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Novel Analytical Procedures for Sample Preparation and Analysis of Environmentally Harmful Compounds

Piia Jõul

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TAL TECH PRESS

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Declaration:
Hereby I declare that this doctoral thesis, my original investigation and achievement, submitted for the doctoral degree at Tallinn University of Technology has not been submitted for doctoral or equivalent academic degree.

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Uudsed analüüsimetoodikad keskkonnakahjulikke ühendeid sisaldavate proovide ettevalmistuseks ja analüüsiks

PIIA JÕUL

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List of Publications

Author's publication list, based on which the thesis has been prepared:

I **P. Jõul**, H. Lees, M. Vaher, E.-G. Kobrin, M. Kaljurand, and M. Kuhtinskaja, "Development of a capillary electrophoresis method with UV detection for the analysis of thiodiglycol and its oxidation products," *Electrophoresis*, vol. 36, no. 9-10, pp. 1202–1207, 2015.

II **P. Jõul**, M. Vaher, and M. Kuhtinskaja, "Evaluation of carbon aerogel-based solid-phase extraction sorbent for the analysis of sulfur mustard degradation products in environmental water samples," *Chemosphere,* vol. 198, pp. 460–468, 2018.

III **P. Jõul**, M. Vaher, and M. Kuhtinskaja, "Carbon aerogel-based solid-phase microextraction coating for the analysis of organophosphorus pesticides," *Anal. Methods*, vol. 13, no. 69, pp. 69–76, 2021.

Author's Contribution to the Publications

Contributions to the papers in this thesis are described as follows:

I The author performed preliminary experiments and was involved in planning all the experiments. The author was responsible for sample collection, planning, and conducting all the sample extraction experiments, as well as the analysis and interpretation of data. The author also contributed toward the preparation of this manuscript.

II-III The author planned all the experiments, participated in the collection of samples, performed all the experimental work (except CA characterisation), as well as analysed and interpreted the data. The author wrote the manuscript and supporting information with contributions from the co-authors and is the first and the corresponding author.

Other Publications by the Author (not discussed in this thesis)

- Book chapter: M. Söderström, A. Östin, J. Qvarnström, R. Magnusson, J. Rattfelt-Nyholm, M. Vaher, **P. Jõul**, H. Lees, M. Kaljurand, M. Szubska, P. Vanninen, J. Bełdowski, "Chemical analysis of dumped chemical warfare agents during the MODUM project," in *Towards the Monitoring of Dumped Munitions Threat (MODUM)*, J. Bełdowski, R. Been, and E. K. Turmus, Eds. Dordrecht: Springer, 2018, pp. 71–103.
- **P. Jõul**, M. Kuhtinskaja, M. Vaher, and M. Koel, "Green Chemistry and reconsidering simple analytical methods," *Chim. Oggi – Chem. Today,* vol. 35, no. 2, pp. 32–34, 2017.
- H. Lees, **P. Jõul**, K. Siilak, and M. Vaher, "Separation of perfluoroalkyl substances by using nonaqueous capillary electrophoresis with conductivity detection," *Sep. Sci. Plus,* vol. 3, no. 7, pp. 313–320, 2020.

Introduction

Sample analysis comprises several steps required for the identification and quantitation of target compounds. Most of the analytical methodologies include sample collection and storage, sample preparation, analyte determination, and data analysis steps to afford reliable results. Sample preparation is the most laborious and time-consuming step, which accounts for nearly two-third of the overall analysis time and contributes to approximately 30% error sources of the total sample analysis process [1]–[3]. Although sample preparation is often a bottleneck in chemical analysis, it is a key step in most sample analysis methodologies, particularly if an efficient detection of low concentrations of target analytes is required in environmental samples with high complexity. It consists of a series of steps to eliminate the matrix effect, exchange the solvent, and concentrate the sample, if required, to subsequently allow the use of appropriate separation and detection methods. Considering green analytical chemistry parameters, sample preparation is considered the most polluting step in sample analysis, owing to the high amount of solvent and energy required [4], [5]. Thus, despite a broad spectrum of available sample analysis methods, novel strategies for sample preparation and analytical procedures are in high demand. One of the aims of sustainable development of novel sample preparation procedures is to decrease the number of steps required to transform a sample into suitable form for analysis. Such sample preparation techniques include solid-phase extraction (SPE) and its miniaturised form, solid-phase microextraction (SPME) [5]. Selecting an appropriate SPE sorbent and SPME fiber coating for a particular sample matrix and target analytes plays a key role in achieving high extraction efficiency. Various types of commercial SPE sorbents and SPME fiber coatings are available, but the need for alternative ones that can afford improved selectivity, robustness, and cost-effectiveness have led to the development and evaluation of novel materials that can be used as SPE and SPME sorbents.

Carbon aerogels (CAs) are promising materials for such applications because of their well-developed pore structures, low densities, large specific surface areas, and physiochemical stability [6]–[9]. In the present study, 5-methylresorcinol (MR), which is an oil shale processing by-product, and formaldehyde (FA)-based CAs were prepared and evaluated as SPE sorbents for the analysis of the degradation products of chemical warfare agents (CWAs), and as *in situ* synthesised fiber coatings for the SPME of organophosphorus pesticides (OPPs); in Estonia, oil shale is the most important local solid fossil fuel, which is a major energy source [10].

The analysis of the degradation products of CWAs in environmental samples, particularly water and sediment samples, has received considerable attention because of the issue of dumped munitions. After World War II, many countries encountered issues related to the disposal of conventional and chemical munitions, which was prohibited according to the Geneva Protocol 1925 [11]. Although it is clearly irrational, but dumping in the sea was considered the most appropriate solution at the time [12]. It is assumed that approximately 50 000 chemical weapons (CWs) containing approximately 15 000 tons of CWAs were dumped in the Baltic Sea. As 63% of the CWAs that were dumped near Gotland and Bornholm contained a mixture of sulfur mustard (HD), it is the most abundant sea-dumped CWA in the Baltic Sea [12], [13].

Due to the corrosion of these shells and possible leakages of the highly toxic CWAs into the marine environment, the entire Baltic marine ecosystem as well as human health might be at serious risk. Thus, in the last 15 years, several European Union (EU)-funded

international projects, including the North Atlantic Treaty Organisation (NATO) Science for Peace and Security (SPS) project, "Towards the Monitoring of Dumped Munitions Threat" (MODUM), have targeted dumped munitions in the Baltic Sea [14]. This project involved scientists from nine different countries, and portion of the current study was carried out as a part of this project, where our research group was responsible for the development of capillary electrophoresis (CE) procedure for the analysis of the degradation products of HD, which involved the development of sample preparation (extraction and derivatisation) and analysis procedures [15].

Another group of environmentally harmful compounds includes the OPPs, which are extensively used as insecticides in agriculture. Although many of these OPPs are not approved as active substances in the EU for use in plant protection products [16], the abuse or inappropriate application of these compounds is often related to poisoning, environmental pollution, and food contamination [17]. Considering the increasing concern, the development of selective and sensitive analytical procedures, including environmentally friendly sample preparation techniques such as SPME [18], for the determination of OPPs in environmental water samples and food is highly desirable.

This dissertation is divided into five sections. Section 1 includes an overview of the literature reports on the need for suitable sample preparation techniques. It also includes a concise introduction of the sea-dumped CWs and CWAs, as well as OPPs, and provides a brief overview of the sample preparation and analysis methods for environmentally harmful compounds investigated in this study. Section 2 outlines the main aims of the thesis, and Section 3 describes the reagents, materials, and samples used for method development, as well as the instrumentation and experimental conditions. Section 4 discusses the results presented in **Publications I-III**, i.e., sample preparation and analysis of environmentally harmful compounds such as the degradation products of HD (**Publications I and II**) and OPPs (**Publication III**). **Publication I** describes the development and optimisation of a pre-capillary derivatisation procedure by employing the CE separation method using ultraviolet absorbance detection for the analysis of acyclic degradation products of HD. **Publication II** focuses on the development and evaluation of SPE procedure using the CA-based sorbent for the simultaneous pretreatment and enrichment of acyclic and cyclic degradation products of HD present in environmental water samples. The second part of Section 4 describes the *in situ* synthesis of a novel CA-based SPME fiber coating on stainless steel wire and evaluation of the applicability of CAs as SPME coating materials for the direct immersion SPME procedure together with gas chromatography-mass spectrometry analysis of selected OPPs in the environmental matrices of natural water and honey samples (**Publication III**).

In addition to the publications, the author presented the research at several international conferences in Estonia, Denmark, Finland, Spain, Italy, Portugal, and Turkey.

Abbreviations

ACN	Acetonitrile
BGE	Background electrolyte
CA	Carbon aerogel
CAR	Carboxen
CE	Capillary electrophoresis
CW	Chemical weapon
CWA	Chemical warfare agent
CWC	Chemical Weapons Convention
DAD	Diode array detector
DFT	Density functional theory
DI	Direct immersion
DTHD	3,5-Dithia-1,7-heptanediol
DTOD	3,6-Dithia-1,8-octanediol
DVB	Divinylbenzene
EU	European Union
FA	Formaldehyde
FPD	Flame photometric detector
GC	Gas chromatography
HD	Sulfur mustard
HPLC	High-performance liquid chromatography
HS	Headspace
ID	Internal diameter
IS	Internal standard
LC	Liquid chromatography
LLE	Liquid–liquid extraction
LOD	Limit of detection
LOQ	Limit of quantification
MEKC	Micellar electrokinetic chromatography
MODUM	Towards the Monitoring of Dumped Munitions Threat
MR	5-Methylresorcinol
MR/C	Molar ratio of 5-methylresorcinol to catalyst (Na_2CO_3)
MR/FA	Molar ratio of 5-methylresorcinol to formaldehyde
MR/W	Molar ratio of 5-methylresorcinol to water
MS	Mass spectrometry
NATO	North Atlantic Treaty Organisation
OP	Organophosphorus
OPCW	Organization for the Prohibition of Chemical Weapons
OPP	Organophosphorus pesticide
PA	Polyacrylate
PDMS	Polydimethylsiloxane

PEG	Polyethylene glycol
R	Resorcinol
R^2	Coefficient of determination
S/N	Signal-to-noise ratio
SEM	Scanning electron microscopy
SIM	Selected ion monitoring
SLE	Solid–liquid extraction
SPE	Solid-phase extraction
SPME	Solid-phase microextraction
SPS	Science for Peace and Security
TDG	Thiodiglycol
TDGO	Thiodiglycol sulfoxide
TDGOO	Thiodiglycol sulfone
UV	Ultraviolet
XPS	X-ray photoelectron spectroscopy

1 Literature overview

1.1 Sample preparation

Considering the complexity of sample matrices, low concentrations of target compounds, presence of interferences, and incompatibility with subsequent analysis steps, sample preparation is a crucial step for the efficient detection of target analytes in environmental samples [2]. However, it must be preceded by suitable sample collection and preservation methods, followed by efficient and correct data management to obtain reliable results. As the most laborious and time-consuming step of chromatographic analysis, sample preparation accounts for nearly two-third of the overall analysis time (Figure 1A), contributing approximately 30% of the error sources, thereby rendering it the most error-prone step of the total sample analysis procedure (Figure 1B) [1]–[3].

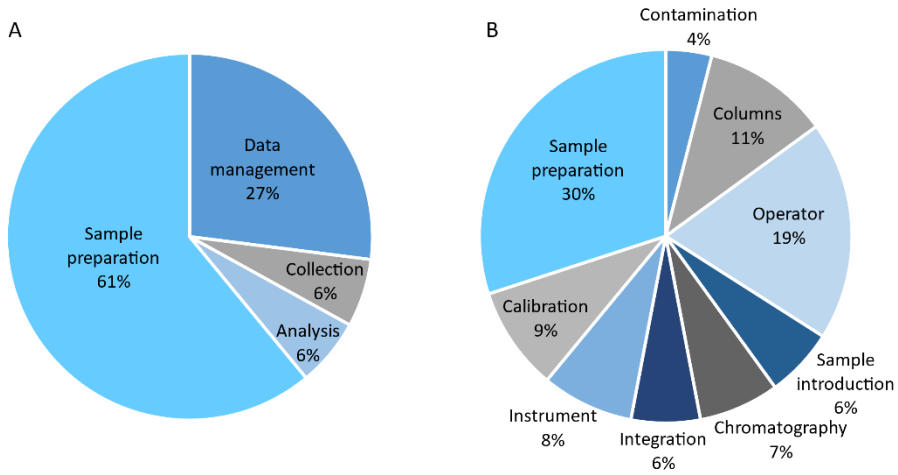


Figure 1. Time spent on typical chromatographic analysis (A) and sources of error generated during chromatographic analysis (B) [3].

Sample preparation comprises several steps that depend on the sample, matrix, analyte concentration, and subsequently applied separation/detection methods. Commonly employed sample preparation steps include homogenisation, size reduction, extraction, concentration, and clean-up, often together with analyte derivatisation for trace analysis [19].

As a frequently used procedure in sample preparation, derivatisation refers to the modification of analyte molecules by chemical reaction to obtain the desired chemical and physical properties of the molecules that can allow the isolation, separation, and/or detection of the derivative. The derivatisation mode in capillary electrophoresis (CE) can be classified as pre-capillary (before separation), in-capillary (during separation), or post-capillary (after separation) derivatisation, and must be carefully selected depending on the analyte, sample matrix, other sample preparation procedures, and separation and detection methods [20], [21].

According to the principles of green analytical chemistry, sample treatment is considered the most polluting step of the analysis procedure because of the high amount of solvent and energy required [4], [5]. However, as sample preparation is indispensable in most analytical methodologies, it is possible to decrease the number of steps to

transform a sample into a suitable form for analysis. Outstanding examples include solid-phase extraction (SPE) and the miniaturised counterpart, solid-phase microextraction (SPME), techniques [5].

1.1.1 Solid-phase extraction

The first application of the SPE-type packed sorbent was reported in 1951, when Braus *et al.* [22] used granular activated carbon in an iron cylinder for the isolation of organics from surface water. Since then, significant progress in SPE technology has been made in terms of miniaturisation, automation, overall simplification of the extraction method, and the development of new formats and sorbents for the procedure [23]. However, the main aims of this extraction technique remain the same – to use isolation, concentration, solvent exchange, and matrix simplification in combination as a procedure to transform the sample into a form that is compatible with the analytical instrument employed for analysis [24]. In addition, this non-equilibrium, exhaustive extraction procedure is the most popular sample preparation technique because it is faster, easier, relatively inexpensive, and greener compared to liquid–liquid extraction (LLE) [25].

Currently, the most commonly used and commercially available configuration of SPE is cartridge (column)-based SPE, where a short disposable column containing a bed of porous particles is immobilised between two porous frits, that is typically used in flow-through extraction mode. The main steps of traditional flow-through SPE include sorbent conditioning, sample introduction, and rinsing/cleaning steps. The analytes are finally recovered from the sorbent by solvent displacement [24], [26].

The main mechanisms of interaction between the analyte of interest and sorbent depend on their hydrophobic, polar, and ionogenic properties. Considering these properties, the most common retention mechanism in SPE involves the van der Waals forces, π–π interaction, hydrogen bonding, dipole–dipole, and ion exchange interactions [23], [27]. Based on the retention mechanism, the sorbents used in SPE can be classified as follows: reversed phase, for example, C8- and C18-bonded phases; normal phase, such as silica, Florisil, alumina, diol, NH_2, and CN-bonded phases; ion exchange, for example, sulfonic–strong cation exchange and ammonium – strong anion exchange bonded phases [23]. The most frequently used groups of sorbents comprise the chemically modified silica gel, polymer sorbents, and graphitised or porous carbon. In addition, mixed-mode SPE sorbents are used, which provide the selectivity that is necessary to obtain clean extracts for the analysis of a wide variety of samples from different research areas, as well as other selective extraction sorbents such as molecularly imprinted polymers and immunosorbents [23], [27].

1.1.2 Solid-phase microextraction

SPME is a commonly used miniaturised SPE method that was introduced by Arthur and Pawliszyn in 1990 [28] to meet the need for a rapid sample preparation method [29]. In this microextraction technique, a small amount of extracting phase on a small-diameter fiber is exposed to the sample, and after a certain period of time, the analytes are distributed into the stationary phase (SPME fiber coating). Finally, the coated SPME fiber is removed from the sample vial, and the target analytes are desorbed either thermally, directly into the gas chromatography (GC) system, or using a desorption solvent [28], [30]. This easy-to-use, versatile, and non-exhaustive microextraction technique allows the elimination of interfering compounds from complex sample matrices using a minimum number of steps to afford a reproducible methodology, which is the main goal of the sample preparation procedure [29].

The law of mass conservation in equilibrium for the sample matrix and fiber coating, if only these two phases are considered, can be described as follows [29]:

$$C_0 V_s = C_s^{\infty} V_s + C_f^{\infty} V_f, \qquad (1)$$

where C_0 is the initial concentration of the analyte in the sample, V_f is the volume of the fiber coating, V_s is the volume of the sample, C_s^{∞} is the equilibrium concentration in the sample, and C_f^{∞} is the equilibrium concentration in the fiber coating.

The distribution coefficient K_{fs} of the analyte between the fiber coating and sample matrix is defined as shown below.

$$K_{fs} = \frac{C_f^{\infty}}{C_s^{\infty}} \qquad (2)$$

Combining and rearranging Equations 1 and 2, the number of moles of analyte n extracted by the coating can be calculated using Equation 3.

$$n = C_f^{\infty} V_f = C_0 \frac{K_{fs} V_s V_f}{K_{fs} V_f + V_s} \qquad (3)$$

Equation 3 shows that the amount of analyte extracted onto the coating (n) is linearly proportional to the analyte concentration in the sample (C_0); thus, SPME can be used for quantitative analysis.

Furthermore, when the sample volume is very large ($V_s \gg K_{fs} V_f$), Equation 3 can be simplified to Equation 4.

$$n = K_{fs} V_f C_0, \qquad (4)$$

This proves that the SPME fiber can be exposed directly to different samples such as flowing blood, ambient air, or water, where the amount of extracted compound corresponds directly to its concentration in the sample matrix and does not depend on the sample volume [29]. Since 1990, SPME has become one of the most widely used microextraction techniques for the analysis of food (wine volatiles), environmental (on-site analysis of soil and water), and biological samples (*in vivo*/*in vitro* metabolomic studies*, in vivo* analysis of pollutants, and pharmaceutical and biomedical analyses) [28], [31].

Compared to other sample extraction techniques including traditional SPE, SPME is relatively inexpensive because of the reduced consumption and disposal of high-purity solvents, rendering it amenable to the implementation of green chemistry principles in practice [5], [31], [32].

There are three basic extraction modes of SPME including direct extraction, headspace (HS) extraction, and extraction involving membrane protection [29]. In the direct extraction–direct immersion (DI) SPME, the coated fiber is inserted into the sample phase. In HS sampling, the coated fiber is inserted into the HS above the sample, and can be applied only to the analysis of relatively volatile analytes [29]. Membrane-protected SPME is used for the analysis of samples containing both non-volatile target compounds and high-molecular-weight interfering compounds, where the coated fiber is inserted into the hollow membrane, which allows the target molecules to diffuse freely through the membrane while excluding the potential interfering compounds [29], [33].

The physicochemical characteristics and thickness of the SPME fiber coating affect the efficiency, selectivity, sensitivity, and reproducibility of the extraction process [31]. The thickness of the coating determines the analyte capacity of the fiber and length of the sorption time required to reach the desired equilibrium [29]. Various commonly

applied commercial SPME fibers with different film thicknesses are available, which include non-polar polydimethylsiloxane (PDMS) extraction phase, as well as polar extraction phases such as polyacrylate (PA) and polyethylene glycol (PEG). Additionally, many mixed (bipolar) phases are also available, such as PDMS-divinylbenzene (PDMS-DVB), Carboxen-PDMS (CAR-PDMS), Carbopack Z-PDMS, and DVB-CAR-PDMS-coated fibers [31], [34]. PDMS, PA, and PEG act as liquid phases, and the extraction mechanism is absorption, while the mixed phases containing solid coating act as adsorbents [29].

Despite the successful utilisation of these commercially available sorbents in various applications, some limitations in terms of the selectivity, robustness, carryover, swelling in solvents, operation temperature, and poor affordability are encountered. Therefore, the development of novel SPME coatings is an active area of research [31], [32]. Several new coating materials have been developed and applied to the extraction of different sample matrices and compounds, including molecularly imprinted polymers, metal organic frameworks, metal or metal oxide nanoparticles, graphene, porous carbon, and ionic liquids [32], [35], [36]. Of these, porous carbon materials have attracted considerable interest as suitable materials for use as SPME fiber coatings. Owing to their well-developed pore structures, high surface areas, physicochemical stability, and densities, these afford excellent sorption and enrichment capabilities [36]–[38].

1.1.3 Carbon aerogel as a potential SPE sorbent and SPME coating material

Aerogels are porous nanostructured materials with high surface areas and low densities, and their production process involves three main stages: sol-gel preparation, aging, and drying [6], [39]. Aerogels were first described by S.S. Kistler in 1931, who confirmed that the liquid phase in a gel, which is composed of independent solid and liquid phases, could be replaced by a gas with little or no shrinkage. This was achieved by supercritical drying; the prepared gel was placed in a closed autoclave, where the temperature and pressure of the solvent were increased to above its critical point [40].

Depending on the precursors and preparation techniques, aerogels exhibit various properties and allow various applications. To date, one of the most studied organic aerogels consists of a framework of resorcinol-formaldehyde (R-FA) resin, which was first described by Pekala *et al.* in 1989 [39]. These gels were prepared by base-catalysed (Na_2CO_3) polycondensation of R with FA using deionised water as a solvent. This is followed by ageing, solvent exchange from water to acetone, and supercritical drying with CO_2 [39].

Carbon aerogels (CAs) were obtained by including an additional pyrolysis step in the production process of organic aerogels. CAs are examples of nanoporous materials composed of covalently bonded nanometre-size particles that are arranged in a three-dimensional network [6], [39], [41]. Well-developed porous structures and desirable properties allow their use in various applications including the preparation of biosensors [42], hydrogen storage [43], preparation of catalyst supports [44], electrodes [45], supercapacitors [46], and adsorbents [41], [47], [48].

In the last few decades, interest in CAs as sorbent materials for sample preparation techniques has consistently increased because these can potentially be used as sorbents in SPE and SPME to achieve high selectivity, sensitivity, and throughput for the analysis of various compounds present in complex matrices [6]–[9].

Pérez-Caballero *et al*. [6] prepared CAs by pyrolysing organic aerogels, which were synthesised by the sol-gel polymerisation of 5-methylresorcinol (MR – an oil shale processing by-product) and FA, in different molar ratios of MR to Na_2CO_3 catalyst (MR/C) and water (MR/W) and different pyrolysis temperatures. At a pyrolysis temperature of

1000 °C as well as MR/C = 92 and MR/W = 43, the highest surface area of CAs (specific surface area of 408 $m^2 g^{-1}$; microporous area of 250 $m^2 g^{-1}$) and low density (0.192 g cm^{-3}) were obtained [6]. Kreek *et al.* [49] prepared CAs doped with ferromagnetic metals, which were tested as magnetic SPE sorbents for the extraction of perfluoroalkyl substances [50].

Meena *et al.* [41] applied R-FA CAs as sorbents for the removal of heavy metal ions from aqueous solutions, whereas Dong *et al.* [9] used R-FA CAs to analyse plant growth regulators by employing micro-SPME and magnetic SPE techniques, which were considered as environmentally friendly methods.

Despite the suitable characteristics, there have been only a few studies on the utilisation of CAs as SPME fiber coating materials. In addition to selecting an appropriate coating material, the choice of a suitable SPME coating support and procedure is also important.

The most frequently used coating methods for carbon-based materials are dip coating, glue, sol-gel, and deposition methods, while employing metal or fused silica supports [36]. One of the first studies of CAs as SPME coating materials was published by Zhu *et al.* [51], who compared the performances of two types of SPME fibers that were coated with CAs and wormhole-like mesoporous carbon using three different coating methods and stainless steel wire as a supporting core to that of a commercial PDMS fiber for the HS-SPME of four non-polar compounds and five polar compounds from water samples. The results indicated that the efficiencies of CA-based SPME fibers were higher than those of other coated fibers for the extraction of both non-polar and polar analytes [51]. Stainless steel wire coated with powdered CA using the glue method was employed by Zheng *et al.* [37] for the HS-SPME-GC-FID analysis of hydrophobic analytes with one or two benzene rings from water samples. Compared to other fibers such as commercial PDMS and PDMS/DVB as well as powdered polymer aerogel-coated fibers, the enrichment factors of the target analytes were higher. Based on these results, factors influencing the enrichment effect include π–π interactions, van der Waals forces, and hydrophobic interactions, as well as surface area and microporosity [37].

In this dissertation, MR-FA-based CAs were successfully applied and evaluated for the first time as SPE sorbents for the simultaneous analysis of acyclic and cyclic degradation products of sulfur mustard (HD) [52], [53] and for the i*n situ* synthesis of CA-based SPME fiber coating onto stainless steel wire. The applicability of the coated fibers for the analysis of selected OPPs in environmental matrices was also evaluated [54].

1.2 Sea-dumped chemical weapons and chemical warfare agents

The Chemical Weapons Convention (CWC) became effective in 1997 and banned the use, development, production, and otherwise acquiring, stockpiling, or retention of chemical weapons (CW), or the transfer, directly or indirectly, of CWs to anyone [55]. To date, 193 countries have become members of the CWC [56] and have established the Organization for the Prohibition of Chemical Weapons (OPCW) to achieve the objective and purpose of CWC and to oversee the global endeavours to permanently and verifiably eliminate the CWs [57]. According to the CWC, the definition of CWs includes toxic chemicals and precursor substances required for the manufacture of chemical warfare agents (CWAs), munitions, and devices specially designed to release toxic substances [55]. These CWAs are toxic chemical compounds that can interfere with the physical functions of human organisms through chemical or biochemical reactions; these occur in gaseous, liquid, or solid form and are mostly contained in shells or bombs [58]. There are many methods to

classify CWAs, but the most common is the classification in terms of the physical effect produced on humans. Therefore, according to the OPCW, there are five main types of CWAs including chocking (pulmonary), blister (vesicants), blood (cyanogenic), nerve, and riot control (tear gases) agents [59].

In recent years, CWs have attracted considerable attention because of the issues related to dumped chemical munitions. According to the Geneva Protocol 1925, the use of CWs in international armed conflict is prohibited, but no restrictions or regulations had been set for the production, storage, or destruction of these munitions [11]. Thus, many countries including the US, UK, USSR, Germany, France, and Canada, which accumulated large quantities of CWs and CWAs after World War I, encountered issues concerning the disposal of conventional and chemical munitions [12]. Dumping at the sea was considered the most appropriate solution at the time; the first dumping of CWs was accomplished in the 1920s in the English Channel, but the catalyst for global sea dumping of munitions as a cheap method of deposal was the Potsdam Agreement in 1945 [12], [60]. This was the time when the main dumping actions started in Europe as well, and sea deposal was a common practice until the 1970s for 40 countries [60].

Due to inadequate documentation and possible destruction of records, the exact amounts of dumped munitions are not known, but it is assumed that approximately 50 000 tons of CWs containing approximately 15 000 tons of CWAs have been dumped in the Baltic Sea on the orders of the Soviet Military Administration in Germany [13]. The main dumpsites in the Baltic Sea are the Little Belt, Bornholm Basin, and Gotland Basin [13].

After approximately 70 years of such sea-dumping activities, the entire Baltic marine ecosystem as well as human health might be at serious risk due to the corrosion of the shells and possible leakage of highly toxic compounds into the marine environment. These sea-dumped CWs mainly contain mustard gas, also known as HD, and lower quantities of arsenic-based agents (Arsine oil, Clark I and II, Adamsite, α-chloroacetophenone, and Lewisite), phosgene, tabun, and nitrogen mustards [15], [61]. HD is the most abundant sea-dumped CWA in the Baltic Sea, and near Gotland and Bornholm, mixtures of HD correspond to approximately 63% of all dumped CWAs [62].

1.2.1 Sulfur mustard and its degradation products

HD is a blistering agent (vesicant), and is the most frequently used and stored CWA. As a powerful alkylating agent, it readily reacts with a wide variety of biological molecules, thereby affecting many processes in the living tissue, causing chemical burns on the skin, temporary or permanent blindness, and respiratory system disorders [63], [64]. HD is in the form of an oily yellowish liquid or vapour and may persist in an open environment for a long time if protected from wind and rain [64]. Other chemicals (e.g., CWAs, benzene, chlorobenzene, and tetrachloromethane) were often added to pure HD to alter its melting point and viscosity as well as enhance its persistence, particularly, the ability to resist hydrolysis, thereby prolonging the duration of ground contamination [62].

In addition to HD, eight more analogues have been included in the CWC Schedule 1 chemicals [55], [65]. The CWC Schedule 1 chemicals constitute the compounds that have been developed, produced, stockpiled, or used as a CW or pose a high risk to the purpose of the CWC. Additionally, it has no or very limited use in applications that are not prohibited by the CWC [55]. However, most of these are longer-chain analogues that are more vesicant and persistent than HD, but because of their lower volatility, these do not produce casualties in the vapour form [66], [67].

In an aqueous environment, HD degradation occurs primarily through hydrolysis. First, HD is converted into a sulfonium ion and then to hemimustard and thiodiglycol (TDG). After rapid hydrolysis, the less toxic TDG may slowly oxidise to thiodiglycol sulfoxide (TDGO) and thiodiglycol sulfone (TDGOO) (Figure 2) [68].

