LAPPEENRANTA UNIVERSITY OF TECHNOLOGY
Faculty of Technology
Degree Program in Environmental Technology

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DETERMINATION OF CHLOROPHENOLS FROM WATER BY SOLID PHASE MICROEXTRACTION – ION MOBILITY SPECTROMETRY (SPME – IMS)

Examiners: Professor, D.Sc. (Tech.) Mika Sillanpää
Lic.Sc. (Tech.) Simo Hammo

Supervisor: M.Sc. Sanna Holopainen

ABSTRACT

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Determination of chlorophenols from water by solid phase microextraction – ion mobility spectrometry (SPME-IMS)

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Chlorophenols have been classified as possible carcinogens for humans. Chlorophenols have been used as pesticides and wood preservatives. In Finland, during 1930 – 1980s, saw mills used KY-5 wood preservative that contained 2,4,6-TCP, 2,3,4,6-TeCP and PCP. Especially in Finland chlorophenols have entered the environment by leaking from contaminated grounds of old saw mills. Although chlorophenol concentrations found in environment do not cause acute concern, long term exposure can increase the risk of cancer.

SPME is relatively cheap and simple sampling method, in which the sample extraction and concentration are performed in a single step. Solvents are not required in SPME. IMS is based on the detection of sample ion drift times. Based on the drift times, reduced mobilities are calculated, which are comparable despite the measurement conditions. SPME-IMS coupling has not been used earlier in the determination of chlorophenols from water samples.

The scope of this work was to study, if SPME-IMS system is suitable for detecting chlorophenols from water samples. The aim was to determine the most optimal extraction conditions, which were then applied to real water samples. Following detection limits were determined: 2,4,6-TCP: 0.33 mg/l; 2,3,4,6-TeCP: 0.63 mg/l and PCP: 1.63 mg/l. Detection limits were high compared to the highest possible chlorophenol concentration that is allowed in Finnish drinking water, 10 µg/l. Detected concentrations from water sample differed from verified concentrations in the case of 2,3,4,6-TeCP by 4.6 % and in the case of 2,4,6-TCP by 48.4 %. Based on the results it can be said that SPME-IMS setup is suitable for preliminary analysis of mg/l chlorophenol concentrations from water samples.

TIIVISTELMÄ

Lappeenrannan teknillinen yliopisto
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Ympäristötekniikan koulutusohjelma

Ville Luukkonen

Kloorifenolien määritys vedestä käyttäen kiinteäfaasimikrouuttoa ja ioniliikkuvuus-spektrometriaa (SPME-IMS)

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Kloorifenolit on luokiteltu ihmisille mahdollisiksi karsinogeeneiksi. Kloorifenoleja on käytetty muun muassa torjunta- ja puunsuoja-aineissa. Suomessa sahat käyttivät vuosien 1930 – 1980 aikana KY-5–puunsuoja-ainetta, joka sisälsi 2,4,6-trikloorifenolia (2,4,6-TCP), 2,3,4,6-tetrakloorifenolia (2,3,4,6-TeCP) sekä pentakloorifenolia (PCP). Kloorifenoleita on päässyt vesiin käytöstä poistettujen saha-alueiden pilaantuneiden maiden kautta. Vaikka löydetyt kloorifenolipitoisuudet eivät aiheuta akuuttia vaaraa, saattaa pitkäaikainen altistus aiheuttaa syöpää.

Kiinteäfaasimikrouutto (SPME) on suhteellisen halpa ja yksinkertainen näytteenottomenetelmä, jossa uutto ja näytteen konsentrointi tapahtuvat yhdellä kertaa. Kiinteäfaasimikrouutossa ei myöskään tarvita liuottimia. Ioniliikkuvuusspektrometria (IMS) perustuu ionimuotoisen näytteen kulkeutuma-aikojen tunnistamiseen. Kulkeutuma-aikojen perusteella näytteelle lasketaan redusoitu liikkuvuus, joka on vertailukelpoinen mittausolosuhteista riippumatta. SPME-IMS–yhdistelmää ei ole aiemmin käytetty kloorifenolien tutkimiseen vesinäytteistä.

