinhart, *Estuar. Coast. Shelf Sci.*, 28 (1989) 117.
56 A. Wenck, L. Mintrop and J. C. Duinker, *Mar. Chem.*, 33 (1991) 1.
57 S. Einarsson, S. Folestad, B. Josefsson and S. Lagerkvist, *Anal. Chem.*, 58 (1986) 1638.
58 W. S. Gardner and P. A. St. John, *Anal. Chem.*, 63 (1991) 537.
59 W. S. Gardner, L. R. Herche, P. A. St. John and S. P. Seitzinger, *Anal. Chem.*, 63 (1991) 1838.
60 K. Kohata, M. Watanabe and K. Yamanaka, *J. Chromatogr.*, 558 (1991) 131.
61 S. Roy, *J. Chromatogr.*, 391 (1987) 19.
62 B. Stancher, F. Zonta and L. G. Favretto, *J. Chromatogr.*, 440 (1988) 37.
63 J. F. Lawrence and C. Ménard, *Fresenius' J. Anal. Chem.*, 339 (1991) 494.
64 L. Ebdon, S. J. Hill and P. Jones, *Analyst (London)*, 110 (1985) 515.
65 L. Ebdon and J. I. G. Alonso, *Analyst (London)*, 112 (1987) 1551.
66 S. Branch, L. Ebdon, S. Hill and P. O'Neill, *Anal. Proc.*, 26 (1989) 401.
67 I. Tolosa, J. M. Bayona, J. Albaigés, L. F. Alencastro and J. Tarradellas, *Fresenius' J. Anal. Chem.*, 339 (1991) 646.
68 B. R. Willeford and H. Veening, *J. Chromatogr.*, 251 (1982) 61.
69 G. Nickless, *J. Chromatogr.*, 313 (1985) 129.
70 D. J. Mackey, *Mar. Chem.*, 13 (1983) 169.
71 D. J. Mackey and H. W. Higgins, *J. Chromatogr.*, 436 (1988) 243.
72 A. Mazzucotelli, R. Frache, A. Viarengo and G. Martino, *Talanta*, 35 (1988) 693.
73 A. Mazzucotelli, A. Viarengo, L. Canesi, F. de Paz, E. Ponzano and P. Rivaro, *Anal. Proc.*, 28 (1991) 79.
74 A. Mazzucotelli, A. Viarengo, L. Canesi, E. Ponzano and P. Rivaro, *Analyst (London)*, 116 (1991) 605.
75 Y. Nagaosa, H. Kawabe and A. M. Bond, *Anal. Chem.*, 63 (1991) 28.
76 H. Ohashi, N. Uehara and Y. Shijo, *J. Chromatogr.*, 539 (1991) 225.
77 J. W. McLaren, K. W. M. Siu, J. W. Lam, S. N. Willie, P. S. Maxwell, A. Palepu, M. Koether and S. S. Berman, *Fresenius' J. Anal. Chem.*, 337 (1990) 721.
78 N. Theobald, *Mar. Pollut. Bull.*, 20 (1989) 134.
79 X. Bo-Xing and F. Yu-Zhi, *Talanta*, 35 (1988) 891.
80 M. D. Núnez and F. Centrich, *Anal. Chim. Acta*, 234 (1990) 269.
81 M. A. Quilliam and P. G. Sim, *J. Chromatogr. Sci.*, 26 (1988) 160.
82 R. Amodio Cocchieri, A. Arnese and A. M. Minicucci, *Mar. Pollut. Bull.*, 21 (1990) 15.
83 M. M. Krahn, G. M. Ylitalo, J. Joss and S.-L. Chan, *Mar. Environ. Res.*, 31 (1991) 175.
84 R. J. van de Nesse, G. J. M. Hoogland, J. J. M. de Moel, C. Gooijer, U. A. Th. Brinkman and N. H. Velthorst, *J. Chromatogr.*, 552 (1991) 613.
85 J. W. Hofstraat, H. J. M. Jansen, G. Ph. Hoornweg, C. Gooijer and N. H. Velthorst, *Int. J. Environ. Anal. Chem.*, 21 (1985) 299.
86 J. W. G. Mastenbroek, F. Ariese, C. Gooijer and N. H. Velthorst, *Chemosphere*, 21 (1990) 377.
87 C. Schlett, *Fresenius' J. Anal. Chem.*, 339 (1991) 344.
88 S. Folestad, B. Josefsson and P. Marstorp, *Anal. Chem.*, 59 (1987) 334.
89 J. Faller, H. Hühnerfuss, W. A. König, R. Krebber and P. Ludwig, *Environ. Sci. Technol.*, 25 (1991) 676.
90 J. Faller, H. Hühnerfuss, W. A. König and P. Ludwig, *Mar. Pollut. Bull.*, 22 (1991) 82.
91 P. Ludwig, W. Gunkel and H. Hühnerfuss, *Chemosphere*, 24 (1992) 1423.
92 S. Einarsson, B. Josefsson, P. Möller and D. Sanchez, *Anal. Chem.*, 59 (1987) 1191.
93 D. Ishii, *Introduction to Microscale High-Performance Liquid Chromatography*, VCH, Weinheim, 1988.
94 K. Abrahamsson and S. Klick, *Chemosphere*, 18 (1989) 2247.
95 K. Abrahamsson and S. Klick, *J. Chromatogr.*, 513 (1990) 39.
96 E. Fogelqvist, *J. Geophys. Res.*, 90 (1985) 9181.
97 E. Fogelqvist and M. Krysell, *Mar. Pollut. Bull.*, 17 (1986) 378.
98 B. Bullister and R. F. Weiss, *Deep-Sea Res.*, 35 (1988) 839.