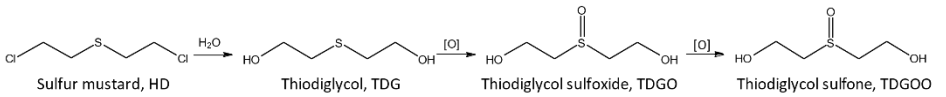


Figure 2. Degradation pathway of HD to acyclic TDG, TDGO and TDGOO.

TDG, as a precursor of HD production, is also included in the CWC Schedule 2 of chemicals, because it can be used as a precursor in the manufacture of mustard agents. However, it is not produced in large quantities for commercial applications that are not prohibited under the CWC regulations, and has only limited applications in fields other than chemical warfare [55]. In addition to these compounds, many more open-chain and cyclic degradation products of HD and its analogues are known [68]. Examples of the degradation pathways of HD into cyclic 1,4-thioxane and 1,4-dithiane are shown in Figure 3.

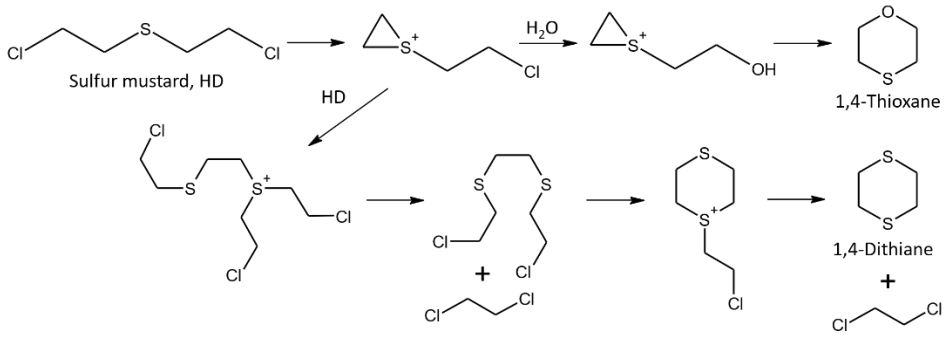


Figure 3. Degradation pathways of HD to cyclic 1,4-thioxane and 1,4-dithiane [12].

HD agents were frequently used during World War I and II as well as in the Iran-Iraq War (1980-1988), and were stockpiled by many countries as CWAs [61]. A recent example of the CW use in the last decade is in the ongoing Syrian Civil War [69], [70]. Thus, there remain reasonable concerns about the possible use of HD by terrorists in a chemical attack against civilians, or it can be involved in industrial accidents. Therefore, it is important to analyse HD and its degradation products in a timely manner and establish the management protocol for HD-exposed population.

Regardless of the current illegal use of CWs, sea-dumped CWs remain a concern. Hydrophobic HD, which has been accumulated on the seabed since decades, has the propensity to form lumps with hard polymer coating, but HD is still extremely dangerous inside the lump shells. There are many examples of the incidents in which these lumps are caught by fishermen during trawling and they have been directly or indirectly exposed to CWAs in the Baltic Sea. According to the HELCOM (The Baltic Marine Environment Protection Commission, also known as the Helsinki Commission) reports, HD-type materials account for 88% of all reported incidents [62]. The former dumping areas are marked on the official sea charts and two small areas around the Danish island

of Bornholm have been closed to traffic [62]. Nevertheless, even though the potential ecological and health risks are associated primarily with HD, the analysis of HD degradation products is important as these can act as markers of HD leakage in various locations. Therefore, interest in the analysis of HD degradation products in environmental water samples has consistently increased.

In the last 15 years, several European Union (EU)-funded international projects (MERCW, CHEMSEA, DAIMON, and DAIMON 2) and the North Atlantic Treaty Organisation (NATO) Science for Peace and Security (SPS) project, "Towards the Monitoring of Dumped Munitions Threat (MODUM)", have targeted dumped munitions in the Baltic Sea, and qualitative and quantitative data for the leaked CWAs and their degradation products have been collected [14].

During the MODUM project expedition to the Bornholm dumpsite, HD was detected in the sediment sample collected near the wreck in the Bornholm Deep at a concentration of 1.3 μg kg^{-1} dw^{-1}. However, different degradation products of HD, including acyclic hydrolysis, oxidation, and cyclic decomposition products, have been found in both the sediment and pore water samples collected from different dumpsites of the Baltic Sea [13], [15], [71]. In the MODUM and CHEMSEA projects, 21 of the 27 (total number) sediment samples collected from the area, which was officially established as a target location for dumping the CWs in the Bornholm Deep (Bornholm Primary dumpsite), contained the degradation products of HD with a mean value of 266 ± 642 μg kg^{-1}, where the minimum and maximum concentrations were 2 and 2887 μg kg^{-1}, respectively. Eighteen of the 20 (total number) sediment samples collected from the Bornholm Secondary dumpsite, where there were fewer munitions that are likely still there because of the bottom currents and accidental trawling by draggers and trawlers, contained the degradation products of HD with a mean value of 28 ± 64 μg kg^{-1}, where the minimum and maximum concentrations were 1 and 274 μg kg^{-1}, respectively. These two areas are characterised by the highest maximum, mean, and median concentration values of the degradation products of HD compared to all other dumpsites in the Baltic Sea [13].

Hydrolysis is considered to be the main breakdown pathway for sea-dumped CWAs, as well as for HD. Therefore, it has been assumed that the concentration of hydrolysis products in the samples should be higher, and these degradation products should be detected more frequently [72]. In fact, acyclic HD degradation products have not been detected in pore water samples collected and analysed in the last decade, but have been found in most sediment samples collected from the dumpsites [13], [15], [73]–[75]. For example, sediment samples collected from the Gdansk Deep and Gotland Deep regions contained 196.8–263.9 μg kg^{-1} dw^{-1} TDGO and 31.6–53.0 μg kg^{-1} dw^{-1} TDG, respectively [74]. However, cyclic degradation products of HD have been detected more often in the Baltic Sea water samples; for example, in the CHEMSEA project, cyclic degradation products were detected at concentrations of 3.4–19 μg L^{-1} in the pore water samples [13], [15], [71]. The undetectability of the acyclic degradation products might be related to the slow dissolution and formation of the polymeric crust and solid precipitate of dumped HD, known as mustard heel [68], [15]. Considering that there are only a few studies on the contamination of pore or deep water samples in comparison to those on the sediment samples, future studies should focus more on the analysis of these water samples, which is important for evaluating the possible spread of leakages and risk to marine biota [13].

1.2.2 Analysis methods for HD degradation products

The most frequently used methods for the analysis of different degradation products of HD employ GC and liquid chromatography (LC), in combination with mass spectrometry (MS) and/or MS/MS detection [61], [75]–[83].

Acyclic sulfur-containing precursors and breakdown products of HD have low or lack of volatility; therefore, it is necessary to include an addition derivatisation step to the sample preparation procedure prior to GC analysis. Derivatisation reagents, which are often used for the modification of the hydroxyl groups of HD degradation products prior to GC analysis, are highly moisture-sensitive, and the derivatisation procedure requires a strict control of the sample preparation conditions, particularly for the analysis of water-containing environmental samples [76]. For example, if the water content in the TDG sample is 0.3% (v/v), the initial trifluoroacetylimidazole-derivatised reaction product concentration is reduced by approximately 30%. By ignoring the unfavourable effect on derivatisation, it is possible to achieve very low limit of detection (LOD) for TDG and TDGO, i.e., 0.01 and 5 ng mL^{-1}, respectively [74]. Water solubility and relatively polar characteristics also allow the analyses of TDG and its oxidation products by LC-MS or LC-MS/MS without derivatisation [76], [78], [84]. Cyclic degradation products which do not require additional derivatisation are mainly analysed by GC-MS [13], [15], [61], [79], [82], [83].

In addition to MS detection, nuclear magnetic resonance [85], ultraviolet (UV) [86], and flame photometric detector (FPD) [87] are also employed in combination with LC for the analysis of hydrolysis and decomposition products of HD-related compounds.

In addition to chromatographic methods, there is a very limited number of CE methods that have been developed for the determination of the degradation products of HD [86], [88], [89], all of which are based on micellar electrokinetic chromatography (MEKC) analysis, which allows the analysis of the neutral degradation products of HD in water environment, but with moderate sensitivity because of a lack of UV chromophores in the analytes. For the sensitive detection of neutral molecules without strong UV chromophores using a CE-UV instrument, it is necessary to use an appropriate derivatisation method prior to sample analysis. The advantages of CE instrumentation and operation procedure as well as simplicity and robustness of the method allow its translation to a portable field analysis method [90].

1.2.3 Sample preparation for the analysis of CWAs

Owing to the low concentrations of the HD degradation products and complex environmental sample matrices, direct introduction into the analytical instrument is not possible in most cases. Thus, the concentration and purification of HD degradation products present in water samples is an important part of the analysis procedure.

The sample preparation techniques recommended by the OPCW for the preparation of samples containing CWA degradation products are LLE, solid–liquid extraction (SLE), and SPE [76].

Both cyclic and acyclic compounds have been extracted from dry sediment samples using solvent extraction with both polar and non-polar solvents. Thereafter, these are chemically modified to afford suitable separation and/or ionisation properties for subsequent GC-MS, GC-MS/MS, or LC-MS/MS analysis [77]. TDG has been extracted from groundwater samples using two different SPE columns in tandem; the reversed-phase C18 sorbent removed extraneous interferences from the sample, and the synthetic carbonaceous sorbent, Ambersorb 572 column, allowed the extraction of TDG with a recovery of ≤ 40%. However, derivatisation was employed prior to GC-MS analysis to

improve the detection limit, and a method detection limit of 3.5 µg L^{-1} for TDG was achieved [91]. TDG was extracted from urine samples using a reversed-phase Oasis HLB SPE cartridge with a recovery of 28% [92] and activated carbon fiber sorbent with a recovery of 63–79% [93].

For the extraction of cyclic degradation products of HD, Lees *et al.* applied a reversed-phase Supelclean LC-18 SPE column with a recovery of 62.9–99.4% [86]. Cyclic degradation products in dry sediment samples were analysed using dynamic HS extraction together with GC-MS; when the obtained recoveries were 60–90%, the analysis allowed automatic identification of these compounds in spiked sediment samples at concentrations of 13–65 µg kg^{-1} dw^{-1} [79]. As microextraction methods are attracting increasing attention, SPME fibers for the HS extraction of cyclic degradation products of HD have been often applied. Nawała *et al.* [94] developed the HS-SPME-GC-MS/MS method using laboratory-generated butyl acrylate fibers for the analysis of CWA degradation products, including the cyclic degradation products of HD, from environmental pore water and sediment samples collected from the Baltic Sea.

1.3 Organophosphorus pesticides

Organophosphorus (OP) compounds were first synthesised in the early 19th century when the toxicity and modes of action of these compounds were not known. In the 1930s, when the toxic effect was well-known, German chemist, Gerhard Schrader, synthesised many OP compounds including tetraethyl pyrophosphate, which was the first commercialised OP insecticide [95]. The fundamental structure of organophosphorus pesticide (OPP), first reported by Schrader in 1937, is shown in Figure 4, where the pentavalent phosphorus is attached via a double bond to sulfur or oxygen. Typically, R_1 and R_2 are the alkoxy groups, and X is the leaving group [95].

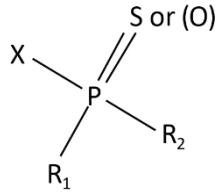


Figure 4. General chemical structure of OPP.

Schrader also discovered that some of the OP compounds were too toxic to be used for agricultural applications, and over the course of World War II, the development of OP compounds was mainly focused on two different applications. One was the production of insecticides, which were less toxic to mammals (parathion, malathion, and diisopropyl fluorophosphate), and the other was the synthesis of compounds with high toxicity to humans and high volatility for use as CWAs (tobun, sarin, and soman) [95], [96]. Although these CWAs were not used during World War II and their production and use is currently prohibited, OP compounds have been used as CWAs in some terrorist attacks and conflicts [55], [96]. OPPs have been heavily used as insecticides in agriculture and their use is still often related to poisoning cases; however, many of these OPPs have not been approved as active substances in the EU for use in plant protection products [16]. The abuse or erroneous application of highly persistent, bio-accumulative, and biocidal

OPPs causes significant environmental issues and affects human health by contaminating food [17]. Thus, the development of selective and sensitive methods for the analysis of OPPs in environmental water samples and food is required.

1.3.1 Analysis methods for OPPs

Environmental degradation and risks to food safety are the main motives for the detection and continuous monitoring of OPPs, as well as the development of novel methodologies for the analysis of these hazardous compounds. Desirable strategies include highly selective and sensitive procedures that can allow simultaneous analysis of different pesticides using minimal sample preparation steps and toxic extraction solvents.

Currently, the most widely applied reliable methods for the multiresidue analysis of OPPs are the chromatographic methods in combination with MS or MS/MS detection [17]. Based on the characteristics of the pesticides of interest, appropriate separation techniques are selected. More volatile and thermally stable compounds can be determined by GC in combination with MS or detectors that are specific for phosphorus- or sulfur-containing compounds to achieve high sensitivity and specificity, such as nitrogen–phosphorus detector [97] and FPD [98]. Highly polar pesticides are usually analysed by high-performance liquid chromatography (HPLC) with MS or MS/MS detection as these are not easily vaporised and/or thermally unstable [17], [99].

1.3.2 Sample preparation for the analysis of OPPs

Owning to the low concentration and distinct chemical properties of pesticides as well as potential interferences in complicated matrices encountered in environmental and food samples, sample preparation is a key step in chemical analysis for sample extraction, purification, and concentration.

The sample preparation for the subsequent separation and detection of OPPs has shifted increasingly toward miniaturised sample preparation methods, leaving behind conventional techniques such as LLE, SLE, and SPE [99], [100]. SPME is an appropriate sample preparation technique for the extraction of OPPs from environmental samples including honey and natural water samples, particularly when GC is used for sample analysis, which allows the direct thermal desorption of target analytes to the analytical instrument [99], [100]. Honey is a complex and diverse environmental sample matrix that contains many potential interferences including carbohydrates, acids, minerals, and vitamins [101], and OPPs in honey may originate from contaminated plants from which bees collect nectar to produce honey, or from pest- and disease-controlled beehives [102]. The key step of SPME is the selection of an appropriate fiber coating for the extraction procedure. For the extraction and subsequent analysis of OPPs in environmental samples, both commercial fibers such as those coated with PDMS [103]–[107], PA [103], [105]–[107], PDMS/divinylbenzene (PDMS/DVB) [106], [107], and carbowax/templated resins [106], as well as lab-made fibers such as polystyrene [108], crown ether [109], methyltrimethoxysilane-tetraethoxysilane [110], porous carbon [111], and graphene [112]-based coatings have been used. Moreover, interest in using different coating materials and technologies in the SPME procedure and potential applications of the extraction method have been continuously increasing in the past few decades.

2 Aims of the study

The aim of the present study was to develop novel procedures for the sample preparation and analysis of environmentally harmful compounds to determine the potential leakage of sea-dumped CWs and environmental pollution caused by the abuse or erroneous application of highly toxic OPPs. Furthermore, the potential of MR-FA CA as a material that can be applied in sample preparation as a SPE and SPME sorbent was explored.

The specific aims of the dissertation are listed as follows:

- Development and optimisation of a pre-capillary derivatisation procedure employing CE separation method using UV absorbance detection for the analysis of acyclic degradation products of HD.
- Development and evaluation of SPE methodology using CA-based sorbent for the simultaneous extraction of acyclic and cyclic degradation products of HD from environmental water samples, followed by CE and HPLC analyses with UV absorbance detection.
- *In situ* synthesis of a novel CA-based SPME fiber coating on stainless steel wire and evaluation of the applicability of CA as SPME coating material for the direct GC-MS analysis of selected OPPs in environmental matrices of natural water and honey samples.

3 Experimental

3.1 Reagents and materials

All chemicals were of analytical grade and used as received. Milli-Q water obtained using a Milli-Q water purification system (Millipore S. A. Molsheim, France) was used throughout the study.

In **Publications I and II**, TDG, TDGO, TDGOO, 1,2,5-trithiepane and 1,4,5-oxadithiepane were synthesised by Envilytix GmbH (Wiesbaden, Germany), while 1,4-thioxane, 1,3-dithiolane, 1,4-dithiane, and 3,5-Dithia-1,7-heptanediol (DTHD) were obtained from Sigma-Aldrich (Germany). 3,6-Dithia-1,8-octanediol (DTOD) was purchased from Alfa Aesar (Germany). All the degradation products are listed in Table 1.

Ethyl acetate, dichloromethane, acetonitrile (ACN), MeOH (HPLC grade; ≥ 99.99%), boric acid, NaOH, NaCl, HCl, sinapinic acid (internal standard (IS)), and imidazole were purchased from Sigma-Aldrich (Germany). Phthalic anhydride and pyridine were purchased from Merck KGaA (Darmstadt, Germany).

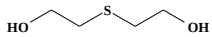
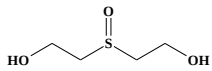
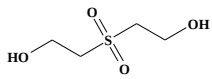
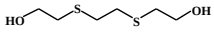
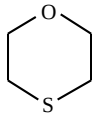
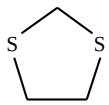
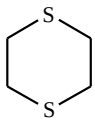
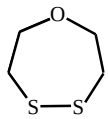
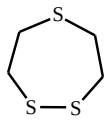
Stock solutions of the analytes were prepared using Milli-Q water or MeOH. The working solutions were prepared daily by appropriate dilution of the stock solutions, and the background electrolyte (BGE) solution was prepared using Milli-Q water. All the solutions were stored at 4 °C.

In **Publication III**, OPPs including heptenophos, paraoxon-ethyl, tetrachlorvinphos, chlorfenvinphos, parathion, and malathion (Table 2), as well as NaCl and NaOH were purchased from Sigma-Aldrich (Germany). Pure OPP standards were refrigerated in their original packaging at 4 °C. MeOH and ACN (HPLC grade; ≥ 99.9%) were purchased from Sigma-Aldrich (Germany). Stock solutions of OPP standards were prepared using MeOH, stored in the dark, and refrigerated at -20 °C. The working solutions were prepared daily by appropriate dilution of the stock solutions and stored at 4 °C.

For the preparation of CAs (**Publications I and II**) and CA-coated SPME fibers (**Publication III**), MR with reported purity of > 99% was provided by AS VKG (Estonia). FA solution (37 wt% in H_2O) and Na_2CO_3 powder (> 99.0%) were purchased from Sigma-Aldrich (Germany). In **Publications I and II**, empty SPE tubes (polypropylene, tube volume: 3 cm^3, Phenomenex) and polyethylene frits (20 µm porosity, Phenomenex) were used for the preparation of CA-based SPE cartridges. Powdered CA (MR/C = 90, W/MR =45, and MR/FA = 0.5) [6] was prepared by our research group (Institute of Chemistry and Biotechnology, TalTech). For the comparative analysis of sorbents, Supelclean LC-18 500 mg (SUPELCO, Bellefonte, PA, USA), Chromabond NH_2 500 mg (Mecherey-Nagel GmbH & Co.), and HyperSep Hypercarb 500 mg (Thermo Scientific) SPE cartridges were used.

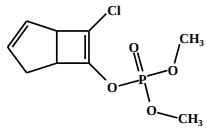
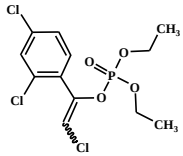
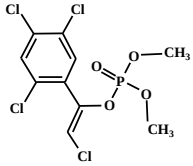
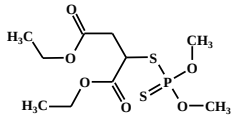
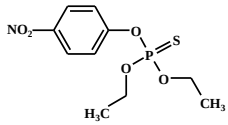
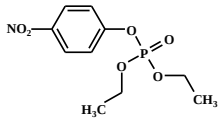
In **Publication III**, a 200 µm-thick stainless steel wire and epoxy glue were purchased from a local hardware store. Tubings with an internal diameter (ID) of 395 µm were purchased from IDEX Health & Science LLC (USA). A manual SPME fiberF holder (Supelco-57330-U) and SPME fibers with 65 µm PDMS/DVB, 50/30 µm DVB/CAR/PDMS, 85 µm CAR/PDMS, and 85 µm PA coatings were purchased from Supelco (Bellefonte, PA, USA).

Table 1. Structures and chemical properties of target HD degradation products.

Name	Structure	MW (g mol^{-1})	Water solubility (mg mL^{-1}) [a]	pKa [a] (± 0.10)	logP [a]
Thiodiglycol, TDG		122.19	371	14.14	-0.715 ± 0.351
Thiodiglycol sulfoxide, TDGO		138.19	1000	13.61	-2.301 ± 0.376
Thiodiglycol sulfone, TDGOO		154.18	598	13.35	-1.856 ± 0.335
3,5-Dithia-1,7-heptanediol, DTHD		168.28	177	14.10	-0.073 ± 0.473
3,6-Dithia-1,8-octanediol, DTOD		182.30	130	14.17	-0.155 ± 0.450
1,4-Thioxane		104.17	23	-	0.772 ± 0.465
1,3-Dithiolane		106.21	14	-	0.555 ± 0.617
1,4-Dithiane		120.24	18	-	0.112 ± 0.644
1,4,5-Oxadithiepane		136.24	4.8	-	1.741±0.245
1,2,5-Trithiepane		152.30	0.76	-	3.012±0.449

[a] Calculated using Advanced Chemistry Development (ACD/Labs) Software V11.02

Table 2. Structures and chemical properties of target OPPs.

Name	Structure	MW (g mol^{-1})	Water solubility at 20 °C (mg L^{-1}) [b]	logP [a]	Mammals (rat) acute oral LD_{50} (mg kg^{-1}) [b]
Heptenophos		250.61	2200	2.56 ± 0.37	96
Chlorfenvinphos		359.57	145	4.51 ± 0.38	12
Tetrachlorvinphos		365.95	11.6	3.86 ± 0.40	> 4000
Malathion		330.36	148	2.93 ± 0.35	1778
Parathion		291.26	12.4	3.84 ± 0.32	2
Paraoxon-ethyl		275.19	3.64	2.31 ± 0.30	1.8

[a] Calculated using Advanced Chemistry Development (ACD/Labs) Software V11.02
[b] PPDB: Pesticide Properties DataBase [113]

3.2 Instrumentation and operating parameters

3.2.1 Capillary electrophoresis

In **Publications I and II**, an Agilent 3D CE instrument (Agilent Technologies, Waldbronn, Germany) with a diode array detector (DAD) was used for the separation and detection of acyclic degradation products of HD. Uncoated fused-silica capillaries (Polymicro Technologies, Phoenix, AZ, USA) with an ID of 50 µm and a length of 52/60 cm (effective length/total length) were employed in the experiments. 30 mM borate as the BGE, pH of 8.90, voltage of 15 kV, and sinapinic acid (IS) were used. The samples were injected hydrodynamically by applying a pressure of 50 mbar for five seconds. Before each sample injection, the capillary was flushed with 1 M NaOH for two minutes, Milli-Q water for two minutes, and finally with the BGE for three minutes. The separation process was monitored at a wavelength of 200 nm. All electropherograms were recorded and integrated using the Agilent ChemStation software.

3.2.2 High-performance liquid chromatography

In **Publication II**, the cyclic degradation products of HD were analysed using an Agilent 1260 Infinity II HPLC system with DAD (Agilent Technologies, Waldbronn, Germany). The separation was carried out using a ZORBAX SB-C18 column (2.1 x 150 mm, 5 µm particle size, Agilent Technologies, Waldbronn, Germany) at a flow rate of 0.2 mL min^{-1} (isocratic water-ACN mobile phase, 1:1). An injection volume of 5 µL, column temperature of 30 °C, and detection wavelength of 200 nm were used. All chromatograms were recorded and integrated using the OpenLAB CDS Chemstation Edition software.

3.2.3 Gas chromatography

In **Publication III,** chromatographic separation of the OPPs was performed using an Agilent 7890A GC system equipped with an ultra-inert splitless liner (Agilent Technologies, type 5190-2293) to eliminate the matrix-induced chromatographic response enhancement. The GC system was coupled to an Agilent 5975C mass spectrometer with an electron ionisation source and quadrupole mass analyser. The separation was performed using a ZB-5MSi capillary column (30 m × ID 0.25 mm, film thickness 0.25 µm, Agilent Technologies). Helium (6.0 purity) was used as the carrier gas at a constant flow rate of 1.3 mL min^{-1}. Sample injection was performed in splitless mode at 275 °C for two minutes. The oven temperature was programmed as follows. The initial temperature of 60 °C was held for one minute, then increased to 180 °C at a rate of 25 °C min^{-1}, where it was held for two minutes, and finally increased to 250 °C at a rate of 15 °C min^{-1} and held for 2.5 min (total run time of 15 min). The analyte ionisation was performed in the electron ionisation mode using an electron energy of 70 eV. The temperatures of the interface, ion source, and mass analyser were set to 280, 230, and 150 °C, respectively. All analytes were monitored using the selected ion monitoring (SIM) mode. Chromatographic peaks were identified by measuring the retention times of individual OPP standards and confirmed by the fragmentation pattern of molecules and matching to the compounds in the database (NIST17). Quantitative analysis was carried out in the SIM mode using a specific quantification ion for each target analyte (Table 6). The instrument was controlled using the MassHunter Acquisition software, and data analysis was performed using the Agilent MassHunter Workstation software.

3.2.4 Preparation and characterisation of CAs

In **Publications II and III**, a supercritical extraction system including a 100-mL double-clamp autoclave (NWA Analytische Meßgeräte GmbH, Germany) was used. For the preparation of CAs (**Publication II**), liquid CO_2 was first flowed through the reactor for two hours (120 bar, 25 °C) and then held under supercritical conditions (100 bar, 45 °C) for four hours. After extraction, the extractor was slowly depressurised to atmospheric pressure, and the organic aerogel was recovered. For the preparation of CA-coated SPME fibers (**Publication III**), the supercritical extraction conditions were as follows. First, acetone in the gel pores was replaced by liquid CO_2 (120 bar, 25 °C for one hour) and then subjected to supercritical drying (100 bar, 45 °C for 1.5 h).

In **Publications II and III**, pyrolysis was carried out in N_2 atmosphere using an MTF 12/38/400 pyrolysis oven (Carbolite, England). The pyrolysis temperature program of the oven was as follows. The initial oven temperature was 25 °C, which was increased to 300 °C at a rate of 10 °C min^{-1} (held for 10 min), followed by an increase to 550 °C at 10 °C min^{-1} (held for 10 min), and finally to 900 °C at 10 °C min^{-1} (held for one hour). After pyrolysis, the furnace was allowed to cool down to room temperature under N_2 atmosphere.

Characterisation of the coating material in **Publication III** was performed by scientists from TalTech. The surface morphology of the CA-coated SPME fiber was examined using high-resolution scanning electron microscopy (HR-SEM, Zeiss Merlin), which was performed by Dr. Olga Volobujeva (Department of Materials and Environmental Technology, Taltech). The N_2 adsorption-desorption isotherms of CA were recorded using a Quantachrome Autosorb iQ-C instrument. Pore size distribution was determined using density functional theory (DFT), and the specific surface area was calculated according to the Brunauer–Emmett–Teller theory. N_2 adsorption-desorption analysis was performed by Dr. Heidi Lees (Department of Energy Technology, TalTech). X-ray photoelectron spectroscopy (XPS, Kratos AXIS Ultra DLD) was used to analyse the obtained coating material and was performed by Dr. Mati Danilson (Department of Material and Environmental Technology, TalTech).

All SPE procedures in **Publications I and II** were accomplished using a 12-position Visiprep SPE Vacuum Manifold (Supelco).

3.3 Sample preparation and samples of the degradation products of HD (Publications I and II)

3.3.1 Derivatisation of acyclic degradation products of HD

The derivatisation mixture was prepared using phthalic anhydride (0.163 g mL^{-1}) and imidazole (0.025 g mL^{-1}) in pyridine (1 mL), following the method described by Vanhoenacker *et al.* [114]. The mixture was sealed with a septum and stored in a desiccator in the dark. Optimised derivatisation conditions included the addition of 100 µL of phthalic mixture to each analyte (2.5 mg) and heating at 45 °C for 20 min.

Thereafter, the mixture was cooled to room temperature, and the same amount of water was added to stop the derivatisation reaction. Finally, the mixture was diluted with Milli-Q water according to the need, and after adding the IS, the sample was used for analysis by employing a CE-UV instrument.

3.3.2 Preparation of CA-based SPE cartridge

The SPE sorbent packing material used in this study was prepared according to a previously published literature protocol [6] using a water solution of MR, Na_2CO_3, and FA (MR/C = 90, W/MR = 45, and MR/FA = 0.5). The mixture was kept overnight at room temperature for sufficient gelation and then placed in acetone, which was changed every 24 h for four days. The gels were then dried using supercritical CO_2 to obtain MR-FA organic aerogels. The CAs were fabricated by pyrolysing these MR-FA organic aerogels in N_2 atmosphere. The resulting monolithic material was powdered and sieved, and the obtained particles had a diameter of 0.063–0.2 mm. The powdered CA (50 mg) was inserted into the empty SPE tubes between the two polyethylene frits and after pressing it with a rod, the density of the obtained sorbent was approximately 157 mg cm^{-3}. Before the first use, the SPE column was conditioned by rinsing with 3 mL of MeOH and 3 mL of Milli-Q water, followed by vacuum drying. The schematic of the CA-based SPE cartridge preparation, followed by SPE-CE/HPLC-UV analysis is shown in Figure 5.