Tämän diplomityön tarkoituksena oli tutkia, kuinka SPME-IMS-yhdistelmä soveltuu kloorifenolien määrittämiseen vesinäytteistä. Tavoitteena oli määrittää parhaat mahdolliset uuttoolosuhteet, jonka jälkeen menetelmää sovellettiin oikeisiin vesinäytteisiin. Kloorifenoleille saatiin määritettyä seuraavat toteamisrajat: 2,4,6-TCP:lle 0,33 mg/l, 2,3,4,6-TeCP:lle 0,63 mg/l ja PCP:lle 1,63 mg/l. Toteamisrajat ovat korkeat verrattuna suurimpaan juomavedessä sallittuun kokonaiskloorifenolipitoisuuteen, joka on 10 µg/l. Vesinäytteessä havaitut pitoisuudet erosivat 2,3,4,6-TeCP:n osalta 4,6 % ja 2,4,6-TCP:n osalta 48,4 % varmistetuista pitoisuuksista. Tutkimustulosten perusteella voidaan sanoa SPME-IMS-yhdistelmän soveltuvan alustavaan, mg/l-kloorifenolipitoisuuksien analysoimiseen vesinäytteistä.

ALKUSANAT

Näitä sanoja kirjoittaessani opintoni alkavat olla loppusuoralla. Viisi vuotta on kulunut mahdottoman nopeasti ja tuntuukin lähes epätodelliselta kirjoittaa näitä alkusanoja, jotka tuntuivat vielä hetki sitten siintävän pitkällä tulevaisuudessa.

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Ville Luukkonen

CONTENTS

SYMBOLS

a	slope	[-]
b	intercept	[V*ms]
c	concentration	[mg/l]
E	electric field gradient	[V/cm]
K	ion mobility	[cm^2/Vs]
K_0	reduced mobility	[cm^2/Vs]
K_{fs}	distribution coefficient between fiber and aqueous phase	[-]
K_{hs}	distribution coefficient between headspace and aqueous phase	[-]
LC	lethal concentration	[mg/l]
LD	lethal dose	[mg/kg]
LOD	detection limit	[mg/l]
p	pressure	[kPa, bar, Torr]
pKa	acid dissociation constant	[-]
Q	flow rate	[ml/min, l/min]
R	extraction recovery	[%]
r	response	[V*ms]
T	temperature	[K, °C]
t_d	drift time	[ms]
V	volume	[l, ml]
v_d	drift velocity	[cm/s]
x	concentration	[mg/l]
y	response	[V*ms]

SUBSCRIPTS

0	initial, standard
1	in, new
2	out, old
bn	background noise
drift	drift gas
f	fiber
h	headspace
peak	peak
ref	reference compound
u	analyte

TERMS AND ABBREVIATIONS

96-h LC_{50}	lethal concentration to kill 50 % of test organisms in 96 hours
baking	a procedure, where temperature of IMS drift tube is elevated to thermally clean contaminants
CAR	carboxen
CAR-PDMS	carboxen-polydimethylsiloxane
CP	chlorophenol
CW	carbowax
DI-	direct immersion
DMS	differential mobility spectrometry
DVB	divinylbenzene
EtOH	ethanol
FAIMS	high-field asymmetric waveform ion mobility spectrometry
HS-	headspace
IMS	ion mobility spectrometry
LD_{50}	lethal dose to kill 50 % of test animals

LOD	limit of detection, a lowest quantity of target analyte that can be detected reliably
LOQ	limit of quantification
MTBE	Methyl *tert*-butyl ether
PA	polyacrylate
PCP	pentachlorophenol
PDMS	polydimethylsiloxane
pKa	a pH value, where half of the substance is in ionized form
PPESK	polyphtalazine ether sulfone ketone
PU	polyurethane
recovery	ability of the measurement system to detect the analyte observed
repeatability	precision of measurements under same measurement conditions
RIN	reactant ion peak in negative mode
sensitivity	smallest difference in analyte concentration that can be detected
SBSE	stir bar sorptive extraction
SPME	solid-phase microextraction
SPDE	solid-phase dynamic extraction
TCP	trichlorophenol
TeCP	tetrachlorophenol
TFME	thin-film microextraction
UCW	ultra clear water, deionized water

1 INTRODUCTION

Approximately 3.5 million Finns use ground water or artificial ground water as a drinking water. The exposure to chemicals via drinking water can be significant due to its large daily consumption. The most typical ground water contaminating chemicals derived from human action are nitrates, oil compounds, MTBE, chlorophenols and chlorinated solvents. (Syke 2009.)