99 Th. Class and K. Ballschmiter, *J. Atmos. Chem.*, 6 (1988) 35.

100 Th. Class and K. Ballschmiter, *Fresenius' Z. Anal. Chem.*, 325 (1986) 1.

101 E. Fogelqvist, B. Josefsson and C. Roos, *Environ. Sci. Technol.*, 16 (1982) 479.

102 E. Fogelqvist, M. Krysell and P. Öhman, *Mar. Chem.*, 26 (1989) 339.

103 M. J. Warner and R. F. Weiss, *Deep-Sea Res.*, 32 (1985) 1485.

104 R. F. Weiss, J. L. Bullister, R. H. Gammon and M. J. Warner, *Nature (London)*, 314 (1985) 608.

105 R. F. Weiss and J. L. Bullister, *Deep-Sea Res.*, 35 (1988) 839.

106 D. P. Wisegarver and R. H. Gammon, *Geophys. Res. Lett.*, 15 (1988) 188.

107 P. Ludwig, H. Hühnerfuss, W. A. König and W. Gunkel, *Mar. Chem.*, 38 (1992) 13.

108 H. Hühnerfuss and K. Weber, unpublished results.

109 B. Bush, R. W. Streeter and R. J. Sloan, *Arch. Environ. Contam. Toxicol.*, 19 (1990) 49.

110 P. de Voogt, D. E. Wells, L. Reutergardh and U. A. Th. Brinkman, *Int. J. Environ. Anal. Chem.*, 40 (1990) 1.

111 V. Zitko, *Mar. Pollut. Bull.*, 20 (1989) 26.

112 R. Lara and W. Ernst, *Chemosphere*, 19 (1989) 1655.

113 A. Sabljić, R. Lara and W. Ernst, *Chemosphere*, 19 (1989) 1665.

114 D. E. Schulz, G. Petrick and J. C. Duinker, *Mar. Pollut. Bull.*, 19 (1988) 526.

115 P. R. Loconto and A. K. Gaind, *J. Chromatogr. Sci.*, 27 (1989) 569.

116 H. Hühnerfuss, H. Dannhauer, J. Faller and P. Ludwig, *Dtsch. Hydrogr. Z.*, 43 (1990) 253.

117 T.-M. Xie, K. Abrahamsson, E. Fogelqvist and B. Josefsson, *Environ. Sci. Technol.*, 20 (1986) 457.

118 K. Ballschmiter and M. Zell, *Fresenius' Z. Anal. Chem.*, 302 (1980) 20.

119 S. Tanabe, N. Kannan, T. Wakimoto and R. Tatsukawa, *Int. J. Environ. Anal. Chem.*, 29 (1987) 199.

120 W. Vetter, B. Luckas and W. Haubold, *Chemosphere*, 23 (1991) 193.

121 A. M. Boganov and I. M. Mikhaleva, *Tr. Gos. Okeanogr. Inst.*, 187 (1987) 20; *C.A.*, 108 (1987) 100932x.

122 E. S. Lebedeva and A. M. Boganov, *Tr. Gos. Okeanogr. Inst.*, 187 (1987) 36; *C.A.*, 108 (1987) 100929b.

123 H. Hühnerfuss, J. Faller, W. A. König and P. Ludwig, *Environ. Sci. Technol.*, (1992) in press.

124 N. Kannan, G. Petrick, D. Schulz, J. Duinker, J. Boon, E. van Arnhem and S. Jansen, *Chemosphere*, 23 (1991) 1055.

125 J. W. Hofstraat, W. J. M. van Zeijl, F. Ariese, J. W. G. Mastenbroek, C. Gooijer and N. H. Velthorst, *Mar. Chem.*, 33 (1991) 301.

126 A. F. McElroy and J. D. Sisson, *Mar. Environ. Res.*, 28 (1989) 265.

127 D. C. G. Muir, C. A. Ford, N. P. Grift, R. E. A. Stewart and T. F. Bidleman, *Environ. Pollut.*, 75 (1992) 307.

128 J. L. Sericano, A. M. El-Husseini and T. L. Wade, *Chemosphere*, 23 (1991) 915.

129 B. Brunström, D. Broman and C. Näf, *Environ. Pollut.*, 67 (1990) 133.

130 F. Galgani, G. Bocquene, M. Lucon, D. Grzebyk, F. Letrouit and D. Claisse, *Mar. Pollut. Bull.*, 22 (1991) 494.

131 B. T. Hargrave, G. C. Harding, W. P. Vass, P. E. Erickson, B. R. Fowler and V. Scott, *Arch. Environ. Contam. Toxicol.*, 22 (1992) 41.

132 A. O. Valkirs, P. F. Seligman, G. J. Olson, F. E. Brinckman, C. L. Matthias and J. M. Bellama, *Analyst (London)*, 112 (1987) 17.

133 M. R. Prescott and J. Merrett, *Mar. Pollut. Bull.*, 22 (1991) 516.

134 M. Oehme, *Ambio*, 20 (1991) 293.

135 R. Kallenborn, H. Hühnerfuss and W. A. König, *Chemosphere*, 24 (1992) submitted for publication.

136 J. C. Duinker, D. E. Schulz and G. Petrick, *Chemosphere*, 23 (1991) 1009.

137 R. C. Fischer, W. Krämer and K. Ballschmiter, *Chemosphere*, 23 (1991) 889.

138 H. Hühnerfuss, *GIT Fachz. Lab.*, 36 (1992) 489.