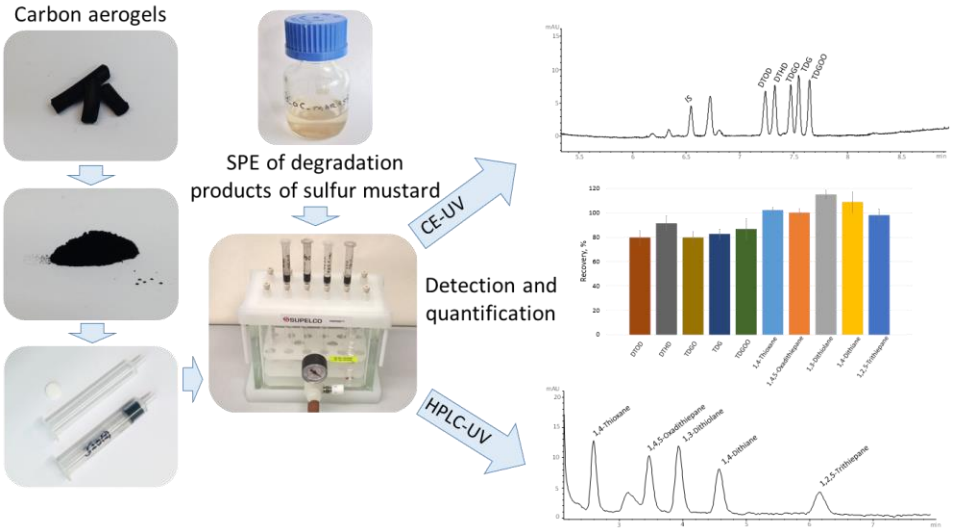


*Figure 5. Schematic of CA-based SPE cartridge preparation followed by SPE-CE/HPLC-UV analysis (**Publication II**).*

3.3.3 SPE procedure and derivatisation prior to CE-UV and HPLC-UV analyses

The SPE cartridge filled with the CA-based sorbent was preconditioned using 3 mL of MeOH and 3 mL of Milli-Q water (1 mL min^{-1}). The optimised procedure for SPE is described as follows. Ten millilitres of the sample (real water sample or sample spiked with all 10 analytes) was loaded into the cartridge at a flow rate of 2 mL min^{-1}. After the sample was passed through it, the sorbent was rinsed with 2 mL of Milli-Q water and kept in vacuum for 10 min to remove residual water. The analytes were eluted with 2 mL of MeOH (1 mL min^{-1}), 1 mL of which was used for the CE analysis of acyclic degradation products and the other 1 mL aliquot for the HPLC analysis of cyclic degradation products. The cyclic degradation products of HD could be analysed instantly using the HPLC-UV method by injecting 5 mL of the eluate [86]. For the acyclic HD degradation products, 1 mL of the eluate was evaporated under a gentle airstream until dryness and 20 µL of the derivatisation mixture was added to the residue and heated at 45 °C for 20 min, after

which the mixture was diluted with Milli-Q water as needed. The derivatised sample was analysed by employing the CE-UV instrument, using 50 µM sinapinic acid as the IS (**Publication I**).

3.3.4 Samples of the degradation products of HD

In **Publication II**, the real sediment samples were collected from the Bornholm Basin, a chemical warfare dumping site in the Baltic Sea, during the MODUM project expedition between 12–16 March, 2016. Three sediment samples were centrifuged for 20 min at 8500 rpm, and the pore water together with deep water was collected and filtered. The liquid part of the sample was analysed by employing the proposed CA-based SPE, derivatisation, as well as CE-UV and HPLC-UV analyses. Whereas another portion of water samples were spiked with target analytes to simulate real matrix conditions and analysed.

To optimise the SPE procedure and evaluate the analytical performance of the CA-based sorbent for the analysis of HD degradation products in **Publication II**, spiked Milli-Q water was used. To evaluate the efficiency of SPE in the optimisation experiments, the extraction recoveries (R) of the target analytes were calculated using the following equation:

$$R = \frac{C \cdot V}{C_0 \cdot V_0} \cdot 100\%, \qquad (5),$$

where C is the analyte concentration in the reconstituted solvent, C_0 is the initial concentration of the analyte in the water sample, and V and V_0 are the volumes of the reconstituted solvent and water sample, respectively.

The matrix effect (Equation 6) [115] was evaluated using MilliQ water and real environmental water samples at two different concentrations of the HD degradation products, which corresponded to the same concentrations used for the recovery experiments.

$$ME(\%) = \left(\left(\frac{response\ in\ matrix}{response\ in\ solvent\ (MQ\ water\ sample)}\right) - 1\right) * 100\% \qquad (6)$$

3.4 Sample preparation and samples of organophosphorus pesticides (Publication III)

3.4.1 Preparation of CA-coated SPME fibers

The CA-based coating onto a stainless steel wire was synthesised *in situ*. Before coating, the 200 µm stainless steel wire was cut into pieces (1.5 cm in length), which were immersed in acetone for 30 min, followed by immersion in 1 M NaOH for additional 30 min, then washed with Milli-Q water for 30 min, taken out and dried at room temperature.

These were then inserted into empty 1.7 cm tubings (ID 0.395 mm) and filled with the initial mixture, a fresh water solution of MR, Na_2CO_3, and FA (MR/C = 90, W/MR = 45, and MR/FA = 0.5) prepared according to the literature protocol [6]. Both ends of the tubings were closed using Parafilm, and the filled tubings were kept overnight at room temperature for gelation. After gelation, both ends of the tubings were opened, and the filled tubings were placed in acetone. Acetone was changed every 24 h over the course of four days. To obtain organic aerogel-coated metal wires, the gel inside the tubes was dried using supercritical CO_2. After supercritical drying, the wires coated with organic aerogels were removed from the tubings.

In the next step, CA-coated wires were fabricated by pyrolysing organic aerogel-coated wires in N_2 atmosphere. After pyrolysis, the coated SPME fibers were fixed with epoxy glue to a commercial fiber assembly (original core from the inner tube was removed). The SPME CA-based fiber coating procedure followed by DI-SPME-GC-MS analysis is illustrated in Figure 6.

3.4.2 DI-SPME procedure prior to GC-MS analysis

The CA-based fiber assembly was attached to the manual SPME holder and conditioned in the injection port of the GC system at 300 °C for 30 min prior to use, while the commercial fibers were conditioned according to the manufacturer's instructions. The CA-coated SPME fibers were then preconditioned using a MeOH:MilliQ water solution (1:1, v/v) for five minutes. For the DI-SPME procedure, the coated fiber protected in the septum piercing needle was inserted into the sample vial, then pushed out from the needle, and immersed into a 0.5 mL sample solution for 20 min at room temperature. Both the preconditioning and sorption procedures were carried out using an ELMI DOS-20M digital orbital shaker (agitation 200 rpm). Before desorption, the fiber was rinsed with Milli-Q water for five seconds to remove the interfering matrix components. The fiber was then withdrawn from the needle and inserted into the GC injection port, where the analytes were thermally desorbed (275 °C for two minutes) for GC-MS analysis.

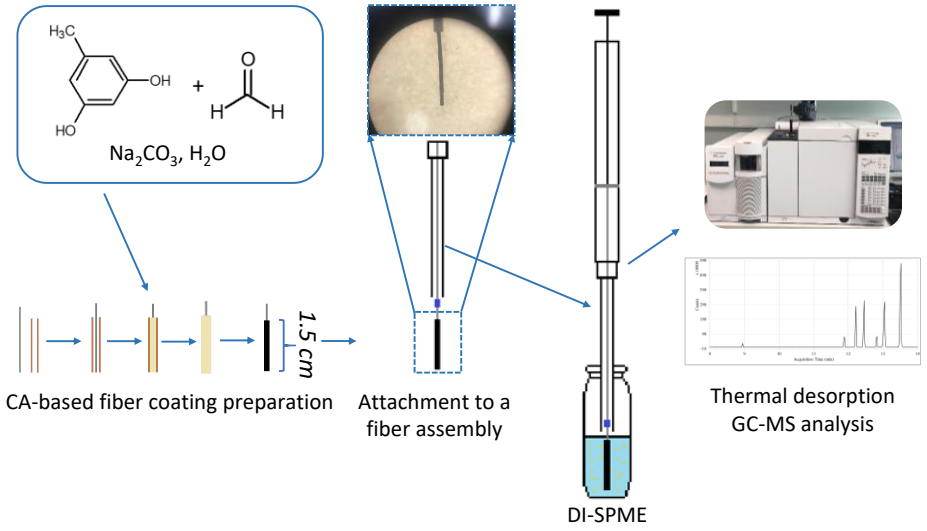


*Figure 6. Schematic of the SPME CA-based fiber coating procedure, followed by DI-SPME-GC-MS analysis (**Publication III**).*

3.4.3 OPP samples

In **Publication III**, natural water samples were collected from the Pirita River, Tallinn Bay, and Baltic Sea (Estonia). Honey sample was obtained from the local beekeeper. Before spiking, all samples were checked for any type of target analyte. Natural water samples were analysed directly by DI-SPME-GC-MS, but a 0.5 mL aliquot of the honey-water mixture (1 mL of the sample was dissolved in 1 mL of MilliQ water) was prepared for the DI-SPME-GC-MS analysis of honey sample. To study the honey matrix effect and determine the recovery of the DI-SPME-GC-MS analysis procedure, a specific amount of

each pesticide standard was added to a 0.5 mL aliquot of the honey-water mixture, mixed for at least 30 min, and then analysed using the above DI-SPME-GC-MS procedure. The spiking approach was used to determine the recovery of the natural water samples.

The matrix effect (Equation 6) was evaluated using Milli-Q water and natural water or honey samples at two different concentrations of OPPs, one of which was the same as that used for the recovery experiments.

For the optimisation of the SPME procedure and evaluation of the analytical performance of the DI-SPME-GC-MS methodology using the CA-coated fibers for the analysis of target compounds in **Publication III**, Milli-Q water samples spiked with target OPP standards were used.

4 Results and discussions

The results discussed in this dissertation are based on three publications (**Publications I-III**). These reports describe the development of sample preparation procedures and analysis of environmentally harmful compounds such as degradation products of HD (**Publications I and II**) and OPPs (**Publication III**).

The first of the following chapters, described in **Publications I-II,** includes the development and optimisation of a pre-capillary derivatisation procedure employing the CE separation method using UV absorbance detection for the analysis of acyclic degradation products of HD such as TDG and its oxidation products (**Publication I**), whereas cyclic degradation products are analysed using HPLC-UV analysis. Furthermore, the development and evaluation of the SPE procedure using a CA-based sorbent is described for the simultaneous extraction of acyclic and cyclic degradation products of HD present in environmental water samples (**Publication II**). The second chapter describes the *in situ* synthesis of a novel CA-based SPME fiber coating on stainless steel wire and evaluation of the applicability of CAs as SPME coating materials for the DI-SPME-GC-MS analysis of selected OPPs in the environmental matrices of natural water and honey samples (**Publication III**).

4.1 Analysis of environmental water samples for the determination of acyclic and cyclic degradation products of HD (Publications I and II)

4.1.1 Derivatisation of acyclic degradation products of HD

Owing to the very high pK_a values ($pK_a > 13$) of the hydrolysis product of HD, named TDG and its oxidation products (TDGO and TDGOO), separation using CE is not possible without employing a derivatisation procedure or utilising MEKC. The analysis of neutral molecules is possible using the MEKC mode; Cheicante *et al.* [88] separated TDG and TDGO, but the sensitivity of the developed analysis procedure was moderate (LOD = 10 µg mL^{-1}), which was due to a lack of UV chromophores in the analyte molecules.

These drawbacks can be overcome by the pre-capillary derivatisation of the target analytes with a strong UV chromophore, phthalic anhydride, using the established method described by Wellons *et al.* [116] and Carey *et al.* [117] for the determination of the hydroxyl content of alcohols. The derivatisation mixture used in the present study for the insertion of a chromophore (phenyl group) and an ionic functionality (carboxyl group) into the target compounds was prepared according to the procedure described by Vanhoenacker *et al.* [114]. It contained phthalic anhydride with imidazole and pyridine to catalyse the phthalation reaction (Figure 7). The derivatisation conditions in terms of the amount of the derivatising reactant (two to four times excess of the phthalic anhydride amount, based on the stoichiometry of the derivatisation reaction), heating time (0–25 min), and temperature (25–75 °C) were carefully investigated. The detailed derivatisation procedure is described in Section 3.3.1.

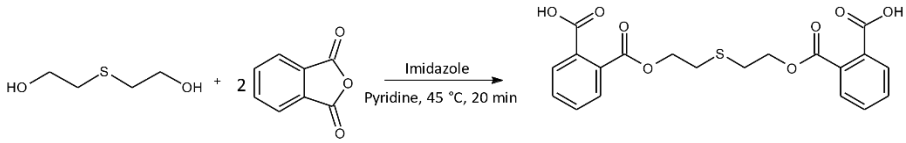


Figure 7. Derivatisation reaction of TDG with phthalic anhydride.

The final derivatisation conditions included 100 µL of reactant per analyte, derivatisation temperature of 45 °C, and derivatisation time of 20 min.

4.1.2 Determination of acyclic degradation products of HD using CE-UV

The optimised derivatisation procedure allows the CE-UV analysis of TDG and its oxidation products because the insertion of the ionic functionality (carboxyl group) into the target compounds enables the formation of derivatives and allows the use of borate as a BGE for the analysis. The separation conditions were optimised by investigating the effects of BGE pH (7.5–10.0), concentration (20–50 mM), capillary temperature (15– 30 °C), and applied voltage (15–25 kV) on the separation efficiency and migration times. In the studied pH range, all the derivatives were negatively charged but were not baseline separated and had asymmetric peak shapes below a pH of 8.0. At pH values of > 9.0, the peak area of the TDGOO derivative decreased rapidly due to the instability of the molecule. Therefore, a pH of 8.5 was selected for further analysis. An increase in the borate BGE concentration led to longer migration times and peak broadening. The best separation efficiency was achieved at a concentration of 30 mM borate BGE (pH 8.5). The effects of the applied voltage and capillary temperature were also investigated, and the best separation was achieved at 15 kV, at which all the peaks were baseline separated. Increasing the temperature up to 25 °C improved the migration times by a quarter without noticeably affecting the separation efficiency.

The optimised conditions for the separation of the three derivatives included the borate BGE concentration of 30 mM, pH 8.5, applied voltage of 15 kV, and capillary temperature of 25 °C. Figure 8 shows the representative electropherogram for the analysis of all the derivatives (25 µM) of the target compounds under optimised derivatisation and separation conditions.

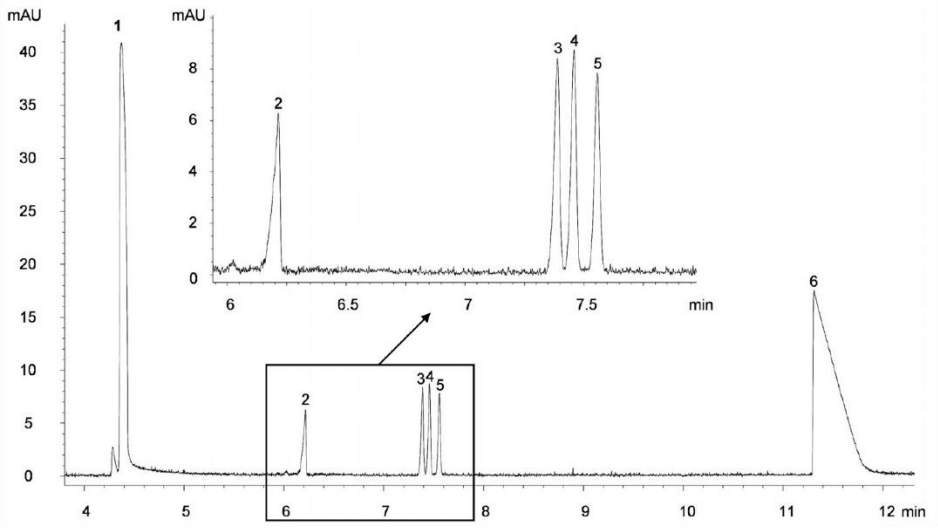


*Figure 8. Electropherogram of 3.45 µg mL^{-1} TDGO (3), 3.05 µg mL^{-1} TDG (4), and 3.85 µg mL^{-1} TDGOO (5) derivatives under optimised separation conditions: 30 mM borate BGE, pH 8.5, 15 kV, capillary temperature of 25 °C, 50 mbar for five-second injection, and detection wavelength of 200 nm. Additional peaks: EOF (1), sinapinic acid as IS (2), and phthalic acid (6) from the hydrolysis of unreacted phthalic anhydride (**Publication I**).*

Appropriate separation and detection were achieved under optimised conditions, demonstrating the possibility of using CE-UV as a reliable analysis method for the quantitative analysis of acyclic degradation products. Furthermore, it is possible to transfer the analysis protocol to a portable platform, which makes the developed analysis methodology attractive for in-field use. The portable format of the developed CE analysis methodology was successfully used during the MODUM expedition aboard the research vessel in the Bornholm Basin in 2016 [15].

4.1.3 SPE procedure using CA-based sorbent for the simultaneous extraction of acyclic and cyclic HD degradation products from environmental water samples

For the analysis of acyclic and cyclic degradation products of HD (Table 1) in environmental water samples, pretreatment for concentration and purification is an important part of the complete procedure. In addition to LLE, SPE is one of the recommended procedures that is suitable for the extraction of CWAs from aqueous environmental samples [76] using C18 and ion exchange SPE cartridges.

CAs are highly porous, low-density materials with high surface areas, which show significant potential for use as SPE sorbents. As CAs are carbon-based nanomaterials, these can interact through non-covalent forces (for example, hydrophobic interactions, hydrogen bonding, π-π stacking, and electrostatic and van der Waals forces) with acyclic and cyclic degradation products of HD [118], [37].

CAs were prepared using the method described by Pérez-Caballero *et al.* [6], and used for further preparation of SPE cartridges for the extraction of HD degradation products from environmental water samples. After SPE, CE-UV analysis together with the derivatisation procedure for acyclic degradation products was adopted, as described in **Publication I**. The analysis of the cyclic degradation products of HD was performed using the HPLC-UV method, which was the modified version of the methodology first proposed by Lees *et al.* [86].

4.1.4 Optimisation of SPE procedure

As the CA-based SPE sorbent shows significant potential for simultaneous extraction of acyclic and cyclic degradation products of HD from water samples, various parameters affecting the extraction efficiency, including the type and volume of the eluting solvent, sample loading flow rate, volume, ionic strength, and reusability of the cartridge, were investigated using one variable at a time approach to achieve the best performance for the target analytes.

The simultaneous desorption of the non-ionic degradation products of HD is a critical issue, particularly because of their hydrophobic and hydrophilic characteristics. The cyclic degradation products of HD are hydrophobic molecules, but the acyclic HD degradation products are rather hydrophilic (Table 1). A suitable elution solvent must have a proper eluting power, ability to desorb and dissolve the target analytes, and should be appropriate for the further sample analysis steps [119]. Four organic solvents (MeOH, dichloromethane, ACN, and ethyl acetate) were explored as potential eluents (Figure 9A). Eluotropic strength evaluation experiments demonstrated that only the high-polarity solvents (MeOH and ACN) afforded high recoveries (> 70%) for the cyclic HD degradation products, while desorption did not occur with dichloromethane and ethyl acetate. However, the best combination of recovery and relative standard deviation (RSD) of all the target analytes was achieved using MeOH, a protonic polar solvent, which was selected as an optimal solvent for further experiments.

Different volumes of MeOH (1.5–4.5 mL) were investigated to achieve the best desorption performance. As shown in Figure 9B, most of the analytes elute with 1.5 mL of the eluting solvent, whereas no more than 10% of the analytes are desorbed with an elution volume of > 1.5 mL.

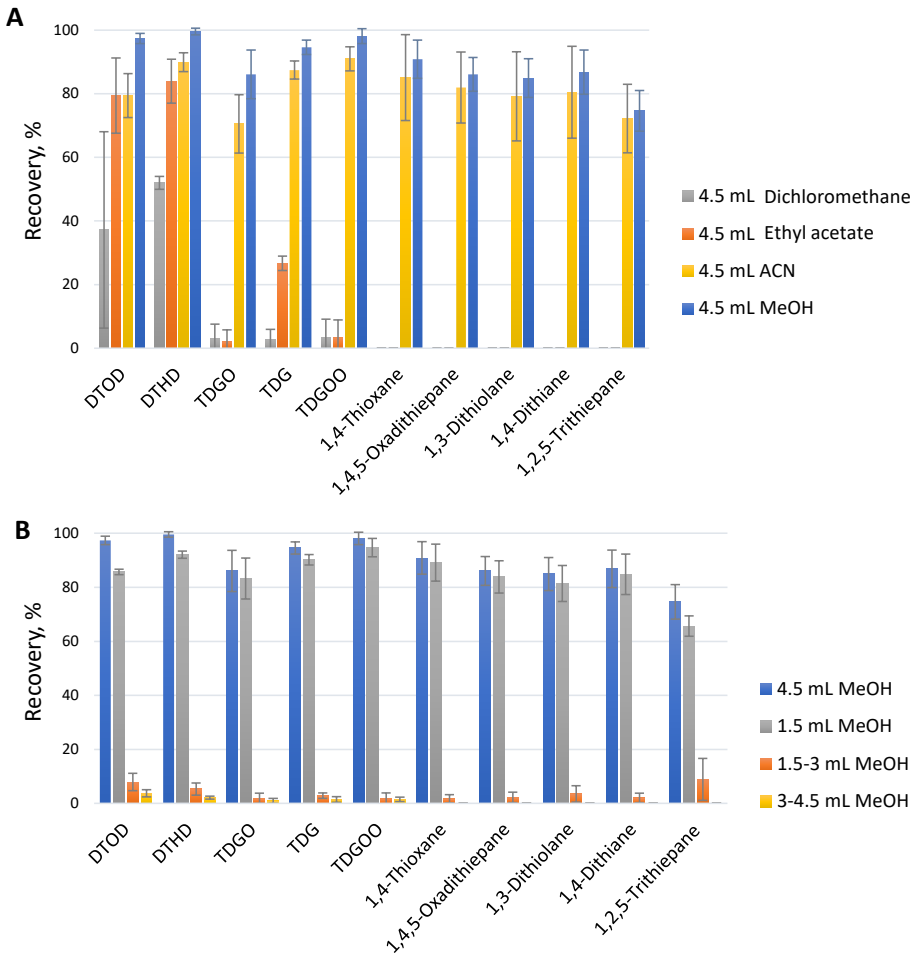


*Figure 9. Effects of eluting solvent (A) and MeOH volume (B) on the recovery of HD degradation products (n = 3). The working solution contains 10 µM of all analytes (**Publication II**).*

Based on the desorption optimisation experiments and considering further sample analysis steps, 2 mL of MeOH was selected as the eluting solvent in subsequent experiments, as it had the highest eluotropic strength for the sorbent and allowed appropriate elution.

Sample loading flow rates in the range of 0.5–5.0 mL min^{-1} were investigated. The results presented in Figure 10, show that high flow rates significantly reduce the recoveries of only the oxidation products of TDG. For all the analytes, a recovery of > 70% was achieved at a flow rate of 2.0 mL min^{-1}. Based on the obtained results, a sample loading rate of 2.0 mL min^{-1} was selected as optimal for further experiments, which afforded acceptable efficiency and reduced the total time of the extraction procedure.

The effect of sample volume (5–100 mL) on extraction recovery (Figure 10B) exhibited a similar trend as that observed for the sample loading flow rate experiments, where the oxidation products of TDG did not interact strongly with the sorbent. When the sample volume was 10 mL, the recoveries were > 70%, but as the sample volume was increased from 10 to 25 mL, the recoveries of TDGO and TDGOO decreased significantly > 10%. For all other degradation products of HD, the recoveries were > 84%, whereas no noticeable variations were observed when the sample volume was increased from 5 to 100 mL. The poor extraction efficiencies of TDGO and TDGOO could be correlated to the low polarities of TDGO and TDGOO, which led to their weak hydrophobic interactions with the sorbent. As the aim of the current study was the simultaneous extraction of all target analytes, 10 mL was selected as the optimal volume of water sample to prevent the loss of target analytes, particularly TDGO and TDGOO.

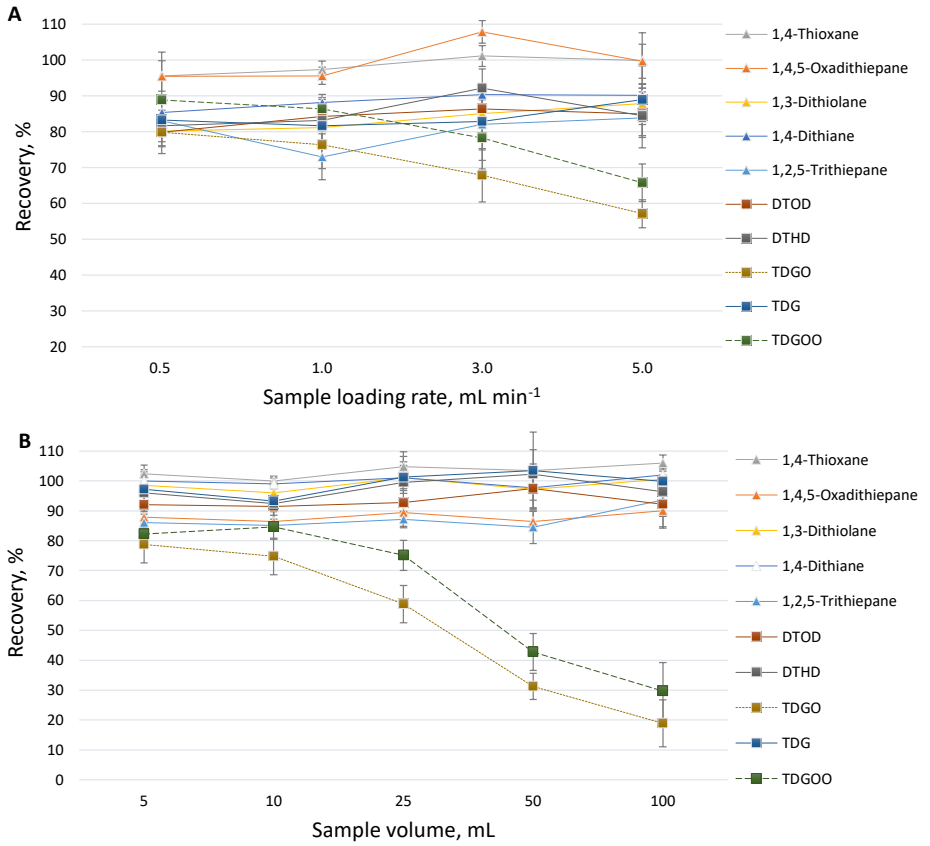


*Figure 10. Effects of sample loading flow rate (A) and sample volume (B) on the recovery of HD degradation products (n = 3). The working solution contains 10 µM of all analytes (**Publication II**).*

As it is necessary to apply CA-based extraction for the analysis of HD degradation products in environmental water samples, particularly seawater samples that contain dissolved salts, the effect of the ionic strength of the sample may affect the SPE procedure, and therefore, it was carefully evaluated. The results showed no noticeable variations in the recoveries of analytes at NaCl concentrations of 0–1.46%, which implied that there was no need to adjust the sample ionic strength in further experiments.

The optimised conditions for the simultaneous SPE of the acyclic and cyclic degradation products of HD included a sample volume of 10 mL, flow rate of 2 mL min^{-1}, and 2 mL of MeOH as the elution solvent.

4.1.5 Analytical performance of CA-based sorbent for HD degradation products (SPE-CE/HPLC-UV) and comparison of CA-based and commercial SPE sorbents

To apply the developed CA-based SPE procedure for the qualitative and quantitative CE-UV and HPLC-UV analyses of HD degradation products, the methodology was evaluated using Milli-Q water spiked with all analytes.

Under the optimised conditions, quantitative parameters such as linear range, coefficients of determination (R^2), LODs and limit of quantifications (LOQs) based on the signal-to-noise ratios of three and ten (S/N = 3 and 10), respectively, precision, and reusability of the cartridge were examined, and the results are presented in Table 3. For the analysis of acyclic compounds, sinapinic acid was used as the IS. All experiments were performed in triplicate, and the values are presented as the average values. The LOD for the acyclic degradation product, TDG, was 300 times lower than that observed in the MEKC analysis, which was previously published by Cheicante *et al.* [88]. However, the obtained LOD values were significantly higher than those obtained by GC-MS method, but were still acceptable based on the concentrations of the degradation products of HD found near the CWA dumpsites. For example, TDGO was found in high concentrations of 196.8–263.9 μg kg^{-1} dw^{-1} in the Gdańsk Deep sediment samples, whereas the LOD for TDGO was 44.2 μg L^{-1} obtained by the developed SPE-CE-UV analysis [74].

The calibration curves exhibited good linearity ($R^2 > 0.99$), and the precision of the methodology was evaluated in terms of intraday and interday precision using spiked standard water solutions with 10 μM target compounds. Intraday RSDs were calculated based on six extractions performed on the same day using the same SPE cartridge (n = 6), while the cartridge was air-dried in between the extractions. Interday RSDs were obtained using three individual CA-based SPE cartridges over three days with three extractions each day (n = 9).

The reusability of CA-based SPE was also evaluated. There were no significant variations in the recoveries (SD ≤ 10%) obtained in 20 consecutive extraction cycles of 10 mL water samples spiked with acyclic and cyclic HD degradation products at final concentrations of 5 and 2 μM. Different matrices of the environmental water samples may affect the sorbent in many ways, but no carry-over effect of the HD degradation products or systematic decrease in recoveries was observed in the present case.