Chlorophenols have been used for many purposes, such as pesticides, fungicides and antiseptics. In addition, chlorophenols may be formed when disinfecting drinking water or bleaching a wood pulp with chlorine. (ATSDR 1999, 2.) Chlorophenols containing chemicals, such as KY-5, were generally used in Finnish saw mills as a weather inhibition substances from 1930s. However chlorophenols were classified as class II toxins in 1946. Despite the classification, KY-5 was not classified as a toxic chemical until 1980. (Etelä-Pohjanmaan ELY 2008.) In 2007, in Mikkeli, Pursiala area, chlorophenols were discovered in the ground water. Based on a research, it seemed obvious that chlorophenols originated from an old saw mill area. The highest detected cholorophenol concentration in ground water was 8 000 µg/l, although the water was not used as a drinking water. (Etelä-Savon ympäristökeskus 2007.)

Chlorophenols have various health effects: irritation of airways, headache and other symptoms of intoxication. Chlorophenols have been classified as possible carsinogenic substances for humans by The International Agency for the Research on Cancer (Morales et al 2012, 1095). Finland's Ministry of Social Affairs and Health imposed that the maximum concentration of chlorophenols in drinking water may not exceed 10 µg/l. (Etelä-Pohjanmaan ELY 2011; A 19.4.2000/461, 18 §.)

In environmental analysis detection limits of the substances have to be low in order to determine pollutants in low concentrations (Patnaik 2010, 21). To achieve these low detection limits preconcentration and sample preparation are usually needed. There are various methods for these purposes, which have their drawbacks: requirement of solvents and time in addition to high costs. Solid-phase microextraction (SPME) can solve some of these problems. SPME can be used to extract various organic pollutants from liquid, gaseous or solid phases. (Djozan & Bahar 2003, 637.) SPME is a solvent-free extraction method which allows sample pre-

concentration and cleanup in a single step. In addition SPME method improves detection limits, shortens time required for sample preparation and reduces disposal costs. These factors make sample preparation with SPME method easy and fast. (Kataoka 2005, 66; Morais et al 2011, 2531; Walendzik et al 2005, 1842.)

Ion mobility spectrometry (IMS) is an analysis method based on determination of ion mobilities in the gas phase under the influence of an electric field. IMS was originally developed to detect and identify volatile and semi-volatile organic compounds. At first it was primarily used to detect explosives and chemical warfare agents. Nowadays it is also used in environmental analysis. Advantages of IMS are its rapidness, low costs, high sensitivity, analytical flexibility, ease of use and suitability for real time monitoring. However there are limitations for the use of IMS in environmental analysis: challenges regarding influences of humidity, complexity of sample matrices and achieving low limits of detection must be solved. (Borsdorf & Eiceman 2006, 323-324; Sun & Ong 2005, 113; Arce et al 2008, 139; Marquez-Sillero et al 2011, 677.)

The scope of this work is to first introduce the basic principles of SPME and IMS. Also chlorophenols are reviewed shortly. The main focus is then to study, if SPME-IMS coupling is suitable for determination of chlorophenols from water samples. The aim of this work is to optimize SPME parameters for optimal extraction and then apply SPME-IMS analysis for actual water samples.

This work is divided into theory part and experimental part. Theory part consists of chapters 2 – 4. Firstly, basic properties of chlorophenols, their sources and their effects to the environment and humans are discussed. Also chlorophenol concentrations found from the environment are discussed shortly. In addition, currently used methods for chlorophenol detection are discussed briefly.

Secondly, the SPME instrument and sampling methods are introduced. The theory behind the SPME is dealt with. After the SPME theory, different structures of SPME instruments are discussed, keeping the main focus in fiber SPME. After that, different sampling methods, HS-SPME and DI-SPME, are reviewed. Lastly a brief review is made on the sensitivity of SPME and already performed studies concerning SPME extraction of chlorophenols.