Table 3. Analytical parameters for the proposed SPE-CE/HPLC-UV methodology.

Analyte	Linear range (µM)	R^2	LOD (µM)	LOQ (µM)	Precision (RSD, %)	
					Intraday	Interday
TDG	1.07–20	0.994	0.32	1.07	3.7	8.5
TDGO	1.07–20	0.999	0.32	1.07	6.7	7.4
TDGOO	1.07–20	0.999	0.32	1.07	2.6	3.9
DTHD	1.07–20	0.998	0.32	1.07	7.7	9.9
DTOD	1.07–20	0.999	0.32	1.07	4.3	8.7
1,4-Thioxane	0.57–20	0.999	0.17	0.57	2.0	2.7
1,3-Dithiolane	0.57–20	0.999	0.17	0.57	2.4	8.9
1,4-Dithiane	0.83–20	0.999	0.25	0.83	3.6	7.4
1,2,5-Trithiepane	1.67–20	0.999	0.50	1.67	6.1	7.6
1,4,5-Oxadithiepane	0.57–20	0.999	0.17	0.57	3.0	5.4

The efficiency of the CA-based sorbent was compared to those of the commercial cartridges such as Superclean LC-18, Chromabond NH_2, and HyperSep Hypercarb for the analysis of the HD degradation products (Figure 11).

Under the described extraction conditions, where the target analytes with high pKa values ($pK_a > 13.0$) were not ionisable, Chromabond NH_2, which is a weak anion exchanger, could only act as a normal-phase sorbent. None of the target compounds were detected using the NH_2 sorbent in the elution fraction (MeOH) because this sorbent exhibited no sorption capability for the target compounds in the water matrix. This was a predictable result because under normal phase extraction conditions, which involve the polar analytes and mid- to nonpolar sample matrix with a more polar elution solvent, no interaction between the target analytes and polar sorbent was observed [27].

Under reversed-phase extraction conditions, the target compounds, sample matrix, and elution solvent should be mid- to nonpolar, polar or moderately polar, and nonpolar, respectively [27]. The results obtained using the reversed-phase Superclean LC-18 SPE cartridge showed that the outcome of the experiment was consistent with the theory. This implied that the cyclic HD degradation products should exhibit strong hydrophobic interactions in the reversed-phase conditions. The results obtained using the Superclean LC-18 cartridges demonstrated that the recoveries of the low-polarity analytes were satisfactory (> 85%), but for more polar analytes such as TDG, TDGO, and TDGOO, this reversed-phase sorbent could not be used.

The performance of HyperSep Hypercarb, which retains highly polar compounds according to the manufacturer's recommendations, was better than that of LC-18 for polar analytes (11.7–24.8%). However, for low-polarity cyclic HD degradation products and DTOD and DTHD, although the recoveries were higher (44.7–113.9%), the RSD values were also high (up to 25%).

A comparison of the performances of the above sorbents and CA-based sorbents indicated that to simultaneously analyse all target HD degradation products, the CA-based sorbent afforded the best overall recoveries (79.8 –115.1%, SD < 9%), despite the wide

range of polarities (logP values ranged from -2.301 to 3.012). Therefore, CA-based SPE allowed simultaneous extraction of the cyclic and acyclic degradation products of HD, which was crucial because there is a very high probability that these compounds can exist together in the same sample.

The superior SPE performance of CA can be explained on the basis of its high specific surface area, microporous area, and low density [6], according to the literature reports. As these are carbon-based nanomaterials, these can interact through various non-covalent forces (for example, hydrophobic interactions, hydrogen bonding, π–π stacking, and electrostatic and van der Waals forces) because the degradation products of HD exhibit different characteristics [118], [37].

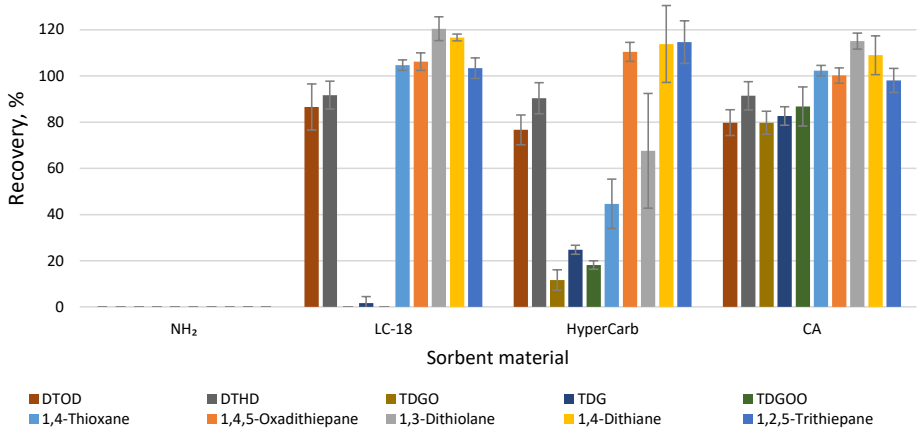


*Figure 11. Comparison of the performances of CA-based and commercially available sorbents for the SPE of HD degradation products under optimised conditions (**Publication II**).*

4.1.6 Environmental water sample analysis

To verify the proposed methodology, real sediment samples were collected from the Bornholm Basin, a chemical warfare dumping site in the Baltic Sea, during the MODUM project expedition between 12 and 16 March, 2016. First, three sediment samples were centrifuged, and the pore water together with deep water was collected and filtered. Thereafter, the liquid part of the sample was analysed by employing the proposed CA-based SPE together with CE-UV and HPLC-UV analyses. To date, the degradation products of HD have been found only in a few samples collected from the Bornholm Basin [13], [15], [71], and the blank samples subjected to analysis show no traces of the target HD degradation products. Thus, to determine the applicability of the SPE methodology for analysing real environmental water samples that may contain the target analytes, the spiking approach was used to simulate real matrix conditions. The samples spiked with target analytes at two different concentrations were analysed, and the overall recoveries and standard deviations (SDs) obtained by the developed CA-based SPE-CE/HPLC-UV methodology are presented in Table 4.

Table 4. Recoveries of environmental water samples spiked with target analytes (n = 3).

Analyte	Concentration added (µM)	Recovery (% ± SD)	Analyte	Concentration added (µM)	Recovery (% ± SD)
TDG	2.5	99.7 ± 2.0	**1,4-Thioxane**	1	91.3 ± 4.0
	10	96.7 ± 2.3		5	94.3 ± 1.3
TDGO	2.5	97.0 ± 4.1	**1,3-Dithiolane**	1	94.3 ± 8.4
	10	83.5 ± 2.9		5	91.1 ± 1.4
TDGOO	2.5	97.4 ± 4.0	**1,4-Dithiane**	1	90.1 ± 6.5
	10	96.1 ± 2.6		5	95.4 ± 2.6
DTHD	2.5	99.4 ± 3.4	**1,2,5-**	2	90.5 ± 8.1
	10	91.2 ± 3.2	**Trithiepane**	5	90.7 ± 5.3
DTOD	2.5	95.3 ± 2.8	**1,4,5-**	1	97.0 ± 4.6
	10	85.4 ± 3.7	**Oxadithiepane**	5	90.0 ± 2.4

As demonstrated in Figure 12, no interfering peaks are observed in the chromatograms and electropherograms obtained by the analysis of non-spiked environmental water samples and the same samples spiked with the analytes. Additionally, no significant matrix effects (< 10.2%, n = 3) were observed using the spiked Milli-Q water and environmental water samples at two different concentrations, using the Equation 6.

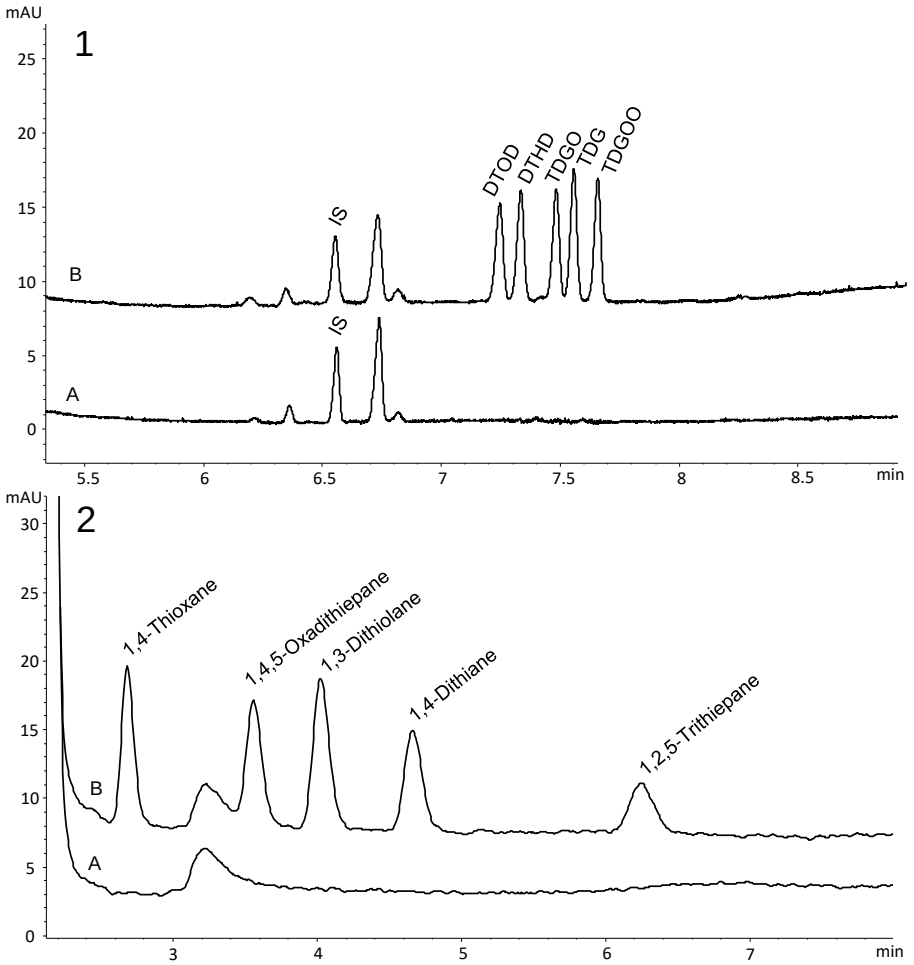


Figure 12. Electropherograms (1) and chromatograms (2) obtained after employing all sample preparation steps and HPLC/CE-UV analysis. A) 10 mL of pore water sample from the Bornholm Deep; B) 10 mL of pore water sample from the Bornholm Deep spiked with acyclic and cyclic HD degradation products at final concentrations of 10 and 5 µM, respectively (50 µM of IS for CE-UV analysis).

4.2 DI-SPME-GC-MS methodology employing on a novel CA-coated SPME fiber for the analysis of OPPs (Publication III)

The evaluation of the CA-based SPE sorbent for the analysis of HD degradation products in **Publication II** showed that MR-FA-based CAs can be also used for other types of sample preparation techniques such as SPME. Since its introduction in the 1990s, SPME has become increasingly popular owing to its simplicity and the possibility of combining sampling, isolation, and enrichment in one step. Additionally, it is rapid, requires little to no solvent, and produces very low laboratory waste, which makes it an environmentally friendly technique [18].

To obtain an appropriate SPME fiber coating, the fiber coating procedure and suitable coating support must be carefully selected, in particular, the types of coating method

and support that are appropriate for the selected sorbent materials. To date, fiber coatings made of porous carbon materials are mainly prepared using sol-gel solution, in which the powder of the material is placed, but possible interactions do not only include the porous carbon-material-based interactions under these conditions [51]. Another often applied coating method is the direct glue method, in which a thin layer of glue is placed on the core surface, and the fiber core is then placed into the CA powder [36], [51]. In **Publication III**, the CA-based coating was *in situ* synthesised on a stainless steel wire. *In situ* synthesis ensured that all the interactions involved were between the analytes and CA-based SPME coating, which afforded high selectivity of the extraction procedure. Detailed description of the pretreatment of the coating support, *in situ* synthesise procedure and fixation to the commercial fiber assembly is provided in Section 3.4.1.

To evaluate the applicability of the CA-coated fibers for the analysis of selected OPPs in environmental water samples, the target compounds were selected based on their structural and thermal stabilities as well as sufficient volatility for thermal desorption procedure, direct transfer of the analytes into the injector of the GC system, and further GC-MS analysis. The target OPPs are the typical pesticides whose residues are monitored in food and feed items as well as in the environment. The target OPPs used in **Publication III** and their chemical properties are listed in Table 2.

Base on the preliminary experiments, the target analytes did not possess desirable properties for sampling from the HS of the sample (HS-SPME mode). However, the CA-based SPME fiber was suitable for the DI to sample (DI-SPME mode); therefore, further studies involved only the DI-SPME sample preparation technique.

4.2.1 Characterisation of coating material

Characterisation of the coating material is important to understand the properties of CAs and evaluating these as extraction sorbents.

The SEM images of the obtained CA-coated SPME fiber showed that the surface of the coating appeared like a solid shell with uniformly distributed pores, whereas the morphology of the cross-section of the coating was similar to that of the fiber surface. The thickness of the coating was approximately 20 μm, which significantly varied for different fibers. The surface morphology of the CA-coated SPME fiber, as shown in Figure 13, indicates that these exposed pores are beneficial for achieving suitable extraction capacity of the coating to adsorb the analytes.

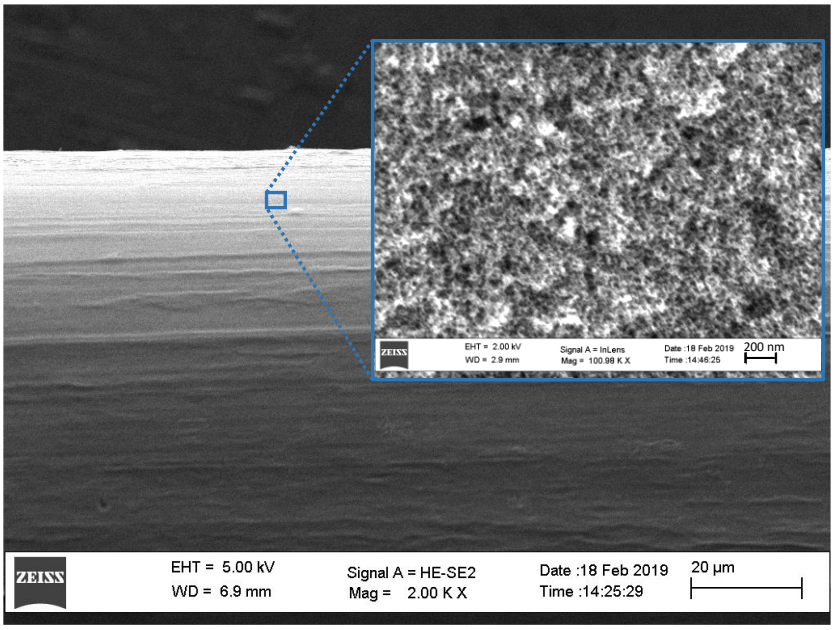


Figure 13. SEM image of the CA-coated SPME fiber surface.

The N_2 adsorption-desorption isotherms of CA were recorded to obtain information on the pore distribution and specific surface area. The pore size distribution was determined using DFT calculations, which confirmed that the fiber coating contained mainly micropores with diameters of < 2 nm, according to the International Union of Pure and Applied Chemistry (IUPAC) classification system (Figure 14) [120]. The specific surface area obtained by Brunauer–Emmett–Teller analysis was 501.157 $m^2 g^{-1}$, providing evidence of the formation of a large adsorption area, which is required for efficient adsorption-based SPE and SPME procedures.

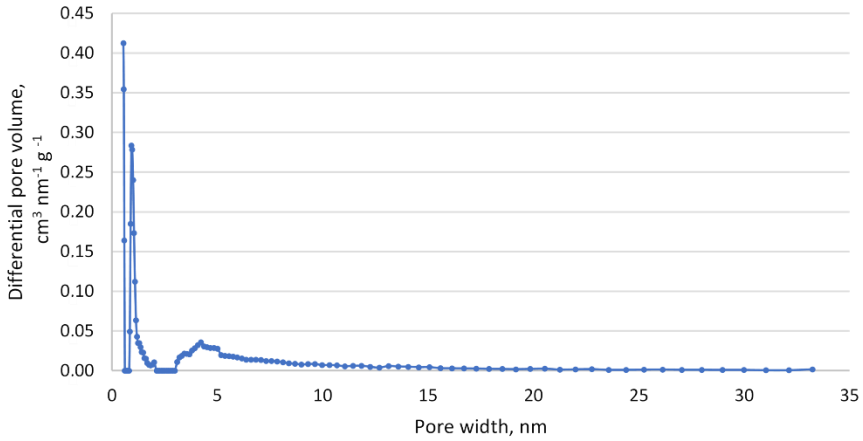


Figure 14. Pore-size distribution of CA-based SPME coating material.

XPS analysis of the obtained coating material mainly showed the presence of sp^2- (66.8%) and sp^3- (12.90%) hybridised carbon atoms, as well as lower amounts of C–O (8.52%), C=O (4.05%), and –O– (2.08%) groups (Table 5 and Figure 15). This was because the pyrolysis procedure (up to 900 °C) for organic aerogels eliminated most of the hydrophilic functional groups such as C=O, C–O, O–H, and C–H groups, resulting in the formation of a relatively hydrophobic material.

Table 5. Quantification data obtained by the XPS analysis of CA-based SPME coating material.

Peak	Position, BE (eV)	Raw Area, cps eV	Atomic Mass	Atomic Conc, %	Mass Conc, %
O 1s oxide	530.533	277.2	15.999	0.56	0.74
O 1s =O	531.656	322.3	15.999	0.66	0.86
O 1s -O-	533.069	779.9	15.999	1.59	2.08
O 1s H_2O	535.872	517.7	15.999	1.05	1.38
C 1s C-C sp^2	284.424	11033.0	12.011	67.65	66.80
C 1s C-C sp^3	285.357	2130.6	12.011	13.06	12.90
C 1s C-O	286.307	1407.9	12.011	8.63	8.52
C 1s C=O	287.506	669.4	12.011	4.10	4.05
C 1s C=O-OR	288.663	440.6	12.011	2.70	2.67

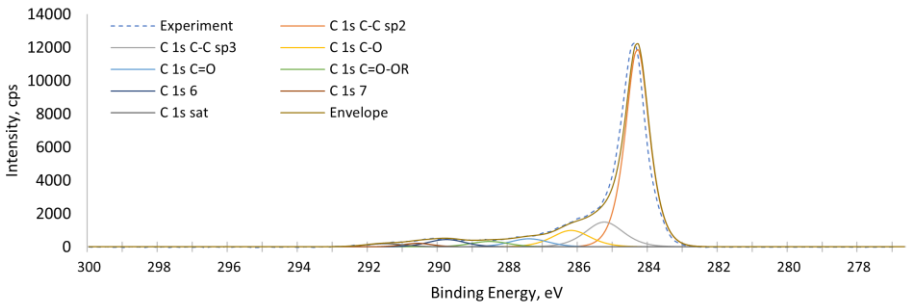


Figure 15. C 1s XPS fitting curve of CA-based SPME coating material.

Considering that the interactions between carbon-based sorbents and analytes involve more than one adsorption mechanism [118], [37], [121] and taking into account the nonpolar and hydrophobic characteristics of both the interacting sides, it was assumed that the hydrophobic interactions played an important role in the sorption process. Furthermore, owing to the presence of conjugated systems in the analytes studied and sp^2-hybridised carbons, the π–π interactions (π-stacking as well as π donor-acceptor interactions) may be an additional supramolecular force that may be involved in the sorption mechanism. In addition, the presence of C–O, C=O, and other oxygen functionalities on the fiber coating surface allows the formation of dipole–dipole interactions between the phosphorus atom of the pesticides and the electronegative oxygen of the sorbent.

4.2.2 Optimisation of DI-SPME procedure

To evaluate the capability of the CA-based SPME fibers for extracting selected OPPs from environmental water samples and achieve the highest extraction efficiency of these lab-made fibers, the optimisation of SPME conditions including the extraction time, desorption time, desorption temperature, and salt concentration was necessary.

The experiments to optimise the extraction time (5–60 min) indicated that 20 min with orbital agitation (200 rpm) was sufficient time to reach the solution-fiber coating equilibrium, which afforded high reproducibility and extraction efficiency. As shown in Figure 16A, no significant changes in the responses of the target analytes are observed after this time.

The target OPPs were recovered from the SPME fiber coating by thermal desorption into the gas phase for analysis. It is essential that the coating maintains its integrity during desorption, which allows the reusability of the fiber. Therefore, the thermal stability of the coating was evaluated (200–350 °C), which was not affected by the temperature in this specific range. This observation is reasonable considering the final preparation step of the fiber coating (pyrolysis up to 900 °C). According to the Le Chatelier principle, temperature is one of the parameters that affects the fiber coating–gas phase equilibrium. An appropriate desorption temperature must be sufficiently high to ensure no condensation of the analytes on the injector walls, such that these can reach the separation column without any losses; however, it should not affect the structures of the analytes. Therefore, based on the physicochemical properties of the target OPPs, the effect of desorption temperature on the extraction efficiency was determined by varying the temperature of the GC injection port in the range of 200–350 °C (Figure 16B).

The desorption time at the optimal temperature should be as short as possible, but long enough to prevent the carryover effect and ensure the complete transfer of pesticides by the carrier gas to the separation column. As 275 °C was found to be the most suitable desorption temperature, the experiments to determine the optimal desorption time (0.5–3 min) were performed at this temperature (Figure 16C). It was confirmed that the efficiency of pesticide extraction was the highest at 275 °C for 2.0 min, affording complete desorption.

Ionic strength is also an important parameter that affects extraction efficiency. The high ionic strengths of the water samples caused by the salting-out effect may present difficulties during the SPME process. Furthermore, high concentrations of salts may mechanically damage the fiber coating; notably, upon the application of DI-SPME, the fiber should be thoroughly rinsed between subsequent analyses. The effect of salting out on extraction efficiency depends on the concentrations of the analytes and salts in the sample. High concentrations of salts increase the ionic strength, which may decrease the solubilities of the analytes in an aqueous solution; thus, their partition coefficients increase [18]. Considering the characteristics of the environmental samples, various concentrations of NaCl (0–10%, w/v) were added to the samples spiked with target compounds to determine the effects of ionic strength on the efficiency of pesticide extraction using DI-SPME. It was expected that high concentrations would decrease the solubilities of the analytes in an aqueous solution and thus affect their sorption on the fiber coating, but the experiments (Figure 16D) revealed that no addition of extra salt into the sample was necessary for further analysis, because the extraction efficiencies of the analytes remained practically unchanged during these experiments.

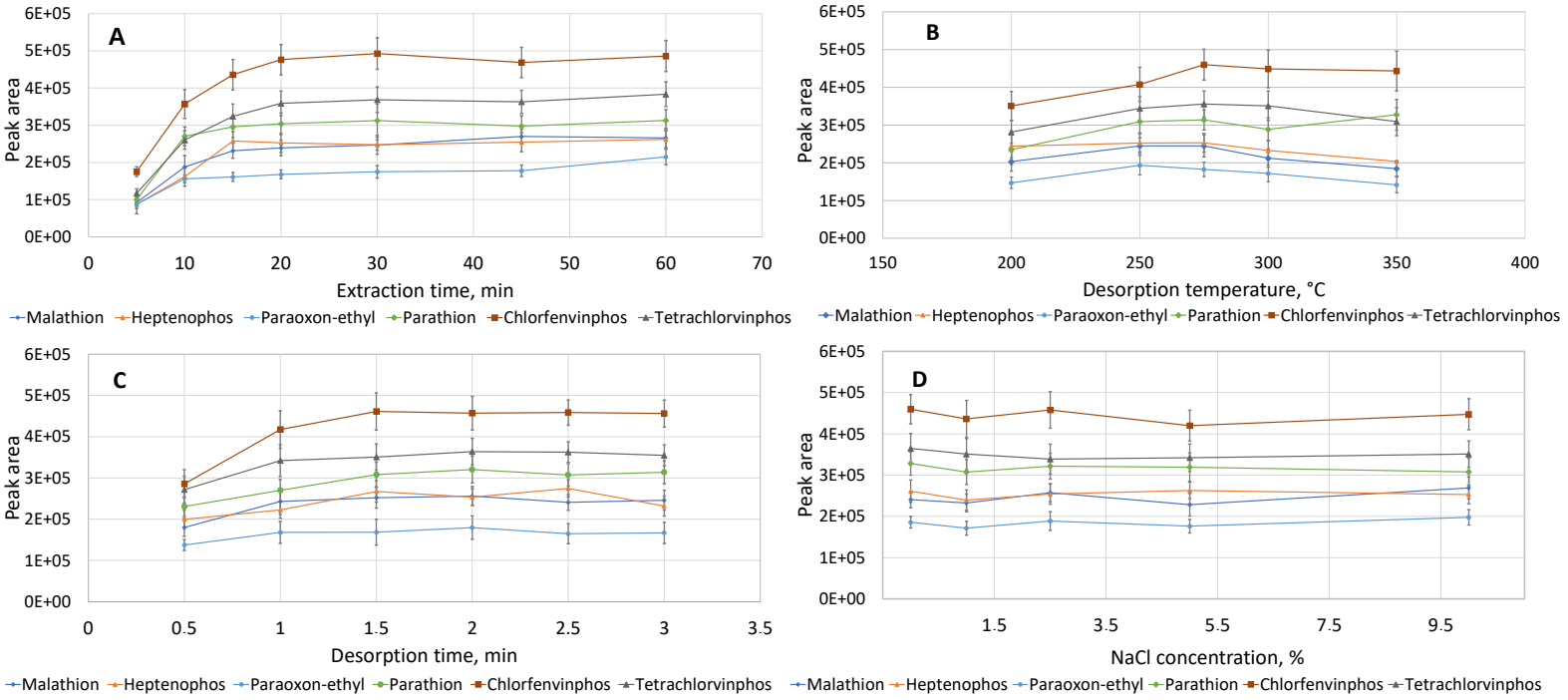


*Figure 16. Effects of (A) extraction time, (B) desorption temperature, (C) desorption time, and (D) NaCl concentration on SPME efficiency. The concentrations in 0.5 mL of working solution include 2.5 mg L^{-1} heptenophos, 2.7 mg L^{-1} paraoxon-ethyl, 3.7 mg L^{-1} tetrachlorvinphos, 2.6 mg L^{-1} chlorfenvinphos, 2.9 mg L^{-1} parathion and 3.3 mg L^{-1} malathion (**Publication III**).*

The optimal SPME conditions for DI-SPME using the CA-coated fiber included an extraction time of 20 min, desorption temperature of 275 °C, and desorption time of 2.0 min. Figure 17 shows the effect of the preconcentration of the CA-coated fiber on the SPME of the OPPs under optimised conditions. Two extracted ion chromatograms were obtained by the DI-SPME-GC-MS analysis of the water samples spiked with OPPs (coloured) and injecting the same concentration of analytes (in ACN) as those in the spiked water sample into the GC-MS system without the use of DI-SPME procedure (black). As shown in Figure 17, CA-based coating exhibits significant binding affinity for all target OPPs, and SPME under optimal extraction conditions allows preconcentration, thereby significantly affecting the sensitivity of the analytical methodology.

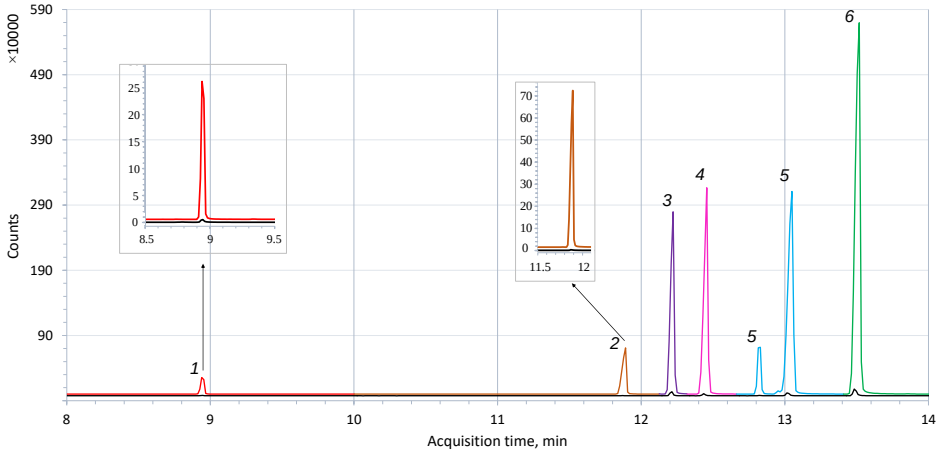


Figure 17. Extracted ion chromatograms obtained by the DI-SPME-GC-MS analysis of water sample spiked with OPPs with final concentrations of 1.3 mg L^{-1} heptenophos (1), 1.4 mg L^{-1} paraoxon-ethyl (2), 1.7 mg L^{-1} malathion (3), 1.5 mg L^{-1} parathion (4), 1.3 mg L^{-1} chlorfenvinphos existing in two isometric forms (5), and 1.8 mg L^{-1} *tetrachlorvinphos (6) (coloured) under optimal extraction conditions and GC-MS analysis of the same concentrations of target OPPs in ACN as those in the spiked water sample (black); (**Publication III**).*

4.2.3 Evaluation of DI-SPME-GC-MS performance and comparison of CA-coated and commercial SPME fibers

The analytical performance of the DI-SPME-GC-MS methodology using the CA-coated fiber was evaluated under optimised extraction conditions and validated using Milli-Q water samples spiked with target OPP standards.

For this purpose, the linearity, R^2, LOD (S/N = 3), LOQ (S/N = 10), repeatability, and RSDs were determined, where all the replicate experiments were performed using a single fiber (n = 5), and the repeatability with three individual CA-coated SPME fibers (n = 3) was evaluated. The data are presented in Table 6.

The fiber reusability experiments showed that the same fiber could be used for at least 80 sorption/desorption cycles for water samples with no significant decrease in the extraction efficiency (Figure 18). However, this quite fragile CA-based fiber coating was prone to damage and required careful handling. This limitation could be addressed by overcoating the fiber with a thin layer of PDMS, which could protect the adsorption phase but may reduce fiber selectivity and affinity for more polar analytes, as well as increase the equilibrium time [122], [123].

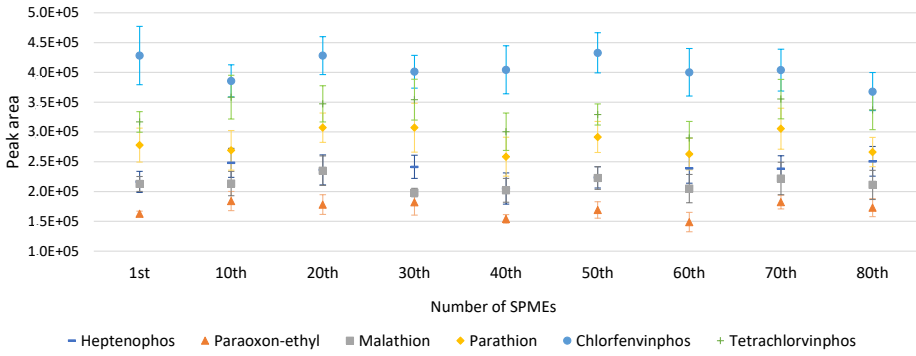


Figure 18. Reusability of CA-coated SPME fiber. The concentrations in 0.5 mL of working solution include 2.5 mg L^{-1} heptenophos, 2.7 mg L^{-1} paraoxon-ethyl, 3.7 mg L^{-1} tetrachlorvinphos, 2.6 mg L^{-1} chlorfenvinphos, 2.9 mg L^{-1} parathion, and 3.3 mg L^{-1} malathion.

The extraction performance of the novel CA-coated SPME fibers for the analysis of target OPPs was compared to those of four commercially available fibers under optimised extraction conditions. One polar polymeric film coating (85 µm PA) for the absorption of analytes and three bipolar SPE coatings, where particles were embedded in the polymeric films (65 µm PDMS/DVB, 85 µm CAR/PDMS, and 50/30 µm DVB/CAR/PDMS), were used for the sorption of analytes. These bipolar coatings contained porous particles (CAR and DVB) with PDMS as a binder, and were found to be more efficient for trace analysis at lower concentrations [122]. The Figure 19 shows the normalised peak areas obtained using CA-coated and commercial SPME fibers, indicating that the extraction capability of the lab-made fibers with CA-based coating for most of the target OPPs is comparable or even higher than those of the commercial ones.

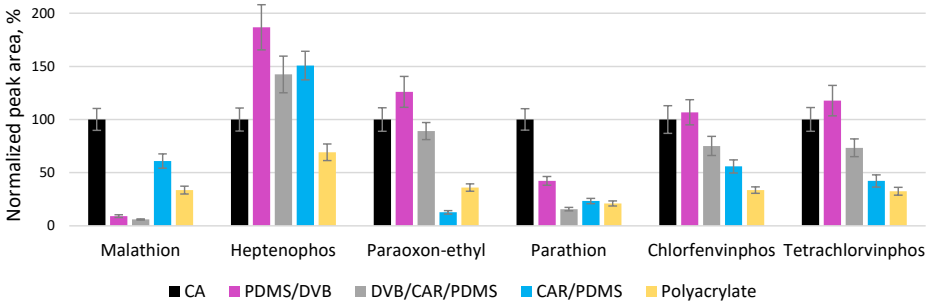


*Figure 19. Extraction of OPPs from water samples using different SPME fibers (**Publication III**).*

*Table 6. Analytical performance of the developed DI-SPME-GC-MS methodology for the analysis of selected OPPs (**Publication III**).*

Pesticide	Quantitation ion, m/z	Linearity, µg L^{-1}	R^2	LOD, µg L^{-1}	LOQ, µg L^{-1}	RSD single fiber (n = 5), %	RSD fiber-to-fiber (n = 3), %
Heptenophos	215.0	0.50–62.7	0.981	0.15	0.50	9.3	11.4
Paraoxon-ethyl	275.0	2.8–68.8	0.985	0.83	2.8	10.3	17.2
Malathion	173.0	0.66–165.2	0.990	0.20	0.66	8.8	16.5
Parathion	291.0	0.58–145.6	0.994	0.18	0.58	10.1	14.9
Chlorfenvinphos	323.0	0.42–103.8	0.990	0.12	0.42	12.3	14.8
Tetrachlorvinphos	331.0	0.37–91.5	0.983	0.11	0.37	9.6	17.1

*Table 7. Recovery of real samples spiked with OPPs (**Publication III**).*

Pesticide	Spiked concentration, µg L^{-1}	Recovery, %		
		River water	Seawater	Honey
Heptenophos	2.5	82.3	96.1	82.5
Paraoxon-ethyl	13.8	85.8	91.8	86.6
Malathion	6.6	83.6	85.1	84.9
Parathion	1.5	85.4	83.9	87.0
Chlorfenvinphos	20.8	89.8	81.8	88.1
Tetrachlorvinphos	1.8	88.6	92.9	85.7

4.2.4 Analysis of OPPs in natural water and honey samples

The applicability of the lab-made CA-coated fibers was examined using real environmental samples. The DI-SPME-GC-MS methodology was applied to the determination of six OPPs in three natural water samples, which were collected from the Pirita River, Tallinn Bay, and Baltic Sea (Estonia). In addition, to demonstrate the applicability of the fiber coating to a more complex sample matrix, target OPPs were also analysed in honey sample as well. None of the analysed blank samples showed traces of the target OPPs. Therefore, to determine the recoveries using the DI-SPME-GC-MS methodology, all the samples were spiked with the initial concentrations of OPPs, as listed in Table 7. The recoveries of the spiked natural water samples were in the range of 81.8–96.1% ($n = 3$, RSD < 14%), and the honey sample recovery was 82.5–88.1% ($n = 3$, RSD < 13%).

The results for the analysis of real samples confirmed that the CA-coated fiber was suitable for the extraction and preconcentration of selected OPPs in environmental matrices such as honey and natural water samples. However, to analyse more complex sample matrices such as honey, more time is required to wash the fiber between the experiments, because the fiber is submerged directly into the matrix during DI-SPME and is more prone to fouling. Matrix effects (Equation 6) evaluated using MilliQ water and natural water or honey samples were < 17% ($n = 3$). Although the observed matrix effects were low, further matrix-matched calibration experiments are required to evaluate the applicability of the developed DI-SPME-GC-MS methodology to the sample preparation and analysis of other complex food and environmental samples.

Conclusions

In this study, novel procedures for the sample preparation and analysis of environmentally harmful compounds were developed to detect the potential leakage of sea-dumped CWs and environmental pollution caused by the abuse or erroneous application of highly toxic OPPs. The potential of MR-FA CA for application to sample preparation procedures as a novel SPE and SPME sorbent was demonstrated.

The main conclusions can be summarised as follows:

- Pre-capillary derivatisation of the acyclic degradation products of HD by phthalic anhydride can be used to separate and significantly reduce the LODs of TDG, TDGO, and TDGOO by employing the CE-UV technique.
- The developed CE-UV methodology is suitable for the analysis of TDG and its oxidation products in seawater by utilising the CA-based sorbent for sample purification and concentration.
- SPE procedure using a CA-based sorbent is developed and evaluated for the simultaneous pretreatment and enrichment of acyclic and cyclic degradation products of HD present in environmental water samples.
- The analysis of spiked real pore and deep water samples collected from the Bornholm Basin, a chemical warfare dumping site in the Baltic Sea, during the MODUM project expedition shows that the developed SPE procedure using the CA-based sorbent is suitable for the simultaneous extraction of HD degradation products from environmental water samples.
- Powdered CA is an appropriate sorbent to achieve high recoveries for both low- and high-polarity degradation products of HD compared to other conventional SPE cartridges.
- CA-based SPME fiber coating is synthesised *in situ* on stainless steel wire for the first time.
- DI-SPME-GC-MS methodology for simultaneous analysis of selected OPPs is suitable for the efficient and sensitive determination of the analytes of interest in the environmental matrices of honey and natural water samples.
- The extraction efficiency of CA-coated SPME fibers is comparable to those of commercial fibers, and is even higher in some cases.

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Minu suurim tänu kuulub minu kallile **perekonnale**, kes on igapäevaselt mulle toeks olnud. Aitäh **ema**, et oled alati olemas ja valmis päriselt kuulama. Aitäh kallid **õed** ja **õdede lapsed**, et oskate mind rõõmsana hoida ja parimal võimalikul moel motiveerida.

Abstract
Novel analytical procedures for sample preparation and analysis of environmentally harmful compounds

In the last few decades, chemical analysis methods greatest developments besides becoming more environmentally friendly, have been towards increasing the reliability of the results. Despite numerous already available sample analysis methods, novel procedures for sample preparation and analyte determination, particularly for the analysis of environmentally harmful compounds, are in high demand. Therefore, traditional analysis methods have been replaced with more sustainable ones. The most problematic, error-prone, labour-intensive, time-consuming, and polluting step of environmental sample analysis is sample preparation.

Solid-phase extraction (SPE) and the miniaturised form of SPE, solid-phase microextraction (SPME), represent these types of sample preparation techniques, which minimise the disadvantages of the sample preparation step, including the high consumption of organic solvents, energy, and waste production. Although various types of commercial SPE sorbents and SPME fiber coatings are available, the need for alternative sorbents that can afford improved selectivity, robustness, and cost-effectiveness have led to the development and evaluation of novel materials, which can be used as SPE and SPME sorbents. Carbon aerogels (CAs) are promising materials with high porosity, large specific surface area, and low density. In this study, CA-based SPE and SPME sorbents were prepared using formaldehyde (FA) and 5-methylresorcinol (MR), which is a by-product of the local oil shale processing industry; in Estonia, oil shale is the most important local solid fossil fuel and a major source of energy.

The analysis of the degradation products of chemical warfare agents (CWAs) in environmental samples has received considerable attention because of the issue of dumped munitions. After World War II, many countries had large quantities of chemical weapons (CWs), which contained highly toxic CWAs. According to the Geneva Protocol 1925, the use of these CWs was prohibited, and dumping at sea was considered to be the most appropriate method for their disposal, which was internationally accepted as a safe and efficient solution at the time. It is assumed that approximately 50 000 CWs containing approximately 15 000 tons of CWAs, mainly sulfur mustard (HD), have been dumped in the Baltic Sea. It has been confirmed that the shells of these dumped munitions have corroded and are presently leaking; thus, the entire Baltic marine ecosystem as well as human health might be at serious risk. Because HD degrades rapidly in an aqueous environment, the determination of the degradation products of HD is important for the identification of possible leakages. Owing to the increasing plans to exploit the seafloor for construction and infrastructure projects in the last 15 years, several European Union (EU)-funded international projects including the project, “Towards the Monitoring of Dumped Munitions Threat” (MODUM), have targeted dumped munitions in the Baltic Sea. The MODUM project involved scientists from nine different countries, and one part of the present study was carried out under this project, where our research group was responsible for the development of CE methodology for the analysis of the degradation products of HD, including the development of sample preparation and analysis procedures.

In the present study, novel sample preparation and analysis procedures for CWAs, particularly for the determination of acyclic (thiodiglycol, thiodiglycol sulfoxide, thiodiglycol sulfone, 3,5-dithia-1,7-heptanediol, and 3,6-dithia-1,8-octanediol) and cyclic

degradation products (1,4-thioxane, 1,3-dithiolane, 1,4-dithiane, 1,4,5-oxadithiepane, and 1,2,5-trithiepane) degradation products of HD in environmental water samples have been developed. SPE procedure using CAs as sorbent materials have been developed and evaluated for the simultaneous extraction of these compounds. The cyclic degradation products of HD were analysed by high-performance liquid chromatography. For the analysis of acyclic compounds, an appropriate pre-capillary derivatisation procedure together with capillary electrophoresis (CE) separation method using ultraviolet absorbance detection was developed. The developed sample preparation methodology was applied during the expedition (MODUM project) at the Bornholm dumping site to detect possible CWA leakages.

Organophosphorus pesticides (OPPs) are another type of environmentally harmful compounds that are widely used as insecticides in agriculture. Although many of these OPPs are not approved as active substances by the EU for use in plant protection products, the abuse or erroneous application of OPPs is often related to environmental pollution, poisoning, and food contamination. Thus, considering the increasing concern, the development of selective and sensitive analysis methodologies, including environmentally friendly sample preparation techniques such as SPME, for the determination of OPPs in environmental water samples and food is highly desirable.

In this thesis, CAs were synthesised *in situ* on stainless steel wires and evaluated as fiber coatings for direct immersion SPME coupled to a gas chromatography-mass spectrometry system for the analysis of OPPs. The developed methodology was applied to the analysis of OPPs (heptenophos, paraoxon-ethyl, malathion, parathion, chlorfenvinphos, and tetrachlorvinphos) in environmental water and honey samples.

The results of this doctoral thesis show the suitability of CE, together with a derivatisation procedure, for the analysis of acyclic degradation products of HD. During the study, MR-FA CAs are demonstrated as suitable materials for application as SPE and SPME sorbents for the analysis of environmentally harmful compounds. The findings about CAs as extraction sorbent materials can allow the continuous application of these materials for further development of novel and sustainable sample preparation and analysis methodologies.

Lühikokkuvõte
Uudsed analüüsimetoodikad keskkonnakahjulikke ühendeid sisaldavate proovide ettevalmistuseks ja analüüsiks

Viimastel aastakümnetel on üha olulisem, et keemilise analüüsi metoodikad muutuksid, lisaks maksimaalselt kõrge usaldusväärsusega tulemuste saavutamisele, aina enam keskkonnasäästlikumaks. Kuigi laialdaselt on kasutusel väga suur hulk nii proovi ettevalmistuse kui ka analüüsi meetodeid, on uute metoodikate väljatöötamine väga oluline just keskkonnakahjulike ühendite määramiseks. Seejuures on analüütilises keemias kasvavaks suunaks traditsiooniliste analüüsimeetodite asendamine keskkonnasäästlikumatega. Kõige problemaatilisemaks peetakse keskkonnaproovide analüüsil proovi ettevalmistust, mis on ka peamine vea allikas, suurima töö- ja ajakuluga ning enim keskkonda saastav etapp.

Tahke faasi ekstraktsioon (SPE) ja eelkõige tahke faasi mikroekstraktsioon (SPME) on just sellised prooviettevalmistuses kasutatavad meetodid, mis võimaldavad minimeerida nimetatud etapi peamisi puudusi, sealhulgas ka orgaaniliste solventide, energia kulu ning jäätmete tekitamist. Kuigi tänapäeval on olemas väga suur hulk erinevaid kaubanduslikult kättesaadavaid SPE kui ka tahkele kandjale seotud SPME sorbente, on uute selektiivsete, soodsate, korduvkasutatavate ja vastupidavate sorbentide väljatöötamine ja rakendamine väga oluline. Üks sellistest, kõrge poorsuse, suure eripinna ning madala tihedusega, materjalidest on süsinikaerogeelid (CA). Antud töös valmistati ning rakendati 5-metüülresortsinoolil (MR) ja formaldehüüdil (FA) põhinevaid CA-sid SPE ja SPME sorbentidena. Seejuures on kasutatav MR pärit Eesti põlevkivitööstusest ning on üheks põlevkivi töötlemise kõrvalsaaduseks.

Käesolevas töös üheks uurimisobjektiks olevate keemiarelvaainete (CWA) analüüsimiseks vajalike metoodikate väljatöötamise vajadus tuleneb aastatetagusest keskkonnakahjulikust tegevusest. Nimelt peale Teist maailmasõda omasid mitmed riigid suurtes kogustes keemiarelvi (CW), mis omakorda sisaldasid ülimalt toksilisi CWA-sid. Kuid kuna CW-de kasutamine oli keelustatud vastavalt Genfi protokollile juba aastast 1925, siis peeti parimaks ja rahvusvaheliselt aktsepteeritud lahenduseks ohtlike CW-de merre uputamist. Kokku uputati ainuüksi Läänemerre hinnanguliselt enam kui 50 000 tonni CW-sid, mis sisaldasid ligikaudu 15 000 tonni ohtlikke keemiarelvaaineid, enim väävel-sinepigaasi (HD). Tänaseks on teada, et merre uputatud CW-d lekivad ning ohtlikud ühendid satuvad merekeskkonda, mis on potentsiaalseks ohuks nii mere ökosüsteemile kui ka inimeste tervisele. Selleks, et avastada võimalikke lekkeid on oluline just laguproduktide tuvastamine, näiteks HD laguneb merekeskkonnas kiiresti mitmeteks atsüklilisteks ja tsüklilisteks ühenditeks. Kuna on kasvav huvi ja reaalne vajadus merepõhja kasutamiseks ehitus- ja taristuprojektideks, on viimase 15 aasta jooksul mitmed teadusprojektid käsitlenud Läänemerre uputatud CW-de teemat, sealhulgas ka MODUM („Uputatud relvastuse ohu monitooring“) projekt. Koos üheksa riigi teadlastega osales projektis ka Taltechi uurimisrühm eesmärgiga töötada välja uusi metoodikaid uputatud CW-dega seotud lekete tuvastamiseks ja võimalike ohtude hindamiseks.

Käesolevas doktoritöös töötati välja proovi ettevalmistus- ja analüüsimetoodikad CWA-de, täpsemalt HD atsükliliste (tiodiglükool, tiodiglükool sulfoksiid, tiodiglükool sulfoon, 3,5-ditia-1,7-heptaandiool ja 3,6-ditia-1,8-oktaandiool) ja tsükliliste (1,4-tioksaan, 1,3-ditiolaan, 1,4-ditiaan, 1,4,5-oksaditiepaan ja 1,2,5-tritiepaan) laguproduktide määramiseks merekeskkonna proovidest. Lisaks CA-del põhinevale SPE-le töötati välja ka atsükliliste HD laguproduktide kapillaarieelse derivatiseerimise ja

edasise analüüsi metoodika, mis võimaldab antud ühendite lahutamist kapillaarelekroforeesi (CE) meetodil ning edasist kõrge tundlikkusega ultraviolett absorptsioon detekteerimist. Tsüklilisi HD laguprodukte analüüsiti kasutades vedelikkromatograafiat. Väljatöötatud proove ettevamistus metoodikaid rakendati ekspeditsioonil Bornholmi CW-de uputuskohas võimalike CWA lekete tuvastamiseks.

Teist tüüpi keskkonnakahjulikud ühendid, mida käesolevas töös käsitleti on organofosfori pestitsiidid (OPP), mis on keskkonnakahjulikud ühendid, mida kasutatakse põllumajanduses tänaseni laiaulatuslikult insektitsiididena. Olenemata sellest, et mitmed OPP-dest ei ole taimekaitsevahendite toimeainetena Euroopa Liidu riikides kasutamiseks heaks kiidetud, esineb tihti nende ühenditega seotud keskkonnareostuse juhtumeid. Seega on äärmiselt oluline uute selektiivsete ja sensitiivsete proovi ettevalmistus- ja analüüsimetoodikate väljatöötamine võimalike reostuste monitoorimiseks, jälgides seejuures keskkonnahoiu põhimõtteid.

Antud doktoritöö teises osas töötati välja metoodika CA-del põhinevate SPME fiibrite valmistamiseks (CA-de *in situ* süntees roostevabast terasest traadile) ning OPP-de analüüsiks kasutades CA SPME fiibreid koos gaasikromatograafia-massispektromeetria (GC-MS) analüüsiga. Väljatöötatud metoodikat rakendati OPP-de (heptenofos, paraoksoon-etüül, malatioon, paratioon, kloorfenvinfos ja tetraklorovinfos) määramiseks looduslike vee ja mee proovide analüüsimisel.

Antud doktoritöö tulemused demonstreerivad CE, koos sellele eelneva kapillaarieelse derivatiseerimis protseduuriga, sobivust HD atsükliliste laguproduktide analüüsimiseks. Käesoleva uurimuse raames tõestati, et MR-FA-l põhinevad CA-d sobivad nii SPE kui ka SPME sorbendiks keskkonnakahjulike ühendite analüüsimisel. Kogutud teadmised CA-de kui ekstraktsioonisorbentide kohta võimaldavad ka edasist uudsete keskkonnasääslikumate proovi ettevalmistus- ja analüüsimetoodikate arendamist.

Appendix

Publication I

P. Jõul, H. Lees, M. Vaher, E.-G. Kobrin, M. Kaljurand, M. Kuhtinskaja, Development of a capillary electrophoresis method with UV detection for the analysis of thiodiglycol and its oxidation products, *Electrophoresis.* 36 (2015) 1202–1207.

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Research Paper

Development of a capillary electrophoresis method with direct UV detection for the analysis of thiodiglycol and its oxidation products

A novel method based on CE with precolumn derivatization and direct UV detection for the determination of thiodiglycol (TDG), TDG sulfoxide, and TDG sulfone in water samples was developed. The lack of a UV chromophore of target analytes was overcome by derivatization with phthalic anhydride. The reactant concentrations, as well as the derivatization dependence on heating temperature and time, were carefully investigated. The baseline separation of three derivatives was achieved in less than 8 min by applying a simple BGE composed of a 30 mM borate buffer at pH 8.5. Several parameters affecting the separation efficiency (buffer pH and concentration, capillary temperature, and applied voltage) were evaluated. Calibration curves of all compounds showed good linear correlations ($R^2 > 0.9994$). The LODs of the TDG and its oxidation products were in the range of 98–154 ng/mL. The precision tests resulted in RSDs for migration times and peak areas of less than 1.2 and 3.6%, respectively. The developed method was successfully applied for the analysis of TDG and oxidation products in seawater, utilizing the carbon aerogel-based adsorbents for sample purification and concentration. Additionally, the method has the potential to be transformed into a portable CE format.

Keywords:
Phthalation / Precolumn derivatization / Sulfur mustard degradation products / UV detection
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1 Introduction

At the end of World War II it was necessary to dispose of large quantities of conventional and chemical munitions left over from German and allied stocks. Dumping at sea was considered the most appropriate solution at this time. Thus, around 50 000 tons of chemical munitions were dumped in the Baltic Sea (mostly bombs and shells) [1]. After almost 70 years of such dumping, human health and the entire Baltic marine ecosystem might be at serious risk due to the corrosion of the shells, which has led to a constantly increasing release of highly toxic compounds into the seawater.

A large number of the dumped munitions contain yperite, commonly known as sulfur mustard (HD). The compound itself is a vesicant that causes chemical burns on skin and is an eye and lung irritant [2]. In an aqueous environment, HD rapidly hydrolyses to nontoxic thiodiglycol (TDG) and then slowly oxidizes to TDG sulfoxide (TDGO) and TDG sulfone (TDGOO). In addition, HD hydrolysis leads to the formation of a variety of degradation products, such as cyclic and open chain compounds [3]. It should be noted that potential ecological and health risks are associated primarily with sulfur mustard itself, and the importance of analysis of HD degradation products is that they act as a markers of the HD leakage locations.

Based on the recommended operating procedure for analysis in the verification of chemical disarmament [4], the most frequently used methods for the identification of a sulfur-containing precursor and breakdown products in aqueous samples are based on GC, in combination with MS and/or MS/MS. Due to the low or nonvolatility of the TDG and its oxidation products, derivatization is an essential step in sample preparation prior to GC analysis. Silylation is a widely used derivatization process for GC and, in the case of HD degradation products, *N*,*O*-bis(trimethylsilyl)trifluoroacetamide [5] and *N*-methyl-*N*-(tertbutyldimethylsilyl)trifluoroacetamide [6] are the most common derivatizing reactants. There are some disadvantages in the application of silylation. The most critical point here is that silyl derivatives tend to be highly moisture sensitive, which leads to derivative decomposition and, thus,

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Abbreviations: CWA, chemical warfare agent; **HD**, sulfur mustard; **TDG**, thiodiglycol; **TDGO**, TDG sulfoxide; **TDGOO**, TDG sulfone

Colour Online: See the article online to view Figs. 2 in colour.

requires strict control under derivatization conditions [7]. Another problem is the appearance of a large number of artificial peaks on the total ion chromatogram caused by the indiscriminate property of these derivatization reactants when they react with hydroxyl as well as carboxylic groups. Moreover, the reactant itself is prone to form clusters and contaminate the total ion chromatogram [8]. 1-(trifluoroacetyl)imidazole also demonstrated its high potential for TDG and TDGO derivatization. Trifluoroacetylation is less demanding, but the derivative is still sensitive to water traces and storage time [9].

Besides GC, LC coupled with MS [10], NMR spectrometry [11] or sulfur flame photometric detection [12] are also used for the analysis of water-soluble degradation products. In this context, the application of new analytical techniques, such as CE, could be the next promising step in the field of chemical warfare agents (CWAs) analysis.

However, the advantages of CE, such as simplicity of instrumentation and operation procedure as well as high separation efficiency, have not fully been realized yet and there is an urgent need to be more closely evaluated regarding, e.g. the screening of seawater quality as pointed out above. To the authors' knowledge, there are a very limited number of scientific articles on the utilization of CE for the analysis of HD and its degradation products. The neutral degradation products of HD can be analyzed by direct UV detection using micellar electrokinetic capillary chromatography (MEKC) [13, 14]. Separation was achieved through a running buffer of 10 mM borate and 100 mM SDS. The moderate sensitivity was due to the lack of UV chromophore sites on analyte molecules.

Moreover, unlike other separation methods (GC and HPLC), the simplicity and robustness of CE (besides its other features) allow for miniaturization of instrumentation and, as a consequence, the design of portable field analyzers [15]. Such instruments can be used in situ, at the point of care. This in turn provides a fast response when information is urgently needed. Although portable GC and HPLC instruments have been developed, the need to use compressed gases or pumps and solvents makes the construction of portable GC and HPLC instruments a difficult task. In contrast, a couple of successful portable CE instruments have been reported [16–18]. In terms of CWA screening, some examples are already available. The excellent separation performance of a portable CE has been confirmed by the separation of alkylphosphonic acids using a contactless conductivity detection system [19, 20]. The separation of other critical compounds of military and forensic interest has been demonstrated by Hauser's group (nitrogen mustard) [21] and Breadmore's group (explosives) [22].

In spite of that the GC-MS/MS protocol still outperforms the CE protocol proposed here on LOD as was pointed above, the portable GC is more difficult to operate in the field and its eventual usefulness of the portable CE instrument will depend on real concentrations that are present at the point of care. The information about the real concentration of TDG and its oxidation products in near-bottom water or sediments is very limited. Thus, the measured concentrations of WW II CWA munitions near the Gotland and Gdansk dumping sites in the Baltic have been reported to be about 20–250 μg/kg of sediment (for sulfur compounds) [9]. In this respect, the proposed CE technology seems to be a very promising and reliable alternative to conventional GC-MS analysis.

In the present study, a CE method with direct UV detection for the analysis of TDG and its oxidation was developed and validated. The proof of the principle was demonstrated on a commercial lab scale instrument (Agilent), with the goal of transforming it further to a portable format. A CZE separation method with direct UV detection after precapillary derivatization with strong UV chromophore (phthalic anhydride) is described. A method validation in terms of specificity, precision, linearity and limits of quantification and detection was performed. The BGE contained only boric acid adjusted with sodium hydroxide, making it simple and easy to use. The developed method was used to determine the low amounts of TDG and its oxidation products in seawater.

2 Materials and methods

2.1 Chemicals

Boric acid, sodium hydroxide, acetonitrile, sinapinic acid (internal standard, IS) and imidazole were purchased from Sigma-Aldrich (Germany). Phthalic anhydride and pyridine were obtained from Merck KGaA (Darmstadt, Germany). TDG, TDGO, and TDGOO were synthesized by Envilytix (Wiesbaden, Germany). All chemicals were of analytical grade and used as received. BGE was prepared in DI water from a Milli-Q water purification system (Millipore S. A. Molsheim, France).

2.2 Instrumentation

An Agilent 3D instrument (Agilent Technologies, Waldbronn, Germany) equipped with a diode array UV/Vis detector (DAD) was used for the separation of TDG and its oxidation products. All electropherograms were recorded and integrated with Agilent ChemStation software. Uncoated fused-silica capillaries (Polymicro Technologies, Phoenix, AZ, USA) with an internal diameter of 50 μm and a length of 52/60 cm (effective length/total length) were employed in the experiments. Samples were injected hydrodynamically by applying a pressure of 50 mbar for 5 s. Separation process was monitored at 200 nm. The pH value of the electrolyte solution was measured with a Metrohm 744 pH meter equipped with a combination electrode (Metrohm, Herisau, Switzerland), which had been calibrated with commercial buffers at pH 7.00 (±0.01), pH 10.00 (±0.01), and pH 12.00 (±0.01) (Sigma-Aldrich). Empty SPE tube (polypropylene, tube volume 3 mL, Phenomenex) was used for SPE cartridge preparation.