The last chapter of the theory part concentrates on IMS. Firstly basic principles of theory in which IMS is based on are introduced. Also components of IMS configuration are discussed, having the main focus on the components used in the practical part of this work. Lastly a brief overview is made at SPME-IMS coupling.

The experimental part consists of chapters 5 and 6. In chapter 5, SPME parameters are optimized to obtain best possible responses for target chlorophenols (2,4,6-TCP, 2,3,4,6-TeCP and PCP) and therefore achieve as low concentrations as possible. After parameter optimization, calibration curves are determined in chapter 6. Chapter 6 also focuses on determination of certain most important validation parameters. After determination of these parameters the SPME-IMS coupling is applied to three actual water samples.

After the theory part, results are presented and discussed in chapter 7. Also sources of errors are discussed and ideas for method development are presented. Lastly, a summary of this work is presented in chapter 8.

2 CHLOROPHENOLS

Cholophenols, (CPs) are phenols with 1 – 5 covalently bonded chlorine atoms. There are nineteen different types of CPs. Eight of them are commercially used and eleven incidentally produced in chlorination of organic materials. Chlorophenols' toxic potency depends on the amount of chlorine atoms in the molecule and the relative position to hydroxyl group. (Pepelko et al 2005, 93-94; WHO 1989.)

Chlorophenols have been used as pesticides, wood preservatives, antiseptics and textile, leather and glue manufacturing purposes, for instance. They are also used as intermediates for producing other compounds and they can be formed as byproducts of water disinfection. CPs enter the environment most commonly by industrial waste discharge but also by leaching from landfills. In addition to point source pollution, most important nonpoint source of pollution is the use of pesticides. (Pepelko et al 2005, 94; ATSDR 1999, 143.)

All of the CPs are solid in room temperature expect 2-chlorophenol which is liquid. CPs have usually strong or medicinal odors. In addition, odor and taste thresholds of CPs are low. Because of these low thresholds, maximum acceptable concentrations of CPs are based more on organoleptic than toxicological criteria. (ATSDR 1999, 133; Inchem 1989.) CPs can bioaccumulate in nature and therefore present in many products. Humans can expose to CPs through daily activities, such as inhalation and ingestion. The most common way to CP exposure is eating or drinking products containing CPs. It is estimated that daily amount of CP intake is around 2.2 – 40 µg/person. (Pepelko et al 2005, 94; ATSDR 1999, 163.)

When CPs enter the environment their transport processes are dependent on various factors. The pH is a key factor: when the pH increases it also increases degree of the compounds ionizing. Also factors such as water solubility, volatilization rate and photolysis rate of CPs affect to transport processes. It has been estimated that when released to environment, 72 % of chlorophenols would remain in water and rest in the sediment. It is also known that the higher CP, the higher sorption to the organic material in soil. (ATSDR 1999, 143, 150.) In the case of CPs in surface waters, adsorption seems to have an important role. If CPs do not degrade, they can accumulate on sediments or be transported for long distances since they are rather persistent. (Inchem 1989.)

Not many CPs have been evaluated in mammalian species. However compounds, such as pentachlorophenol, 2,4-dichlorophenol and 2,4,6-trichlorophenol have been listed as group 2B carcinogens. Toxicity of CPs is based on cellular membrane destruction and oxidative phosphorylation. (Pepelko et al 2005, 94.) In extreme cases an exposure to chlorophenols can lead to death. Acute exposure to chlorophenols can cause headache, dizziness, convulsions, decreased or increased body temperature and exhaustion. However these kinds of exposures are normally linked to industrial accidents. (Inchem 1989.)

If the sorption capacity of the soil is exceeded, chlorophenols can contaminate ground water. Concentrations of CPs have been found even in unpolluted areas; 2,4,6-TCP was found in concentrations of 1 – 12 ng/l in Lake Vattern. Concentrations varied depending on the distance of the bleaching plant. Samples taken from the Baltic sea contained less than 1 ng/l of 2,4,6-TCP. Also maximum concentrations of monochlorophenol measured in European rivers were 2 – 6 µg/l. Concentrations of 65 ng/l of 2-CP, 127 ng/l of 4-CP, 72 ng/l of 2,4-CP and 148 ng/l of 2,4,6-TCP have been measured from Canadian tap water. (ATSDR 1999, 152, 160-161.) In Finland, chlorophenols most usually enter the environment by leaching from old saw mills. Until 1980s chlorophenol containing KY-5 weather inhibition substance was used. In point source pollution cases like in Mikkeli, chlorophenol concentrations in ground water can be as high as 8 000 µg/l (Etelä-Savon ympäristökeskus 2007.)