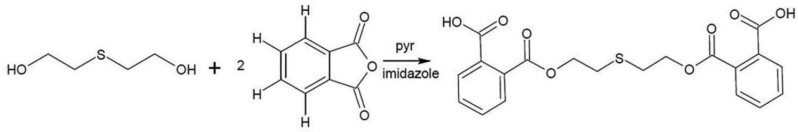

Figure 1. Derivatization reaction of TDG with phthalic anhydride.

2.3 Sample preparation

One liter of seawater (from the Baltic Sea) was spiked with TDG, TDGO, and TDGOO standard solutions to obtain the final concentrations of 0.12, 0.14, and 0.15 μg/mL, respectively. For the target analytes extraction, an SPE cartridge based on powdered carbon aerogels [23] was used (100 mg of powder per cartridge). Fifty milliliters of spiked seawater was run through the SPE cartridge using a vacuum system, then the sorbent was washed with 10 mL of DI water and, finally, the compounds of interest were eluted with 1.5 mL of acetonitrile. Then the solvent was evaporated under a gentle steam of nitrogen till dryness. Finally, 20 μL of derivatizing mixture was added to the solid residue and the sample was treated as described below.

2.4 Derivatization procedure

The derivatizing mixture was prepared in accordance with the literature [24]. Briefly, 1.61 g of phthalic anhydride was dissolved in 10 mL of pyridine, and then 0.24 g of imidazole was added to catalyze the reaction. The mixture was sealed with septum and stored in a desiccator in the dark. For derivatization, 100 μL of phthalic mixture was added to each 2.5 mg of analyte of interest, sealed and heated at 45°C for 20 min. Then the mixture was cooled and the same amount of water was added to stop the derivatizing reaction. Finally, the mixture was diluted by DI water in accordance with need, an IS of sinapinic acid was added, and the sample was introduced into the CE system (Fig. 1).

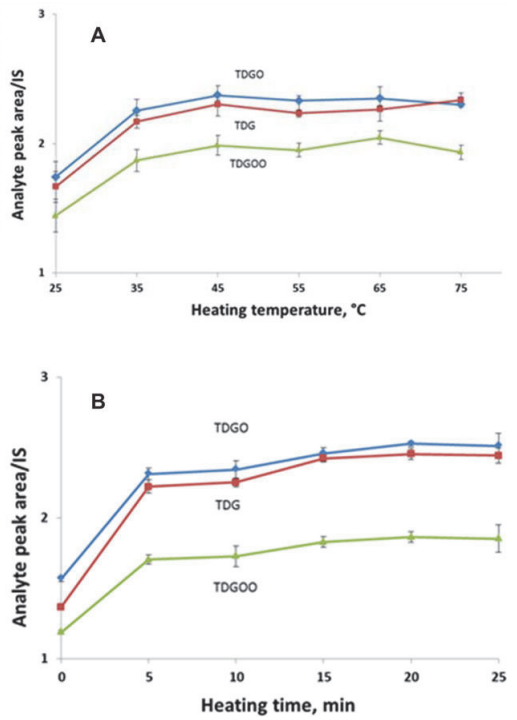

Figure 2. Effect of heating temperature (A) and time (B) on derivatization efficiency.

3 Results and discussion

3.1 Sample derivatization

To obtain reproducible results from the sample derivatization procedure, a careful optimization of several reaction parameters was needed. The effects of the amount of the derivatizating reactant, and the heating time and heating temperature were examined. Each time sample was injected into an electrophoretic system, the average peak area ($n = 3$) was measured and the condition that gave maximum response (peak area) was selected. Based on the stoichiometry of the derivatizing reaction, the required minimum amount of reactant was calculated (~40 μL per 2.5 mg of TDG). Then two-, three-, and fourfold volume excess of reactant was applied for the derivatization of each analyte. Based on the average peak area measurements, the maximum response was achieved applying the twofold volume excess of phthalic mixture. The appearance of the phthalic acid peak on an electropherogram (unreacted phthalic anhydride) acted as an indicator of a sufficient excess of derivatizing reactant. For future experiments, 100 μL of reactant per each 2.5 mg of analyte was selected to avoid the lack of derivatizing reactant in samples.

In the presence of imidazole, the derivatization reaction was quite fast. To find the optimal derivatization temperature and heating time, a set of additional experiments was carried out, varying the temperature in a range of 25–75°C and the heating time in a range of 0–25 min. Keeping the temperature constant (85°C, in accordance with the literature source [24]), the maximum response was achieved within 5 min (Fig. 2A). The effect of the heating temperature was evaluated, keeping the reaction time (20 min) constant. The best response was obtained at 45°C and further temperature increases did not

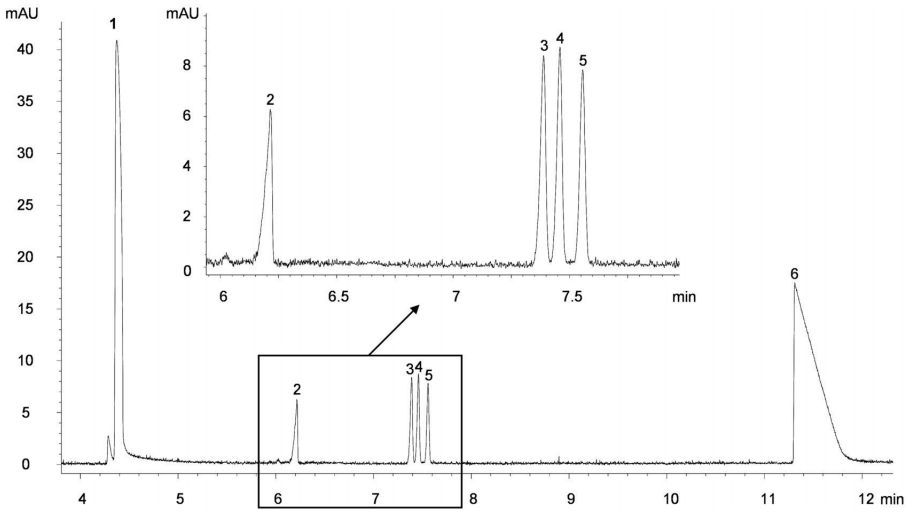

Figure 3. Representative electropherograms obtained after derivatization of 3.05 μg/mL of TDG (4), 3.45 μg/mL of TDGO (3), and 3.85 μg/mL of TDGOO (5) under optimized separation conditions: borate buffer concentration 30 mM, buffer pH 8.5, applied voltage 15 kV, and capillary temperature 25°C. Additional peaks: EOF (1), IS (2), and phthalic acid (6).

affect the result (Fig. 2B). Finally, the derivatization conditions were as follows: the amount of reactant 100 μL per each analyte, derivatization temperature 45°C, and derivatization time 20 min.

The stability of derivatives was investigated. For this, target analytes (3.1, 3.5, and 3.9 μg/mL for TDG, TDGO, and TDGOO, respectively) were derivatizated as described above and after IS addition the mixture was divided into two equal aliquots. The first aliquot was stored at room temperature (23 ± 1°C) and under light (mostly halogen lamps), and the second aliquot was kept in a refrigerator (4°C, in darkness). Systematic sampling over 5 days was performed to measure the peak areas of the derivatives. In the case of TDG and TDGO, there were no systematic changes in the peak areas and shapes during the evaluation period for the first aliquot (kept at room temperature) or for the second one (kept at 4°C). The RSD of the peak areas did not exceed 3.2% ($n = 5$, one analysis per day), which indicated the high stability of the derivatives. TDGOO samples kept at room temperature showed a slight peak area decrease (RSD = 5.7%), but the refrigerated samples demonstrated stability.

3.2 Choice of BGE

Underivatized TDG and its oxidation products are neutral at pH below 9 and, thus, can be analyzed using the MEKC separation technique. Applying 10 mM borate buffer with 100 mM SDS and direct UV detection at 200 nm, TDG and TDGO could be separated within 6 min. The calculated LOD for TDG and TDGO was 10 μg/mL [14]. The high values of LOD are logically justified due to the absence of strong UV-absorbing sites. In this work, an improved method for the analysis of target sulfur compounds involved derivatization with phthalic anhydride incorporating chromophore sites into the analyte structure and, at the same time, affecting the pK_a value of the formed derivatives and giving a charge to the molecules that, in own turn, allows to use borate buffer for analyte separation. The optimization of the separation conditions was carried out by the investigation of the effect of buffer pH, concentration, capillary temperature, and applied voltage on separation efficiency.

It is well known that buffer pH plays the key role in optimization of a separation process affecting the EOF velocity and degree of analyte ionization. So, in the present work, a 30 mM borate buffer with a pH range of 7.5–10.0 was investigated to evaluate the impact on separation results. At buffer pH values below 8.0, the derivatized analytes were unresolved and the peak shapes were asymmetric. An increase in the buffer pH value to 8.5 led to improvement in analysis time and all analytes were baseline separated. Further, pH change to 9.5 resulted in an increase in the electrophoretic mobility of the analytes. Baseline separation was still achieved and the analysis time was the shortest. Unfortunately, starting at pH 9.0, the sulfone peak area was prone to rapid decrease and at pH 10 almost disappeared, which is, obviously, associated with instability of TDGOO at pH 9 and above.

The influence of borate buffer concentration on separation was studied in the range of 20–50 mM at pH 8.5. By increasing the buffer concentration from 20 to 50 mM, the migration times of all analytes were also significantly increased. Nevertheless, the best separation efficiency was achieved at 30 mM and further concentration increase led to peak broadening without a remarkable change in peak separation.

Table 1. Regression data for the calibration curves

Derivative	Linearity range (μg/mL)	Regression equation	Regression coefficient (R^2)	LOD (μg/mL)	Limit of quantification (μg/mL)
TDG	0.10–2.44	$y = 0.1517x + 0.0017$	0.9996	0.10	0.31
TDGO	0.14–2.76	$y = 0.1359x - 0.0161$	0.9997	0.14	0.42
TDGOO	0.15–3.08	$y = 0.1233x - 0.0304$	0.9994	0.15	0.46

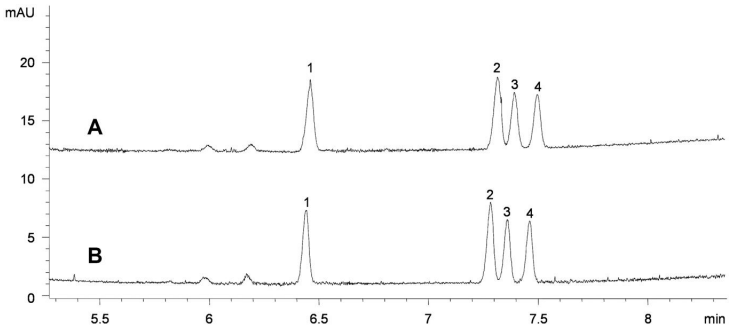

Figure 4. Electropherograms of (A) distilled water and (B) seawater samples. Separation conditions: borate buffer concentration 30 mM, buffer pH 8.5, applied voltage 15 kV, and capillary temperature 25°C. Peaks: IS (1), TDGO (2), TDG (3), TDGOO (4).

Additionally, the effect of the applied voltage over the range 15–25 kV and capillary temperature (15–30°C) was also investigated in terms of separation efficiency and migration times. Voltage values above 20 kV resulted in faster migration times of analytes, but the separation efficiency was not sufficient. All derivatives were baseline resolved at 15 kV, but the analysis time was extended by several minutes. The increase in capillary temperature also noticeably improved the migration time of the derivatives. Thus, the temperature increase from 15 to 30°C decreased the analysis time by a quarter, keeping the separation efficiency at a reasonable level.

Finally, the optimized separation conditions for the separation of three derivatives were as follows: borate buffer concentration 30 mM, buffer pH 8.5, applied voltage 15 kV, and capillary temperature 25°C. The representative electropherogram is shown in Fig. 3.

3.3 CE method validation

The precision of the developed method was investigated. All precision tests were based on optimized BGE and a standard mixture of derivatives. The tests were performed for the run-to-run and day-to-day variations of the migration times and peak areas. Run-to-run precision resulted in maximum RSD values of 0.6% ($n = 6$) and 3.1% ($n = 6$) for the migration time and peak area, respectively. Additionally, day-to-day results showed RSD values of 1.2% ($n = 6$) for the migration times and 3.6% ($n = 6$) for the peak areas. There were no systematic changes in peak shape during the precision tests.

The linearity was evaluated in the range of 0.10–2.44 μg/mL for TDG, 0.14–2.76 μg/mL for TDGO, and 0.15–3.08 μg/mL for TDGOO. Calibration curves were constructed using five concentration levels and were based on the ratio of the corresponding derivative to IS peak area versus concentration. The linearity range, regression equations, and regression coefficients are shown in Table 1.

The LOD and limit of quantitation (LOQ) were obtained experimentally by measuring the S/N. The lowest LOD and LOQ were obtained for TDG and calculated as 98 ng/mL (S/N = 3) and 305 ng/mL (S/N = 10), respectively.

To evaluate the specificity of the developed method, a blank sample was treated as described in Sections 2.3 and 2.4 and then injected into the CE system. There were no interfering peaks observed in the resulting electropherogram.

3.4 Spiked seawater analysis

To demonstrate the method applicability for real sample analysis, seawater was spiked with a standard mixture of free underivatized analytes (TDG, TDGO, and TDGOO) to get the final concentrations of 0.12, 0.14, and 0.15 μg/mL, respectively. The extraction of analytes and the derivatization process are described Sections 2.3 and 2.4. The same sample preparation procedure was carried out for spiked distilled water to evaluate the seawater as a matrix influence on the extraction and derivatization processes. Figure 4 shows the electropherograms of the separation performance of seawater as well as distilled water samples. In all cases, TDG, TDGO, and TDGOO were baseline separated. The RSD of the migration times between all analytes in spiked sea and distilled water samples resulted in 0.4% and the RSD of the peak areas was below 5%. These results show no evident influence of seawater matrix on the extraction and derivatization processes.

4 Concluding remarks

The results of the study show that precolumn derivatization by phthalic anhydride can be used for the significant reduction of LOD of TDG, TDGO, and TDGOO by applying capillary zone electrophoresis with direct UV detection. It also was demonstrated that sample purification and concentration on carbonaceous adsorbent allows for the quantitative analysis of mustard gas hydrolysis and oxidation products in seawater. Nevertheless, it should be noted that the obtained detection limits are less reliable than GC-MS results, but can still be considered acceptable. Moreover, the miniaturization benefits of CE allow translation onto a truly portable instrument, thus making the method more attractive for in-field use.

Transforming the protocol to a portable instrument involves another problem. Contemporary LED sources can generate radiation with wavelengths above 240 nm while the protocol reported in this study requires radiation of 200 nm. Thus, the UV detector implemented in the portable CE instrument must employ a miniature deuterium lamp which somewhat reduces the robustness of the instrument. We believe, however, that this is not a serious obstacle to its design.

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The authors have declared no conflict of interest

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Publication II

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Evaluation of carbon aerogel-based solid-phase extraction sorbent for the analysis of sulfur mustard degradation products in environmental water samples

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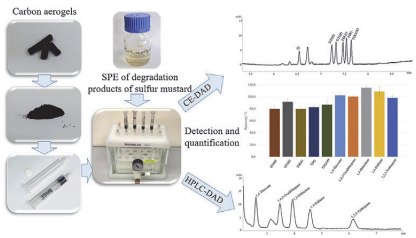
HIGHLIGHTS

- A method was developed for extraction of 10 degradation products of sulfur mustard.
- Carbon aerogel-based sorbent was evaluated for extraction of target compounds.
- The obtained recoveries were at least 80%.
- Applied for analysis of target compound in environmental water samples.

GRAPHICAL ABSTRACT



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ABSTRACT

In this study, SPE method using a carbon aerogel(CA)-based sorbent was developed and evaluated for the simultaneous extraction of sulfur mustard (HD) degradation products from environmental water samples. Applied CAs proved to be very promising materials for use as SPE sorbents, due to their high porosity, very low density and a large specific surface area. 10 degradation products of HD, both aliphatic and cyclic (thiodiglycol (TDG), TDG sulfoxide, TDG sulfone, 3,5-dithia-1,7-heptanediol, 3,6-dithia-1,8-octanediol, 1,4-thioxane, 1,3-dithiolane, 1,4-dithiane, 1,2,5-trithiepane, and 1,4,5-oxadithiepane) were extracted on a CA-based SPE cartridge. The concentrations of target analytes in the eluate were determined by HPLC-DAD and CE-DAD. Several parameters affecting the extraction efficiency, including the kind and volume of the eluting solvent, sample loading flow rate, volume and ionic strength as well as the reusability of the cartridge, were investigated and optimized to achieve the best performance for the analytes. A series of quantitative parameters such as linear range, coefficient of determination, LOD, LOQ and precision were examined under the optimized conditions. High sensitivity (LODs 0.17–0.50 μM) and high precision (intraday RSD = 2.0–7.7% and interday RSD = 2.7–9.9%) for all the analytes were achieved. The performance of the CA-based sorbent was compared with that of commonly used SPE sorbents. Applied for the analysis of spiked pore water samples collected from the Bornholm Basin, one of the largest chemical warfare dumping sites in the Baltic Sea, the proposed method allowed high SPE recoveries of all the analytes ranging from 83.5 to 99.7% to be obtained.

1. Introduction

Sulfur mustard (HD) (bis-(2-chloroethyl)sulfide) is a blister

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Abbreviations

HD	sulfur mustard
CWA	chemical warfare agent
TDG	thiodiglycol
TDGO	thiodiglycol sulfoxide
TDGOO	thiodiglycol sulfone
CWC	Chemical Weapons Convention
CA	carbon aerogel
MR/C	5-methylresorcinol to catalyst molar ratio
DTHD	3,5-dithia-1,7-heptanediol
DTOD	3,6-dithia-1,8-octanediol
ACN	acetonitrile
MeOH	methanol
R^2	coefficient of determination

agent (vesicant), and, as an alkylating agent, readily reacts with a wide variety of different biological molecules and thereby affects many processes in the living tissue (Ghazanfari and Hassan, 2013). HD was used during World War I and the Iran-Iraq War and stockpiled by countries as a chemical warfare agent (CWA). The Chemical Weapons Convention (CWC) prohibits the development, production, stockpiling and use of CWAs ("Organisation for the Prohibition of Chemical Weapons"; Szinicz, 2005). In addition to HD itself, there are eight more analogs which have been included in the CWC Schedule 1 of chemicals ("Organisation for the Prohibition of Chemical Weapons: Annex on Chemicals"). Longer-chain analogs are more vesicant and persistent than the parent compound, however, due to their lower volatility, the analogs do not produce casualties by the action of the vapor (Lemire et al., 2007; Timperley et al., 2003).

In dilute aqueous solutions, degradation of HD occurs primarily through hydrolysis. HD is converted first to a sulfonium ion and then to the hemimustard and thiodiglycol (TDG). Then TDG may slowly oxidize to thiodiglycol sulfoxide (TDGO) and thiodiglycol sulfone (TDGOO). There are known many more open-chain and cyclic degradation products of HD and its analogues (Munro et al., 1999). TDG as a precursor of HD production is also included in the CWC Schedule 2 of chemicals ("Organisation for the Prohibition of Chemical Weapons: Annex on Chemicals").

There is still concern about that HD could be used by terrorists in a chemical attack against civilians or it could be involved in industrial accidents, as a consequence of which emergency physicians must treat persons having been exposed to HD (Davis and Aspera, 2001). Therefore, the physicians must recognize HD and its degradation products and know how to manage HD-exposed patients, while in such cases timely information about the accident is of critical importance (Weibrecht et al., 2012).

In recent years, chemical weapons (CWs) have received much attention because of the problem of dumped chemical munitions. After World War II at least 50 000 tons of CWs was dumped in the Baltic Sea because their use had been prohibited. These weapons, most of which contained HD, have been lying at the bottom of the sea for more than 70 years. The munition shells have corroded and toxic HD has already been leaked into the seawater and decomposed (Bełdowski et al., 2016; CHEMSEA FINDINGS: Results from the CHEMSEA project). Therefore, interest in determination of HD degradation products in environmental water samples has been increasing in recent times.

In real environmental samples from the dumpsite area there have been detected both aliphatic hydrolysis and oxidation products, and cyclic decomposition products of HD (Christensen et al., 2016; Mazurek et al., 2001). It has been assumed that the concentration of hydrolysis products in the samples should be higher and the products should be more easily detectable because hydrolysis is expected to be the main breakdown pathway for sea-dumped CWAs (Greenberg et al., 2016). Despite that cyclic degradation products of HD have been detected much more frequently in the Baltic Sea water samples than aliphatic hydrolysis products (Christensen et al., 2016; Söderström et al., 2018). Unlike other CWAs, HD and its degradation products have been detected only in a limited number of samples from the known dumpsites, and in small quantities (Greenberg et al., 2016). The undetectability might be related to the slow dissolution and formation of the polymeric crust (Munro et al., 1999). Aliphatic HD degradation products have been detected in sediment samples, not in pore water samples (Missiaen et al., 2010; Popiel et al., 2014; Söderström, 2014). Cyclic degradation products of HD have been found in both, sediment samples and pore water samples (Magnusson et al., 2016; Røen et al., 2010a; Söderström, 2014). The maximum levels of HD degradation products established in pore water samples during implementing the Baltic Sea-related research projects in the past decade have been quite low, 19 $\mu g\,L^{-1}$ for 1,4,5-oxadithiepane and 3.4 $\mu g\,L^{-1}$ for 1,2,5-trithiepane. However, much higher concentrations of five target analytes, 35–610 $\mu g\,kg^{-1}$, have been detected in sediment samples (Christensen et al., 2016). In the next decades, more and more chemical weapons will corrode, target compounds may leak into the sediments and even more into the seawater.

At present days, various analytical methods are being employed to analyze different degradation products of HD including mainly LC and GC using MS detection (Bełdowski et al., 2016; D'Agostino et al., 2004; Magnusson et al., 2016; Ohsawa et al., 2004; Pardasani et al., 2004; Røen et al., 2010a, 2010b; Östin, 2012). In addition to the above mentioned analytical techniques, also CE-DAD methods are being used for determination of some of HD degradation products (Cheicante et al., 1995a, 1995b; Jõul et al., 2015; Lees et al., 2017).

To analyze real environmental water samples, pretreatment for the concentration and purification of HD degradation products in water samples is an important part of the whole procedure. The techniques recommended by the Organisation for the Prohibition of Chemical Weapons for the preparation of CWA degradation products-containing samples are liquid-liquid, liquid-solid and solid-phase extractions (Vanninen, 2011). The degradation products of HD are non-ionic compounds and thus the widely-spread ion-exchange SPE phase for the determination of ionic nitrogen mustards degradation products, cannot be used (Kanaujia et al., 2008). Both cyclic and aliphatic compounds have been determined using solvent extraction and subsequent GC–MS, GC–MS/MS or LC–MS/MS analysis (Bełdowski et al., 2016). Cyclic degradation products have been determined using headspace extraction, the recoveries were up to 60–90% (Magnusson et al., 2016). Nawała et al. (2016) applied SPME fibers for the extraction of cyclic degradation products from environmental samples. D'Agostino et al. (2004) applied solvent extraction for the determination of hydrolysis products of HD in aqueous extracts of soil. Tomkins and Sega (2001) used two SPE columns in tandem to extract TDG from groundwater samples: the reversed-phase C18 removed extraneous interferences from the groundwater sample and a synthetic carbonaceous sorbent Ambersorb 572 column enabled extraction of TDG with the recovery not more than 40%. Boyer et al. (2004) extracted TDG from urine samples by using a reversed-phase Oasis HLB SPE cartridge and achieved a recovery of 28%. Leong et al. (1998) evaluated the adsorptive capacity of four carbonaceous sorbents towards four organosulfur compounds, including

TDG and 1,4-thioxane, in water samples. The results showed that the higher polarity of the adsorbates led to the decrease of the adsorptive capacity of sorbents (Leong et al., 1998).

Carbon aerogels (CAs), as an example of nanoporous materials, are sorbent materials composed of covalently bonded nanometer-sized particles that are arranged in a three-dimensional network (Meena et al., 2005). CAs have been used as sorbents for the removal of heavy metal ions from aqueous solutions (Meena et al., 2005). CA-based sorbents have a great analytical potential as an effective SPE sorbents because of their high porosity, very low density and a large specific surface area (Pérez-Caballero et al., 2008), while CAs doped with ferromagnetic metals could be applied as magnetic SPE sorbents (Kreek et al., 2014). Dong et al. (2015) applied CAs as sorbents for the analysis of plant growth regulators by employing SPME and magnetic SPE techniques.

Pérez-Caballero et al. (2008) obtained CAs by pyrolyzing organic aerogels, which were prepared by the sol-gel polymerization of 5-methylresorcinol and formaldehyde. Aerogels can be prepared at different molar ratios of 5-methylresorcinol to catalyst (MR/C) and water, and at different pyrolysis temperatures. At a pyrolysis temperature of 1000 °C and MR/C = 90 the obtained surface areas of CAs were the highest (specific surface area 408 m^2/g, microporous area 250 m^2/g) and low density (0.192 g/cm^3) (Pérez-Caballero et al., 2008).

To date, CA-based sorbents have not been used for the extraction of HD degradation products and no studies have been reported on the standard SPE extraction of as many as 10 cyclic and aliphatic degradation products of HD simultaneously at all.

In this paper, a SPE method using a CA-based sorbent was developed and evaluated for the simultaneous extraction of HD degradation products from environmental water samples. Altogether 10 HD degradation products were subjected to study, including aliphatic TDG, TDGO, TDGOO, 3,5-dithia-1,7-heptanediol (DTHD), 3,6-dithia-1,8-octanediol (DTOD), and cyclic 1,4-thioxane, 1,3-dithiolane, 1,4-dithiane, 1,2,5-trithiepane, and 1,4,5-oxadithiepane. Structures and chemical properties of target HD degradation products are listed in Supporting Information Table S1. The analytes extracted by the CA-based sorbent were analyzed by HPLC-DAD and CE-DAD (Jõul et al., 2015; Lees et al., 2017). Several parameters affecting the extraction efficiency, including the kind and volume of the eluting solvent, sample loading flow rate, volume and ionic strength as well as the reusability of the cartridge, were investigated and optimized to achieve the best performance for the target analytes. A series of quantitative parameters such as the linear range, coefficient of determination (R^2), LODs, LOQs and precision were examined under the optimized conditions. The performance of the CA-based sorbent was compared with that of commonly used SPE sorbents and the proposed method was successfully applied for the determination of HD degradation products in spiked real pore water samples collected from the Bornholm Basin, a chemical warfare dumping site in the Baltic Sea.

2. Materials and methods

2.1. Reagents and materials

TDG, TDGO, TDGOO, 1,2,5-trithiepane (99%) and 1,4,5-oxadithiepane (99%) were synthesized by Envilytix GmbH (Wiesbaden, Germany). 1,4-Thioxane (98% purity), 1,3-dithiolane (97%), 1,4-dithiane (97%) and DTHD were obtained from Sigma-Aldrich (Germany). Ethyl acetate, dichloromethane, acetonitrile (ACN) and methanol (MeOH) (HPLC grade ≥ 99.99%), boric acid, sodium hydroxide, hydrochloric acid, sinapinic acid and imidazole were purchased from Sigma-Aldrich (Germany). Phthalic anhydride and pyridine were purchased from Merck KGaA (Darmstadt, Germany). DTOD (97% purity) was purchased from Alfa Aesar (Germany).

All the chemicals were of analytical grade and were used as received. Deionized water from a Milli-Q water purification system (Millipore S. A. Molsheim, France) was used throughout the study. Stock solutions of the analytes at a concentration of 10 mM were prepared in Milli-Q water, the working solutions were prepared daily by an appropriate dilution of the stock solutions.

The individual solutions of the named standards and the background electrolyte solution were prepared by dissolving accurately the weighed amounts in Milli-Q water and stored at 4 °C.

Empty SPE tubes (polypropylene, tube volume 3 cm^3, Phenomenex), 20 μm polyethlene frits (20 μm, Phenomenex) and powdered organic CA MR/C = 90 (Pérez-Caballero et al., 2008) were used for the preparation of CA-based SPE cartridges.

For the comparative analysis of sorbents Superclean LC-18 500 mg (SUPELCO, Bellefonte, PA, USA), Chromabond NH_2 500 mg (Mecherey-Nagel GmbH & Co) and HyperSep Hypercarb 500 mg (Thermo Scientific) SPE cartridges were used.

All the samples were extracted using a 12-position Visiprep SPE Vacuum Manifold (Supelco).

2.2. Instrumentation and operating parameters

2.2.1. HPLC conditions

Analysis of cyclic degradation products of HD was performed on a HPLC-DAD system Agilent Technologies 1260 Infinity II (Agilent Technologies, Waldbronn, Germany). The separation was carried out on a ZOEBAX SB-C18 column (2.1 × 150 mm, 5 μm particle size, Agilent Technologies, Waldbronn, Germany) at a flow rate of 0.2 mL min^{-1} (isocratic water-ACN mobile phase, 1:1). The injection volume was 5 μL, column temperature 30 °C and detection wavelength 200 nm.

All chromatograms were recorded and integrated with OpenLAB CDS Chemstation Edition software.

2.2.2. CE-DAD conditions

An Agilent 3D CE-DAD instrument (Agilent Technologies, Waldbronn, Germany) was used for the separation of aliphatic degradation products of HD. Uncoated fused-silica capillaries (Polymicro Technologies, Phoenix, AZ, USA) with an ID of 50 μm and a length of 52/60 cm (effective length/total length) were employed in the experiments. A background electrolyte was 30 mM borate, pH = 8.90, 15 kV voltage, and sinapinic acid was used as an IS. The samples were injected hydrodynamically by applying a pressure of 50 mbar for 5 s and before each sample injection, the capillary was flushed with 1 M sodium hydroxide for 2 min, with Milli-Q water for 2 min and finally with the background electrolyte for 3 min. The separation process was monitored at 200 nm.

All electropherograms were recorded and integrated with Agilent ChemStation software.

2.3. Preparation of the CA-based SPE cartridge

The SPE packing material used in this study, CA MR/C = 90, was prepared according to the method described by Pérez-Caballero et al. (2008). The material was obtained by pyrolysing organic aerogels, which were prepared by sol-gel polymerisation of 5-methylresorcinol and formaldehyde. The carbonization was carried out in N_2 atmosphere by raising the temperature to 900 °C. The resulting monolithic material was powdered and sieved, the obtained particles had a diameter of 0.063–0.2 mm. 50 mg of the powdered CA was inserted in the empty SPE tubes between two polyethylene frits and after pressing it by a rod, the density of the

obtained sorbent was about 5.5 $mm^3\ mg^{-1}$. Before the first use the SPE column was rinsed with 3 mL MeOH and 3 mL Milli-Q water and then vacuum dried.

2.4. Sample preparation and analysis

Before each SPE operation, the cartridge was preconditioned with 3 mL MeOH and 3 mL Milli-Q water (1 mL min^{-1}). The optimized procedure for SPE is described as follows. 10 mL of sample (a real pore water sample or a sample spiked with all 10 analytes) was loaded into the cartridge at a flow rate of 2 mL min^{-1}. After the sample was passed through, the sorbent was rinsed with 2 mL Milli-Q water to remove potential interferences and kept in vacuum for 10 min to remove residual water. The analytes were eluted with 2 mL MeOH (1 mL min^{-1}) and then 1 mL of it was used for the CE analysis of aliphatic degradation products and the other 1 mL aliquot for the HPLC analysis of cyclic degradation products.

The analysis methods for the identification of the degradation products had been employed by our research group already previously. While the cyclic degradation products of HD can be analyzed instantly using HPLC-DAD, by injecting 5 μL of the eluate (Lees et al., 2017), in case of the aliphatic HD degradation products the 1 mL of the eluate was evaporated under a gentle airstream till dryness and 20 μL of the derivatizing mixture was added to the residue and heated at 45 °C for 20 min, after which the mixture was diluted with Milli-Q water in accordance with need. The derivatized sample was analyzed using the CE-DAD instrument, together with 50 μM sinapinic acid as IS (Jõul et al., 2015). The derivatizing mixture was prepared from phthalic anhydride (0.163 g mL^{-1}) and imidazole (0.025 g mL^{-1}) in pyridine according to the method described by Vanhoenacker et al. (2001).

To evaluate the efficiency of SPE, the extraction recoveries (R) of the target analytes were calculated according to the following equation:

$$R = \frac{C \times V}{C_0 \times V_0} \times 100\% \quad (1)$$

where C is the analyte concentration in the reconstituted solvent, C_0 is the initial concentration of analyte in the water sample, V and V_0 are the volumes of the reconstituted solvent and water sample, respectively.

2.5. Water samples

To verify the proposed method, the real sediment samples were collected from the Bornholm Basin, a chemical warfare dumping site in the Baltic Sea, during performing the project “Towards the Monitoring of Dumped Munitions Threat” (MODUM) expedition between March 12 and 16, 2016. The obtained sediment samples were centrifuged for 20 min at 8500 rpm, the pore water was collected and filtered. The pore water samples were analyzed and then spiked with target analytes to simulate real matrix conditions. For the optimization of the SPE procedure and evaluation of the analytical performance of the CA-based sorbent for HD degradation products, the spiked Milli-Q water was used.

3. Results and discussions

3.1. Optimization of SPE procedure

To evaluate the CA-based sorbent for the simultaneous extraction of HD degradation products from environmental water samples, several parameters affecting the extraction efficiency, including the kind and volume of the eluting solvent, sample loading flow rate, volume and ionic strength as well as the reusability of the cartridge, were investigated and optimized step by step to achieve the best performance for the target analytes. To avoid matrix effects and have a clear picture of the influence of each parameter, Milli-Q water was used as a sample matrix. All the optimization experiments were performed in triplicate and as described in Section 2.4, the recoveries were calculated according to Eq. (1). The applicability of CAs for the preconcentration and determination of HD degradation products was investigated using HPLC-DAD and CE-DAD (Section 2.2).

3.1.1. Effect of type and volume of the eluting solvent

After the analytes are adsorbed, desorption is a critical issue, especially if target molecules have very different properties. Cyclic degradation products of HD are hydrophobic molecules (logP values 0.112–3.012, estimated by ACD/Labs Software V11.02), but aliphatic HD degradation products are hydrophilic analytes rather. Consequently, to find a suitable solvent for elution, different factors must be considered. In the current study, four organic solvents (MeOH, dichloromethane, ACN and ethyl acetate) were tested as potential eluents. Due to the fact that HD degradation products are non-ionic compounds, different levels of the pH of eluent were not investigated. The elution of target compounds was carried out using 4.5 mL of the eluting solvent, while for sorbent conditioning the same type of solvent was used together with Milli-Q water. To evaluate the eluotropic strength of the solvents, 10 mL water samples spiked with aliphatic and cyclic HD degradation products at final concentrations of 10 and 5 μM, respectively, were investigated. The results demonstrated that only higher-polarity solvents MeOH and ACN gave high recoveries for the cyclic HD degradation products. At the same time, when dichloromethane was used as an eluent, the repeatable recoveries of the aliphatic degradation products of HD remained low, while the recoveries of TDG, TDGO and TDGOO were less than 30% when using ethyl acetate as an eluent. The results are shown in Fig. 1A and it is clearly evidenced that desorption using MeOH and ACN gave quite similar recoveries. However, when ACN was used, RSD values were higher for all the analytes. Considering the maximum recoveries and RSD values of all the target analytes obtained in parallel experiments, MeOH as a protonic polar solvent was selected as an eluent to be used in subsequent experiments.

The volume of the eluent was also optimized to achieve its best desorption performance. To this end, different volumes of MeOH (between 1.5 and 4.5 mL) were investigated and as shown in Fig. 1B, most of the analytes eluted with 1.5 mL of the eluting solvent, but the extraction recoveries still increased with the increase of the volume of MeOH. No more than 10% of the analytes desorbed after 1.5 mL of elution volume and after 3 mL of the elution volume no cyclic degradation products were detected. It should also take into account the further extract evaporation time for the aliphatic HD degradation products and compatibility with the HPLC-DAD instrument. 2 mL MeOH was selected as the eluting solvent in subsequent experiments because based on desorption optimization experiments, MeOH had the highest eluotropic strength toward the sorbent, which ensured the most successfully accomplished elution with minimum elution volume, maximizing thus the effect of SPE preconcentration.

3.1.2. Effect of sample loading flow rate

The effect of sample loading flow rate on the recoveries of 10 mL of sample containing 10 μM of aliphatic and 5 μM of cyclic HD degradation products was studied in its range of 0.5–5.0 mL min^{-1}. The effect of higher flow rates was not investigated due to the limitations of the SPE vacuum manifold. If the flow rate was high enough, the analysis time was reduced and therefore, unless the

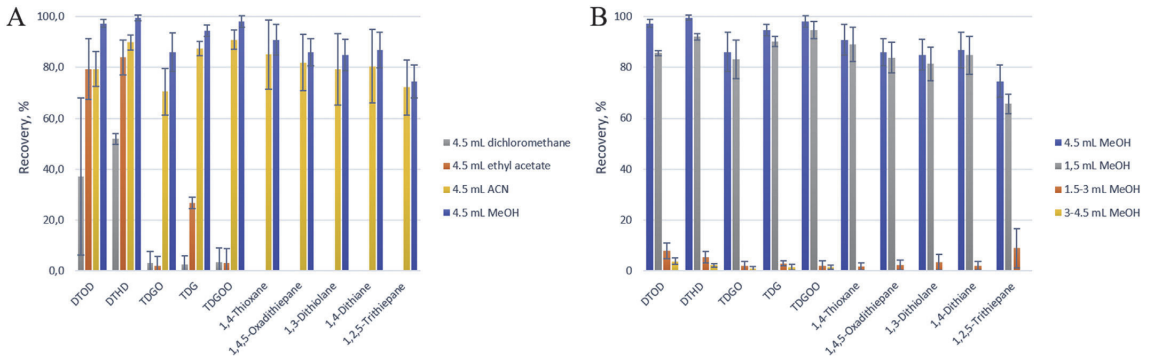

Fig. 1. The effect of eluting solvents (A) and volume of MeOH (B) on the recoveries of analytes.

sample volume was limited, the high rate allowed us to analyze larger sample volumes and samples which may degrade during the introduction into the column. The experimental results illustrated in Fig. 2A show that the sample loading flow rate significantly affected only the oxidation products of TDG, while at a flow rate higher than 1 mL min^{-1}, the extraction efficiency of TDGO and TDGOO was reduced. For all the other analytes, the recoveries did not decrease when the sample loading flow rate increased from 0.5 to 5.0 mL min^{-1}. For all the analytes the recovery higher than 70% was achieved at a flow rate of 2 mL min^{-1}, and this was selected as an optimal sample loading flow rate to achieve a satisfactory efficiency and save total time of the SPE procedure.

3.1.3. *Effect of sample volume*

To analyze as large volumes of samples as possible with satisfactory recoveries, five samples (5, 10, 25, 50 and 100 mL) were spiked with the same amount of each target analyte and all the samples were loaded into the cartridge at the same flow rate (2 mL min^{-1}) and eluted with 2 mL MeOH (1 mL min^{-1}). The obtained recoveries are shown in Fig. 2B. When the sample volume was 10 mL, the recoveries were higher than 70%, but with the sample volume increasing from 10 to 25 mL, the recoveries of TDGO and TDGOO decreased drastically – more than 10%. At the same time, for all the other degradation products of HD, the recoveries were higher than 84%, whereas no obvious variations were noticed

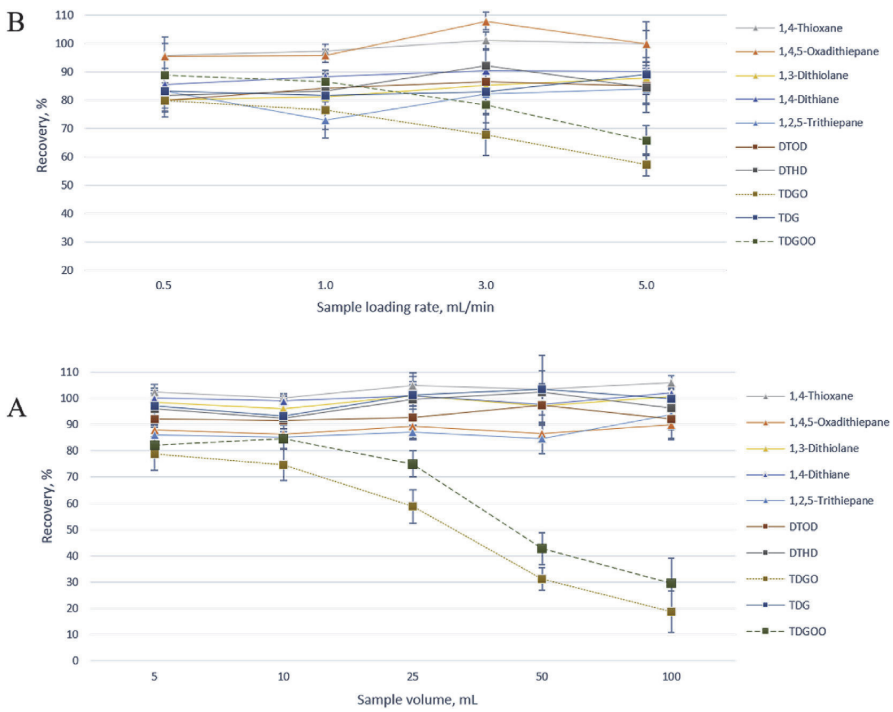

Fig. 2. The effect of sample loading flow rate (A) and sample volume (B) on the recoveries of HD degradation products.

when the sample volume increased from 5 to 100 mL. It was probably due to that the oxidation products of TDG did not interact with the sorbent strongly enough, which tendency was observed during the sample loading flow rate experiments as well. The low polarity of TDGO and TDGOO may lead to their weak hydrophobic interaction with the sorbent. To avoid the loss of target analytes, especially TDGO and TDGOO, 10 mL was chosen as an optimal volume of the water sample.

3.1.4. Effect of the NaCl concentration of the sample

Seawater is an example of environmental water that contains dissolved salts. This means that the ionic strength of its sample may influence the SPE procedure. Thus, the effect of the NaCl concentration of the water sample was evaluated. No obvious variations in the recoveries of analytes were observed at NaCl concentration in the sample between 0 and 0.25 M, which means that there was no need to adjust the sample ionic strength.

3.2. Analytical performance of the CA-based sorbent for sulfur mustard degradation products

Under the optimized conditions quantitative parameters such as linear range, R^2, LOD, LOQ, precision and reusability of the cartridge were examined. The developed CA-based SPE for the CE-DAD and HPLC-DAD determination of HD degradation products was evaluated using Milli-Q water spiked with all the analytes, the results are presented in Table 1. For the aliphatic compounds sinepinic acid as IS was used. All the experiments were performed in triplicate and the given values are averages. The LODs and LOQs of the analytes for the degradation products of HD were obtained experimentally by determining the S/N of 3 and 10, respectively. The calibration curves of cyclic HD degradation products were constructed by plotting the peak areas (y) versus concentration of the corresponding analytes (x). For the aliphatic compounds the peak area of the corresponding analyte was divided by that of IS (50 μM). The precision of the method was evaluated as intraday and interday precision by using spiked standard water solutions with 10 μM of target compounds. Intraday RSDs were calculated from six extractions carried out on the same day by using the same SPE cartridge ($n = 6$), while between the extractions the cartridge was air-dried. Interday RSDs were obtained using three individual CA-based SPE cartridges over three days, three extractions each day ($n = 9$). To investigate the reusability of the CA-based SPE cartridge, 10 mL water samples spiked with aliphatic and cyclic HD degradation products at final concentrations of 5 and 2 μM, respectively, were examined, whereas between the experiments the cartridge was dried for 10 min under vacuum. The results showed that the CA-based SPE cartridge could be reused at least 20 times with no significant variations of recoveries (SD ≤ 10%).

3.3. Comparison of the performances of CA-based and commercially available sorbents

The performance of the CA-based sorbent was compared with that of commonly used SPE sorbents – Superclean LC-18, Chromabond NH_2, and HyperSep Hypercarb cartridges.

All the sorbents were studied using 10 mL of Milli-Q water together with aliphatic and cyclic HD degradation products at their respective final concentrations of 10 and 5 μM under the optimized SPE procedure conditions for the CA-based sorbent (Section 2.4). The results are shown in Fig. 3.

Chromabond NH_2 is a weak anion exchanger, but the target HD degradation products having too high pKa values (pKa >13.0) were not ionizable in the given conditions of the SPE procedure, which means that Chromabond NH_2 could only act as a normal-phase sorbent. None of the target compounds were detected using the NH_2 sorbent in the fraction of elution. Analysis of both loading and washing fractions containing a high amount of target compounds revealed that the Chromabond NH_2 sorbent exhibited no sorption capacity towards the target compounds in the water matrix.

Superclean LC-18 is a reversed-phase SPE cartridge and since all target compounds exist in a neutral form, then especially the cyclic HD degradation products should exhibit a strong hydrophobic interaction with it in reversed-phase conditions. The results obtained using the Superclean LC-18 cartridges demonstrated that the recoveries of less polar analytes were satisfactory, higher than 85%, but for more polar analytes such as TDG, TDGO and TDGOO this reversed-phase sorbent could not be used.

The performance of HyperSep Hypercarb, which should retain highly polar compounds, was better than that of LC-18 for polar analytes, but was still only 11.7–24.8%. However, for less polar cyclic HD degradation products and DTOD and DTHD the recoveries were higher, ranging from 44.7 to 113.9%.

The recovery achieved by using the CA-based sorbent varied

Table 1
Analytical parameters for the proposed SPE method and recoveries of environmental water samples spiked with target analytes ($n = 3$).

Analyte	Linear range (μM)	R^2	LOD (μM)	LOQ (μM)	Precision Intraday	(RSD, %) Interday	Concentration added (μM)	Recovery (% ± SD)
TDG	1.25-20	0.994	0.32	1.07	3.7	8.5	2.5	99.7 ± 2.0
							10	96.7 ±2.3
TDGO	1.25-20	0.999	0.32	1.07	6.7	7.4	2.5	97.0 ±4.1
							10	83.5 ±2.9
TDGOO	1.25-20	0.999	0.32	1.07	2.6	3.9	2.5	97.4 ±4.0
							10	96.1 ±2.6
DTHD	1.25-20	0.998	0.32	1.07	7.7	9.9	2.5	99.4 ±3.4
							10	91.2 ±3.2
DTOD	1.25-20	0.999	0.32	1.07	4.3	8.7	2.5	95.3 ±2.8
							10	85.4 ±3.7
1,4 -Thioxane	1.0-20	0.999	0.17	0.57	2.0	2.7	1	91.3 ±4.0
							5	94.3 ±1.3
1,3-Dithiolane	1.0-20	0.999	0.17	0.57	2.4	8.9	1	94.3 ±8.4
							5	91.1 ±1.4
1,4-Dithiane	1.0-20	0.999	0.25	0.83	3.6	7.4	1	90.1 ±6.5
							5	95.4 ±2.6
1,2,5- Trithiepane	2.0-20	0.999	0.50	1.67	6.1	7.6	2	90.5 ±8.1
							5	90.7 ±5.3
1,4,5-Oxadithiepane	1.0-20	0.999	0.17	0.57	3.0	5.4	1	97.0 ±4.6
							5	90.0 ±2.4

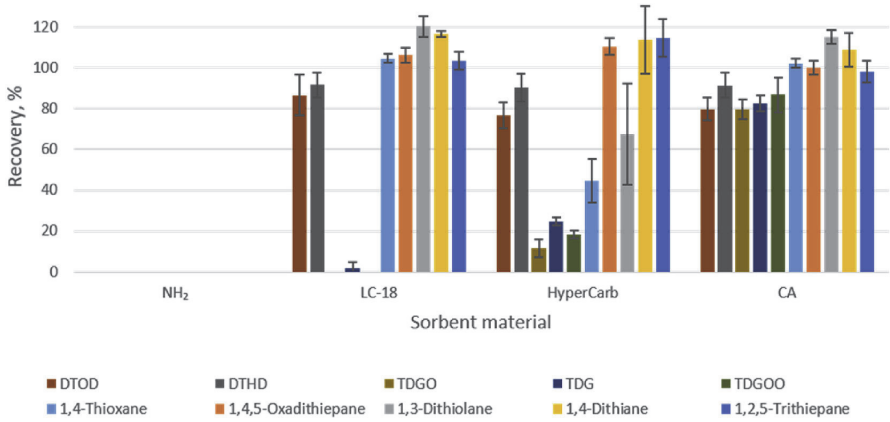

Fig. 3. Comparison of the performances of CA-based and commercially available sorbents for the SPE of HD degradation products under optimized conditions.

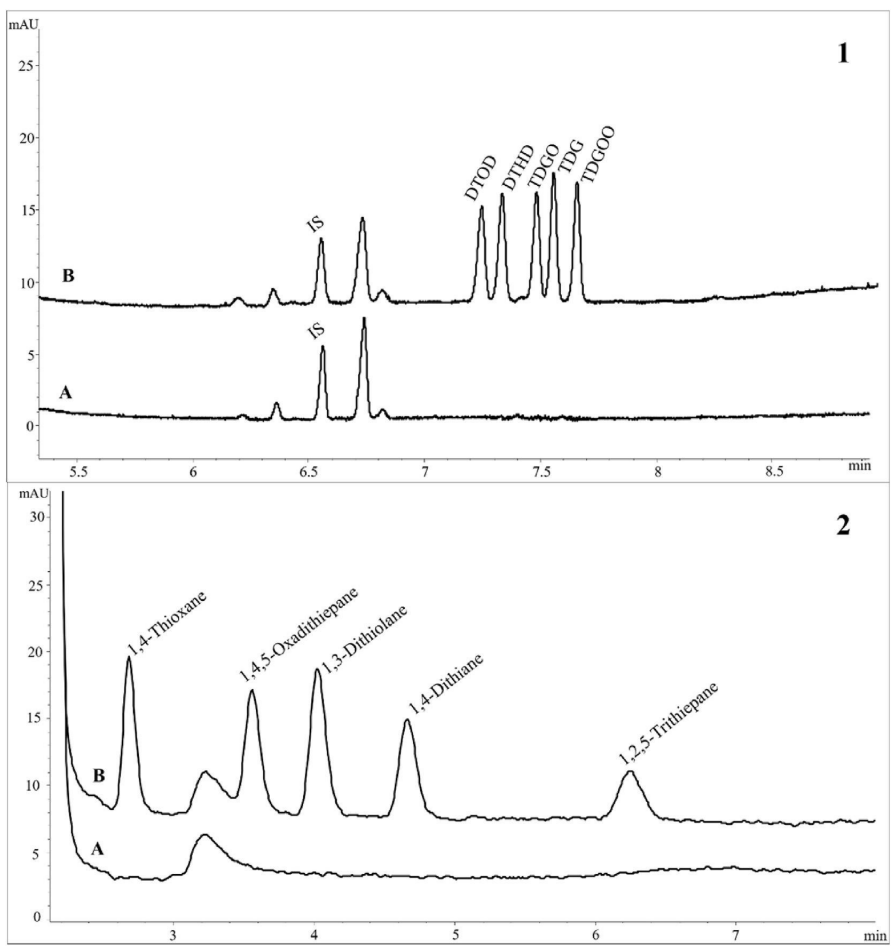

Fig. 4. Electropherograms (1) and chromatograms (2) obtained after applied all the sample pretreatment steps and HPLC/CE-DAD analysis: A) 10 mL pore water sample from the Bornholm Deep; B) 10 mL pore water sample from the Bornholm Deep spiked with aliphatic and cyclic HD degradation products at final concentrations of 10 and 5 μM, respectively (50 μM of IS for CE-DAD analysis).

between 79.8 and 115.1%, while the SD values were lower than 9%. These results demonstrated that it was possible to use the CA-based sorbent for analyzing the degradation products of HD with very different polarities (logP values range from −2.301 to 3.012, as estimated by ACD/Labs Software V11.02).

Comparison of the performances of the above sorbents indicated that to analyze all target HD degradation products simultaneously, the CA-based sorbent afforded the best overall recoveries. The high SPE performance of CAs can be explained by their nature as carbon-based nanomaterials and thus they can interact, through hydrophobic interactions, hydrogen bonding, π-π stacking and electrostatic and van der Waals forces, with molecules having various properties (Pyrzynska, 2013).

3.4. Analysis of sulfur mustard degradation products in environmental water samples

As the degradation products of HD have been found only in a few samples collected from the Bornholm Basin and at low concentrations (Christensen et al., 2016; Söderström et al., 2018), then in order to find out the applicability of the SPE method to analyzing real environmental water samples which may contain target analytes, the spiking approach was used.

The applicability of the optimized sample preparation method was evaluated using environmental water samples (Section 2.5). Each 10 mL sample was extracted (see Section 2.4) and analyzed by HPLC-DAD and CE-DAD (see Section 2.2). The blank sample subjected to analysis showed no traces of target HD degradation products. The environmental water samples spiked with analytes at two different concentrations were studied (Table 1). All the experiments with spiked samples were performed in triplicate. The example of the obtained electropherograms and chromatograms of unspiked environmental pore water samples and the same samples spiked with the analytes are presented in Fig. 4. From the figure it can be seen that possible interfering substances have been removed, which allowed us to achieve a clean baseline. Comparison of the electropherograms, chromatograms and recoveries of Milli-Q water, blank and spiked environmental pore water samples at two different concentrations, revealed no significant matrix effect. Furthermore, such sample pretreatment and analysis afforded us to obtain high recoveries of the compounds of interest. The extraction recoveries and SD of the spiked samples are presented in Table 1.

4. Conclusions

In this work, a method was developed for pretreatment and enrichment of five aliphatic and five cyclic degradation products of HD contained in environmental water samples using powdered CA as sorbent. The analysis of spiked real pore water samples showed that the developed SPE method using the CA-based sorbent was suitable for the simultaneous extraction of HD degradation products from environmental water samples. The obtained recoveries were at least 80% with the RSD values lower than 9%, while the extraction decreased matrix effects, as well as allowed a further analysis of target compounds. It was proved that the powdered CA was a very convenient sorbent to achieve high recoveries for both less and more polar degradation products of HD compared to other conventional SPE cartridges. The parameters of the developed method under optimized conditions were satisfactory, especially low LOD and LOQ values, high precision and reusability.

The results demonstrate that the CAs are suitable to be used as sorbents for SPE of degradation products of CWAs and possesses great potential for applying CAs also in SPME devices.

Conflicts of interest

The authors have declared no conflict of interest.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.chemosphere.2018.01.157.

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Carbon aerogel-based solid-phase microextraction coating for the analysis of organophosphorus pesticides†

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The current study is focused on the *in situ* synthesis of a carbon aerogel (CA)-based solid-phase microextraction (SPME) fiber coating on stainless steel wire and evaluation of the suitability of CAs as SPME coating materials for the analysis of selected organophosphorus pesticides (OPPs) contained in environmental samples. A CA-based coating was obtained by pyrolyzing organic aerogels, which were prepared by the sol–gel polymerization of formaldehyde and 5-methylresorcinol, an oil shale processing by-product. The results demonstrated, for the first time, the *in situ* synthesis of a CA-based SPME fiber coating on stainless steel wire and its suitability for the extraction and preconcentration of six OPPs. Main parameters affecting the extraction efficiency were investigated and optimized. The direct immersion (DI)-SPME procedure combined with gas chromatography-mass spectrometry (GC-MS) for the simultaneous analysis of selected OPPs was successfully applied to the efficient and sensitive determination of analytes of interest in environmental matrices of honey and natural water samples. The developed CA-coated SPME fiber showed good linearity (R^2 = 0.981–0.994), low detection limits (0.11–0.83 μg L^{-1}) and satisfactory single fiber and fiber-to-fiber reproducibilities (8.8–12.3%, n = 5 and 11.4–17.2%, n = 3). The performance of the CA-coating was compared with that of commercially available SPME fiber coatings.

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1. Introduction

Solid-phase microextraction (SPME) is an extraction and preconcentration technique, which was introduced by Arthur and Pawliszyn[1] to address the need for a rapid sample preparation method.[2] In this microextraction technique, a small amount of extracting phase on a small diameter fiber is exposed to the sample and after a defined period of time the analytes are partitioned into the stationary phase, which combines the processes of sampling, isolation and enrichment in a single step. Then the coated fiber is removed from the sample and the analytes are desorbed either by thermally directly into a gas chromatograph or using a desorption solvent.[1,6]

The main goal of sample pretreatment is to eliminate interfering compounds from the complex matrix by using a minimum number of steps, to give a reproducible methodology.[2] Being an easy-to-use method for analyzing compounds in complicated matrices and due to the miniaturized design of SPME fibers, it precisely serves the purpose. It enables the analysis of wine volatiles, *in vivo* analysis of pollutants, on-site analysis of soil and water samples and food analysis; in addition, it makes *in vitro* and *in vivo* metabolomic studies and pharmaceutical and biomedical analyses possible, and even allows for analyses without separation techniques, for example direct coupling of SPME to MS (mass spectrometry).[3] Moreover, this versatile and nonexhaustive sample preparation technique has been gaining wide interest, because in comparison to other sample extraction methods, including traditional solid-phase extraction (SPE), it is rather inexpensive due to the reduced consumption of high-purity solvents. As a result, the need for solvent deposal is diminished and implementation of Green Chemistry principles in analytical practice is supported.[3–5]

Affecting the efficiency, sensitivity and selectivity of extraction, the use of an appropriate SPME fiber coating for a particular application is highly important.[3] There are several kinds of commercial SPME fibers available, including polydimethylsiloxane (PDMS), polyacrylate (PA), polyethyleneglycol, divinylbenzene (DVB), DVB-PDMS, Carbopack Z-PDMS, DVB-CAR-PDMS, Carboxen (CAR)-PDMS and DVB-CAR-PDMS coated fibers.[7] However, despite their successful utilization in various applications, commercial SPME fibers have also some limitations in relation to operation temperature, selectivity, robustness, carryover, swelling in solvents, and poor affordability. Considering this the development of alternative SPME

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† Electronic supplementary information (ESI) available: SEM image of the surface of the uncoated stainless steel wire and CA-coated SPME fiber; the image of the prepared coated SPME fibers fixed by epoxy glue to a recycled commercial SPME fiber assembly; XPS C1s curve fitting, the quantification data, N_2 adsorption and desorption isotherms and DFT pore-size distribution of the CA-based SPME coating material. See DOI: 10.1039/d0ay02002h

coating materials presents a very active area of research.[3,4] Graphene, porous carbon, molecularly imprinted polymers, metal–organic frameworks, polymers, metal or metal oxide nanoparticles and ionic liquids are only a few examples of new coating materials, which have been already applied for the extraction of different sample matrices and compounds.[4,8,9] Among these materials, porous carbons have attracted a lot of attention. High surface area, physicochemical stability and density as well as well-developed pore structure make them suitable materials to be used as SPME fiber coatings, providing excellent sorption and enrichment capability.[8,10,11]

Carbon aerogels (CAs) are one of those porous carbon materials, which have great potential for use as sorbents in different sample preparation techniques, including SPME to achieve high selectivity, sensitivity and throughput of analysis of various compounds contained in complex matrices.[12,13] Aerogels are porous nanostructured materials whose production process involves three main stages: sol–gel preparation, aging and drying. CAs can be produced by an additional stage of organic aerogel production process, carbonization or pyrolysis.[12,14] Due to their well-developed porous structure and advantageous properties, CAs can be used in various applications, for example, preparation of biosensors,[15] storage of hydrogen,[16] preparation of catalyst supports,[17] electrodes and supercapacitors,[18,19] adsorbents,[20–22] *etc.* In the last few decades, interest in using CAs as sorbent materials in sample preparation techniques, especially SPE,[23,24] has been increasing.

CAs as SPE sorbents have been successfully applied for the simultaneous extraction of sulfur mustard degradation products from environmental water samples.[25,26] A CA-based SPE sorbent was obtained by pyrolyzing organic aerogels, which were prepared by the sol–gel polymerization of formaldehyde (FA) and 5-methylresorcinol (MR), an oil shale processing by-product.[12] Dong *et al.*[23] used CAs for the analysis of plant growth regulators by employing micro-SPE and magnetic SPE techniques, referred to as environmentally friendly methods. However, there have been but a few studies on the use of CAs as coating materials of SPME fibers.

In addition to using a suitable SPME coating material, the choice of a suitable coating support and method is similarly important. The most common supports are metal or fused silica ones, while dip coating, glue method, sol–gel and deposition outweigh other coating methods for carbon-based materials.[8]

Zhu *et al.*[27] were one of the first researchers to use CAs as SPME coating materials. The investigators compared the performance of two kinds of SPME fibers coated with porous carbon materials, namely CAs and wormhole-like mesoporous carbons, with that of a commercial PDMS fiber for the headspace solid-phase microextraction (HS-SPME) of four non-polar compounds and five polar compounds from water samples. Six types of fibers were prepared by three different coating methods using stainless steel wire as a supporting core. The results indicated the efficiency of CA-based fibers to be higher than those of other fibers in the extraction of both non-polar and polar analytes.[27] Zheng *et al.*[10] coated stainless steel wire with powdered CA by using the glue method and obtained a suitable fiber for the HS-SPME of hydrophobic analytes with one or two benzene rings from water samples. Compared to other fibers such as commercial PDMS and PDMS/DVB, as well as powdered polymer aerogel-coated fibers, the enrichment factors of target analytes obtained by HS-SPME-GC-FID using powdered CA-coated fibers were higher. By that, factors influencing the enrichment effect most included π–π interactions, van der Waals forces and hydrophobic interactions, as well as surface area and microporosity.[10]

Organophosphorus pesticides (OPPs) are widely used as insecticides in agriculture, but quite often, this use is related to poisoning cases. The abuse or erroneous application of pesticides, including highly persistent, bioaccumulative and biocidal OPPs, causes harm to the environment and contaminates foodstuff.[28] Thus, the development of a selective and sensitive method for the determination of OPPs in environmental water samples and foodstuff is highly required. The most widely applied analytical instruments for the determination of OPPs are gas chromatography (GC) and high-performance liquid chromatography (HPLC) coupled with various detectors, but the complex and diversity of environmental sample matrices make the direct analysis of pesticides very difficult, requiring, an appropriate sample pretreatment technique.[28,29] Honey is one of such matrices and OPPs in honey may originate from contaminated plants where bees collect nectar to make honey, or from pest- and disease-controlled beehives.[30] SPME is considered a suitable sample pretreatment technique for the extraction of OPPs from environmental samples, including honey and natural water.[29,31] For this purpose, the method uses both commercial[32–37] and lab-made SPME fibers.[38–42] However, to the best of the authors' knowledge, CAs have not been applied in SPME as fiber coating materials to analyze OPPs in complex sample matrices.

Hence, the current study is focused on the *in situ* synthesis of a CA-based SPME fiber coating on stainless steel wire, as well as evaluation of the applicability of coated fibers for the analysis of selected OPPs in environmental matrices, such as honey and natural water samples. The direct immersion solid-phase microextraction (DI-SPME) procedure coupled with gas chromatography-mass spectrometry (GC-MS) is optimized for the analysis of six OPPs: heptenophos, paraoxon-ethyl, tetrachlorvinphos, chlorfenvinphos, parathion and malathion. These OPPs were chosen for this study as model compounds based on their structural and thermal stability as well as volatility for the thermal desorption procedure. Moreover, these OPPs are the typical pesticides the residues of which are monitored in food/feed items and in the environment. The performance of novel SPME fibers is compared with that of the most common commercial SPME fibers.

2. Experimental

2.1 Reagents and materials

Organophosphorus pesticides heptenophos, paraoxon-ethyl, tetrachlorvinphos, chlorfenvinphos, parathion and malathion, and chemical compounds sodium chloride (NaCl) and sodium hydroxide (NaOH) were purchased from Sigma-Aldrich (Germany). Pure OPP standards were refrigerated in their original

packages at 4 °C. Methanol and acetonitrile (HPLC grade ≥ 99.9%) were purchased from Sigma-Aldrich (Germany). All the chemicals were of analytical grade and were used as received. Deionized water from a Milli-Q water purification system (Millipore S. A. Molsheim, France) was used throughout the study.

For the comparative analysis of SPME fibers with 65 μm PDMS/DVB, 50/30 μm DVB/CAR/PDMS, 85 μm CAR/PDMS and 85 μm PA coatings were purchased from Supelco (Bellefonte, PA, USA).

MR with a reported purity of >99% was provided by AS VKG (Estonia). The FA solution (37 wt% in H_2O) and sodium carbonate (Na_2CO_3) powder (≥99.0%) were purchased from Sigma-Aldrich (Germany). Stainless steel wire 200 μm thick and epoxy glue were purchased from a local hardware store. Tubings with ID of 395 μm were purchased from IDEX Health & Science LLC (USA).

2.2 Sample preparation

The stock solutions of OPP standards were prepared with MeOH, then stored in the dark and refrigerated at −20 °C. The working solutions were prepared daily by appropriate dilution of the stock solutions and stored at 4 °C.

Natural water samples were taken from the Pirita River and the Tallinn Bay, Baltic Sea (Estonia), spiked and analyzed directly by DI-SPME-GC-MS. Honey samples were obtained from local beekeepers. In order to study the honey matrix effect and the recovery of the DI-SPME-GC-MS analysis procedure, the samples were spiked with a certain amount of each pesticide standard to the 0.5 mL aliquot of the honey–water mixture (1 mL of the sample was dissolved in 1 mL of MilliQ water), mixed at least for 30 min and then analyzed following the above DI-SPME-GC-MS procedure. Before spiking the samples were checked for any kind of target analytes. The concentrations of OPPs for the spiked samples, as well as optimization and calibration standards were carefully selected to ensure adequate sensitivity to all the analytes.

2.3 Preparation of carbon aerogel-based solid-phase microextraction fibers

The CA-based coating was *in situ* synthesized on stainless steel wire. Before the coating procedure, 200 μm stainless steel wire was cut to pieces, each 1.5 cm in length. The wire pieces were immersed in acetone for 30 min, then in 1 M NaOH for 30 min and finally washed with MilliQ water for 30 min. Thereafter they were taken out and dried at room temperature.

After that the stainless steel wire pieces were inserted into empty 1.7 cm tubings (ID 0.395 mm) and filled with the initial mixture, a fresh water solution of MR, Na_2CO_3 and FA (molar ratios MR/Na_2CO_3 = 90, water/MR = 45 and MR/FA = 0.5) prepared according to the literature.[12] Then both ends of the tubings were closed with Parafilm and the filled tubings were kept overnight at room temperature for proper gelation. After gelation, both ends of the tubings were opened and the filled tubings were placed in acetone. Acetone was changed every 24 hours in the course of four days. To obtain organic aerogel-coated metal wires, the gel inside the tubes had to be dried with supercritical CO_2. Thus, at first, acetone in the gel pores was replaced by liquid CO_2 (120 bar, 25 °C for 1 h) and then subjected to supercritical drying (100 bar, 45 °C for 1.5 h). For this purpose a supercritical extraction system including a 100 mL double-clamp autoclave (NWA Analytische Meßgeräte GmbH, Germany) was used. After the supercritical drying the wires coated with organic aerogel were removed from the tubings.

In the next step, the CA-coated wires were fabricated by pyrolysing organic aerogel-coated wires. The pyrolysis was carried out in a N_2 atmosphere by using an MTF 12/38/400 pyrolysis oven (Carbolite, England). The carbonization temperature program of the oven was as follows. The initial oven temperature was 25 °C, then the temperature was increased to 300 °C at a rate of 10 °C min^{-1} (held for 10 min), thereafter increased to 550 °C at 10 °C min^{-1} (held for 10 min) and finally increased to 900 °C at 10 °C min^{-1} (held for 1 h). After pyrolysis, the furnace was allowed to cool down to room temperature in a N_2 atmosphere. The prepared coated SPME fiber was fixed with epoxy glue to a commercial fiber assembly, after removing the original core from the inner tube (Fig. S1 and S2, ESI†).

2.4 DI-SPME procedure

The CA-based fiber assembly was attached to the manual SPME holder and conditioned in the injection port of the gas chromatograph at 300 °C for 30 min prior to use. Commercial fibers were conditioned according to the manufacturer's instructions. The CA-coated SPME fibers were preconditioned using a MeOH : MilliQ water solution (1 : 1, v/v) for 5 min. For the DI-SPME procedure the coated fiber protected in the septum piercing needle was inserted into the sample vial, then pushed out from the needle and immersed into the sample solution (0.5 mL) for 20 min at room temperature. Both procedures, preconditioning and sorption, were carried out using an ELMI DOS-20M Digital Orbital Shaker (agitation 200 rpm). Before desorption, the fiber was rinsed with MilliQ water for 5 s to remove possible interfering matrix components. After that the fiber was withdrawn from the needle and inserted into the GC injection port where the analytes were thermally desorbed for the GC-MS analysis.

2.5 GC-MS analysis

Chromatographic separations were performed on an Agilent Technologies 7890A GC system equipped with an ultra inert splitless liner (Agilent Technologies, type 5190-2293) to eliminate matrix-induced chromatographic response enhancement. The gas chromatograph was coupled to an Agilent 5975C mass spectrometer with an electron ionization source and a quadrupole mass analyzer. Instrument control was carried out with MassHunter Acquisition software. The separation of pesticides from the samples was performed on a ZB-5MSi capillary column (30 m × ID 0.25 mm, film thickness 0.25 μm, Agilent Technologies). Helium (6.0 purity) was used as a carrier gas at a constant flow rate of 1.3 mL min^{-1}. Sample injection was performed in splitless mode at 275 °C for 2.0 min (an optimized value for SPME thermal desorption). The oven temperature programming was performed as follows. The initial temperature, 60 °C,

was held for 1 min, then increased to 180 °C at a rate of 25 °C min^{-1}, held for 2 min and finally increased to 250 °C at a rate of 15 °C min^{-1} and held for 2.5 min. The total analysis time was 15 min starting from fiber introduction into the injector block.

The analyte ionization was performed in electron ionization mode using the electron energy of 70 eV. The temperatures of the interface, ion source and mass analyzer were set at 280, 230 and 150 °C, respectively. All analytes were monitored using selected ion monitoring (SIM) mode. Chromatographic peaks were identified by measuring the retention times of individual OPP standards and confirmed by the fragmentation pattern of molecules and database results (NIST17). Quantitative analysis was carried out in SIM mode using a specific quantifying ion for each target analyte (Table 1). Data treatment was performed with Agilent MassHunter Workstation software.

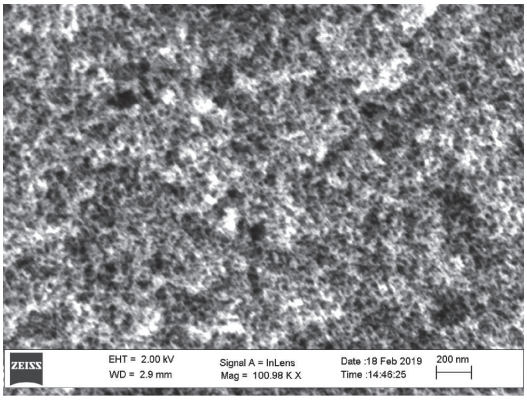

Fig. 1 SEM image of the surface of the CA-coated SPME fiber.

3. Results and discussion

3.1 Characterization of the coating material

The surface morphology of the CA-coated SPME fiber was examined with a high resolution scanning electron microscope (HR-SEM Zeiss Merlin). The SEM image of the surface of the obtained carbon coated fiber is shown in Fig. 1. As can be seen, the fiber surface looks like a solid shell with evenly distributed pores. The morphology of the cross-section of the obtained fiber was similar to that of the fiber surface.

The nitrogen adsorption–desorption isotherms of CA were recorded using a Quantachrome Autosorb iQ-C instrument. The results of the pore size distribution analysis, which was based on the density functional theory (DFT), showed that the fiber coating contained mainly micropores, diameter < 2 nm according to IUPAC (Fig. S4 and S5, ESI†). The thickness of the coating was about 20 μm, which varied between different fibers insignificantly. The specific surface area obtained by Brunauer–Emmett–Teller (BET) analysis was 501.157 m^2 g^{-1}, giving evidence of the formation of a large adsorption area.

It is known that carbon-based sorbent/analyte interactions involve more than one adsorption mechanism.[10,43] During the pyrolysis of organic aerogels most of the hydrophilic functional groups, such as C═O, C–O, O–H and C–H, are eliminated under the influence of high temperature, as a result of which a relatively highly hydrophobic material is formed. The X-ray photoelectron spectroscopy (XPS, Kratos AXIS Ultra DLD) analysis of the obtained coating material indicated the dominance of carbon atoms in the sp^2 (66.8%) and sp^3 (12.90%) hybridization as well as a minor presence of C–O (8.52%), C═O (4.05%) and –O– (2.08%) groups (Fig. S3 and Table S1, ESI†). Taking into account the nonpolar and hydrophobic nature of both interacting sides, we assume that hydrophobic interactions play one of the most important roles in the sorption process. Moreover, due to the existence of conjugated systems in the analytes under study and sp^2 carbon, π–π interactions (π-stacking as well as π donor–acceptor interactions) may be considered as another strong supramolecular force responsible for the mechanism of sorption. In addition, a considerable amount of C–O, C═O and other oxygen functionalities on the fiber surface allows the occurrence of dipole–dipole interactions between the phosphorus atom of pesticides and the electronegative oxygen of the sorbent.

3.2 Optimization of extraction conditions

To achieve the highest extraction efficiency of the lab-made CA-based fiber, optimization of SPME conditions such as extraction time, desorption time, desorption temperature and salt concentration was necessary. All the experiments were performed in triplicate with 0.5 mL water samples spiked with target OPPs at a final concentration of 2.5 mg L^{-1} heptenophos, 2.8 mg L^{-1} paraoxon-ethyl, 3.3 mg L^{-1} malathion, 2.9 mg L^{-1} parathion, 2.6 mg L^{-1} chlorfenvinphos, and 3.7 mg L^{-1} tetrachlorvinphos.

Table 1 Analytical performance of the developed DI-SPME-GC-MS method in the analysis of selected OPPs

Pesticide	Quantitation ion, *m/z*	Linearity, μg L^{-1}	R^2	LOD, μg L^{-1}	LOQ, μg L^{-1}	RSD single fiber ($n = 5$), %	RSD fiber-to-fiber ($n = 3$), %
Heptenophos	215.0	0.50–62.7	0.981	0.15	0.50	9.3	11.4
Paraoxon-ethyl	275.0	2.8–68.8	0.985	0.83	2.8	10.3	17.2
Malathion	173.0	0.66–165.2	0.990	0.20	0.66	8.8	16.5
Parathion	291.0	0.58–145.6	0.994	0.18	0.58	10.1	14.9
Chlorfenvinphos	323.0	0.42–103.8	0.990	0.12	0.42	12.3	14.8
Tetrachlorvinphos	331.0	0.37–91.5	0.983	0.11	0.37	9.6	17.1

3.2.1 Extraction time (sorption time). Extraction time needs to be optimized because before reaching the solution-fiber equilibrium, the efficiency of extraction increases and the reproducibility of analysis results may be poor (higher standard deviations). The extraction time was varied from 5 min to 60 min to determine its effect on extraction efficiency. All the experiments were performed at room temperature 23 ± 2 °C. As shown in Fig. 2a, 20 min proved to be the most efficient time to extract most pesticides. No significant changes in the response of target compounds were observed after that time. So, the extraction time of 20 min was used for all further experiments.

3.2.2 Desorption conditions (temperature and time). The effect of desorption temperature on extraction efficiency was investigated by varying the temperature of the GC injection port. According to Le Chatelier's principle, temperature is one of the parameters affecting the fiber coating-gas phase equilibrium. The right desorption temperature does not influence the structure of analytes, however, the temperature has to be high enough to ensure that all the analytes would not condense onto the injector walls and would reach the separation column without losses. Based on the physicochemical properties of target analytes, the temperature was studied in its range of 200–350 °C (Fig. 2b).

The desorption time at optimal temperature should be as short as possible to decrease the analysis time. However, a very short time may lead to a carryover effect and may not enable a complete transfer of pesticides by the carrier gas to the separation column. The desorption time of 0.5–3 min was used to investigate the desorption of analytes from the CA-coated SPME fiber to the gas phase (Fig. 2c). The experimental results demonstrated that the efficiency of pesticide extraction was the highest, at a temperature of 275 °C during 2.0 min with their complete desorption. Moreover, no carry over effect of pesticides was observed, which allowed the fiber to be used repeatedly.

3.2.3 Ionic strength. The high ionic strength of water samples caused by the salting-out effect may pose a problem during their analysis. It may decrease the solubility of analytes in an aqueous solution and thus affect their sorption on the fiber coating. To elucidate which effect ionic strength may exert on the efficiency of pesticide extraction in the current DI-SPME procedure, NaCl at various concentrations (0–10%, w/v) was added to the water samples spiked with pesticides of similar concentrations, to increase the ionic strength of the samples. The experiments showed (Fig. 2d) that the extraction efficiency of analytes remained practically unchanged during the above analysis and extra salt was not added to the sample.

3.3 Performance of DI-SPME-GC-MS under optimal extraction conditions

Based on the results of experiments, the extraction time of 20 min, desorption temperature of 275 °C, desorption time of 2.0 min proved to be the most optimal for DI-SPME. The effect of the preconcentration of the CA-coated fiber on pesticides extraction under optimized conditions was investigated using a solution of target OPPs (1.3 mg L^{-1} heptenophos, 1.4 mg L^{-1} paraoxon-ethyl, 1.7 mg L^{-1} malathion, 1.5 mg L^{-1} parathion, 1.3 mg L^{-1} chlorfenvinphos, and 1.8 mg L^{-1} tetrachlorvinphos). Fig. 3 compares two extracted ion chromatograms obtained by DI-SPME-GC-MS of water samples spiked with OPPs at the final concentration of 1.3 mg L^{-1} heptenophos (1), 1.4 mg L^{-1} paraoxon-ethyl (2), 1.7 mg L^{-1} malathion (3), 1.5 mg L^{-1} parathion (4), 1.3 mg L^{-1} chlorfenvinphos-exists in two isometric forms (5), and 1.8 mg L^{-1} tetrachlorvinphos (6) (colored) and injecting the test solution, the same concentration of target

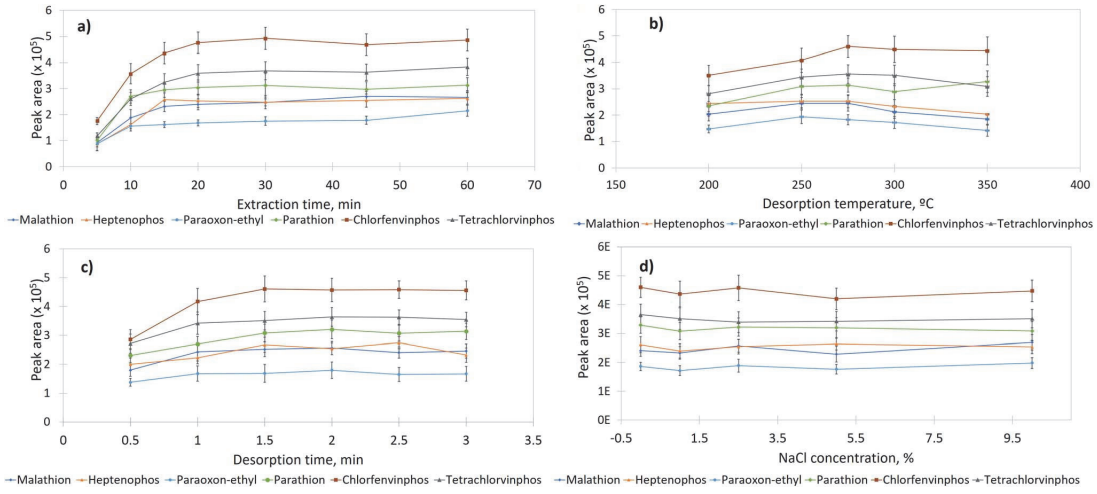

Fig. 2 The effect of different factors on SPME efficiency. (a) Extraction time, (b) desorption temperature, (c) desorption time, (d) NaCl concentration. The concentration of OPPs in 0.5 mL of working solution, 2.5 mg L^{-1} heptenophos, 2.7 mg L^{-1} paraoxon-ethyl, 3.7 mg L^{-1} tetrachlorvinphos, 2.6 mg L^{-1} chlorfenvinphos, 2.9 mg L^{-1} parathion and 3.3 mg L^{-1} malathion.

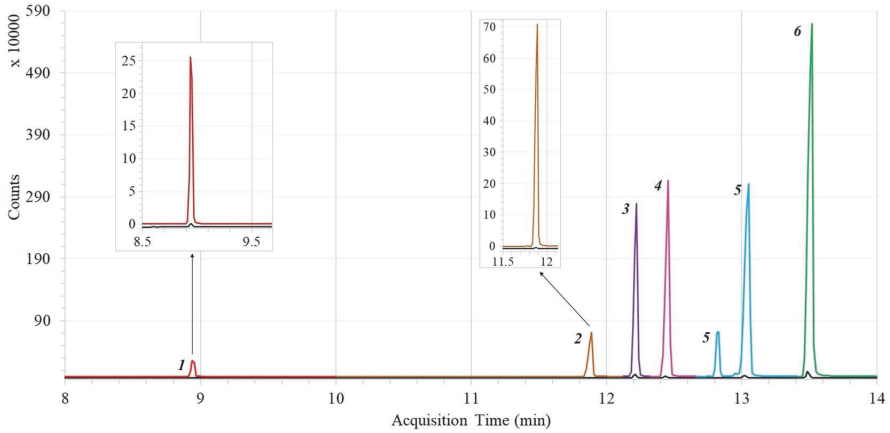

Fig. 3 Extracted ion chromatograms of DI-SPME-GC-MS of water sample spiked with OPPs at the final concentration of 1.3 mg L^{-1} heptenophos (1), 1.4 mg L^{-1} paraoxon-ethyl (2), 1.7 mg L^{-1} malathion (3), 1.5 mg L^{-1} parathion (4), 1.3 mg L^{-1} chlorfenvinphos exists in two isometric forms (5), and 1.8 mg L^{-1} tetrachlorvinphos (6) (colored) under optimum extraction conditions and of GC-MS of the same concentration of target OPPs, as in the spiked water sample, in acetonitrile (black).

OPPs as in the spiked water sample, in acetonitrile, into GC-MS without the DI-SPME procedure (black).

In the latter case, the recommended maximum volume of solution to be injected was 2 μL to avoid the carryover effect. As seen from Fig. 3, the coating material has a significant binding affinity for all target analytes, demonstrating its ability towards preconcentration of analytes and improvement of the method sensitivity.

3.4 Evaluation of the performance of DI-SPME-GC-MS

The analytical performance of the DI-SPME-GC-MS method using the CA-coated fiber was evaluated under optimized extraction conditions and validated using MilliQ water samples spiked with target pesticide standards. For this purpose, the chromatographic peak area of each pesticide was plotted against the corresponding concentration. The linearity, coefficients of determination (R^2), limits of detection (LODs), limits of quantification (LOQs) and repeatability as relative standard deviation (RSD) of single fiber and fiber-to-fiber were determined. As revealed by Table 1, for all pesticides the linearity R^2 ranged from 0.981 to 0.994. LODs based on the signal-to-noise ratio of three (S/N = 3) for the analytes varied from 0.11 to 0.83 μg L^{-1}, and LOQs (S/N = 10) of the method were in the range of 0.37–2.8 μg L^{-1}. Under the optimized conditions of DI-SPME-GC-MS, RSDs varied from 8.8 to 12.3%, while all the replicate experiments were performed using a single fiber (n = 5). The repeatability for the three CA-coated SPME fibers was in the range of 11.4–17.2%. The thermal and solvent stabilities of the coating were determined as well. The experiments showed that the same fiber could be used at least in 80 sorption/desorption cycles with no significant decrease in extraction efficiency. Nevertheless, the fragile coating material is prone to damage and requires caution in handling. The obtained results show that the CA-based fiber coating provides satisfactory accuracy and precision of extraction and is suitable to be used for sample pretreatment and further GC-MS analysis of target OPPs.

3.5 Functionality of the CA-based coating on real samples

To evaluate the applicability of the CA-based fiber coating for the analysis of selected OPPs in actual environmental samples, the novel DI-SPME-GC-MS analysis method was applied to the determination of six OPPs in three different samples. Two natural water samples were collected from the Pirita River and

Table 2 Recovery of real samples spiked with OPPs

		Recovery, %		
Pesticide	Spiked concentration, μg L^{-1}	River water	Seawater	Honey
Heptenophos	2.5	82.3	96.1	82.5
Paraoxon-ethyl	13.8	85.8	91.8	86.6
Malathion	6.6	83.6	85.1	84.9
Parathion	1.5	85.4	83.9	87.0
Chlorfenvinphos	20.8	89.8	81.8	88.1
Tetrachlorvinphos	1.8	88.6	92.9	85.7

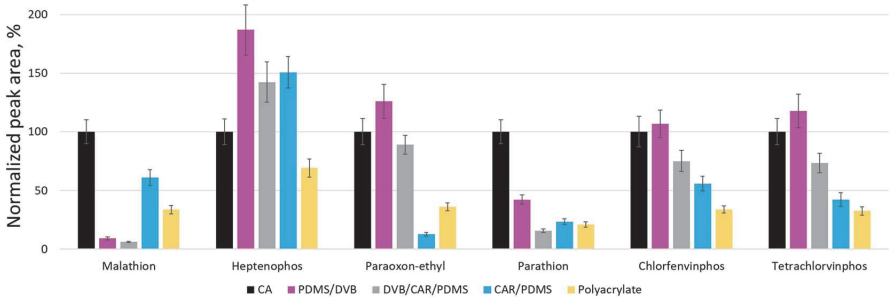

Fig. 4 Extraction of OPPs from water samples using different SPME fibers.

the Tallinn Bay, Baltic Sea (Estonia) and their recoveries were measured by spiking the samples with the initial concentrations of OPPs, as listed in Table 2. The recoveries recorded were in the range of 81.8–96.1% ($n = 3$, RSD < 14%). Another promising application of the developed method, which was tested to demonstrate the fiber coating applicability, was the analysis of OPPs in a more complex sample matrix, honey. In which case the recovery was 82.5–88.1%, $n = 3$, and RSD < 13%. The recoveries of the spiked natural water and honey samples are given in Table 2. All the blank samples subjected to analysis showed no traces of target OPPs. The results of analysis of real samples demonstrated that the CA-coated fiber was suitable for this kind of extraction and preconcentration. No such matrix effect in the determination OPPs in natural water and honey samples was observed. However, further matrix-matched calibration experiments will be required to estimate the applicability of the novel CA-based DI-SPME-GC-MS method to the analysis of other complex food and environmental samples.

3.6 Comparison of CA-coated and commercial fibers

The extraction performance of CA-coated fibers in relation to six OPPs was compared with that of four commercial fibers (65 μm PDMS/DVB, 50/30 μm DVB/CAR/PDMS, 85 μm CAR/PDMS, 85 μm PA) under the optimized extraction conditions. The normalized peak areas obtained using the CA-coated and commercial fibers are shown in Fig. 4. The figure displays that the ability of CA-coated fibers to extract most target OPPs was similar to or even higher than that of commercial fibers. However, in the case of parathion and malathion, the extraction performance of CA-coated fibers was significantly higher compared to commercial ones. At the same time, commercial CAR-coated fibers exhibited a higher capacity to extract one of the pesticides, heptenophos. The high binding affinity of CA-coated SPME fibers for target OPPs may be due to the high surface area and porosity of CA, as well as strong π–π interactions and other sorption mechanisms typical of carbon-based materials.

4. Conclusions

The results obtained verify the suitability of wire-supported CAs as SPME fiber coatings for the extraction and preconcentration of six OPPs. The DI-SPME-GC-MS method was successfully applied to the simultaneous determination of analytes of interest in complex environmental matrices such as honey and natural water samples. The extraction efficiency of CA-based SPME fibers was comparable to that of commercial ones, being in some cases even higher. The current study gives evidence of the high analytical potential of this type of coating procedure and sorbents for microextraction techniques and micro total analysis systems of samples of interest. Further studies of CA-based sorbents in microextraction techniques are in progress, including the improvements of coating durability and some additional aspects affecting the extraction process and applicability.

Conflicts of interest

There are no conflicts to declare.

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