2.1 2,4,6-TCP, 2,3,4,6-TeCP and PCP

2,4,6-TCP, 2,3,4,6-TeCP and PCP were the main components of wood preservation agent KY-5. Therefore they are the main interest of this study. Chlorophenols mentioned above had the following deviation in KY-5:

-78 – 83 % 2,3,4,6-TeCP
-7 – 15 % 2,4,6-TCP
-6 – 9 % PCP (Aspholm & Rajala 2003, 7.)

2,4,6-TCP was used for preservation purposes of wood, leather and glue in addition for antiseptics. Nowadays the use of 2,4,6-TCP has been restricted in many countries, including USA, Finland and other European countries. 2,4,6-TCP consists of a benzene ring with three chlorine atoms covalently bonded to it. 2,4,6-TCP is solid at room temperature and has a yellow color. 2,4,6-TCP has a sweet, intense odor. 2,4,6-TCP's chemical formula is $C_6H_3Cl_3O$ and molecular weight 197.46 g/mol. Its melting point is 69 °C and boiling point 246 °C. (ATSDR 1999, 135, 137; US EPA 2007.) The structure of 2,4,6-TCP is presented in figure 1.

2,4,6-TCP can be either in ionized or un-ionized form in water. The ionized form is 2,4,6-trichlorophenate and depends on the pH of water. (Halappa et al 1985, 188.) It is estimated that photolysis half-life of 2,4,6-TCP in midday sun is 62 hours (ATSDR 1999, 154). It has been studied that 96-h LC_{50} for blue gills is 0.32 mg/l. LD_{50} value was also studied. It was found out that for rats LD_{50} was 820 mg/kg. (Halappa et al 1985, 188-191.) In addition, it has also been found in animal tests that 2,4,6-TCP is carcinogenic. Therefore 2,4,6-TCP is classified as a possible carcinogen for humans. (US EPA 2007.)

The concentration of 2,4,6-TCP in environment has been declining since 1980s. In 1980 the amount of 2,4,6-TCP in sediments of Lake Ketelmeer was 1.9 µg/kg on a dry basis. The concentration of Rhine River has declined from 205 ng/l, measured in 1987, to less than 50 ng/l, measured in 1988. 2,4,6-TCP decomposes completely in soils in 5 days. (Vershueren 2001a.)

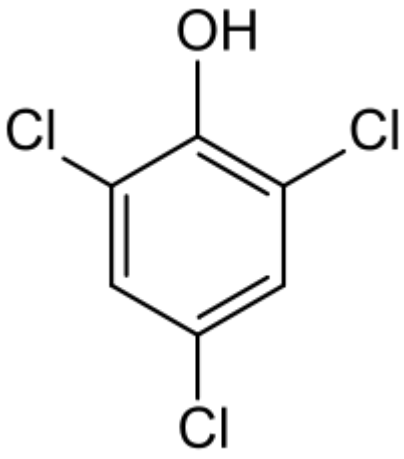

Figure 1. Structure of 2,4,6-TCP.

2,3,4,6-TeCP has been used as fungicide. Tetrachlorophenols are probably in the water in ion form. It is however not known, whether the ionized tetrachlorophenol reacts more easily in the environment than the un-ionized form. 2,3,4,6-TeCP consists of a benzene ring with four chlorine atoms covalently bonded to it. 2,3,4,6-TeCP is solid at room temperature, having a light brown color and an intense odor. Its chemical formula is $C_6H_2Cl_4O$ and molecular weight 231.89 g/mol. 2,3,4,6-TeCP melts at 70 °C and boils at 164 °C. (ATSDR 1999, 135, 137, 154.) The structure of 2,3,4,6-TeCP is presented in figure 2.

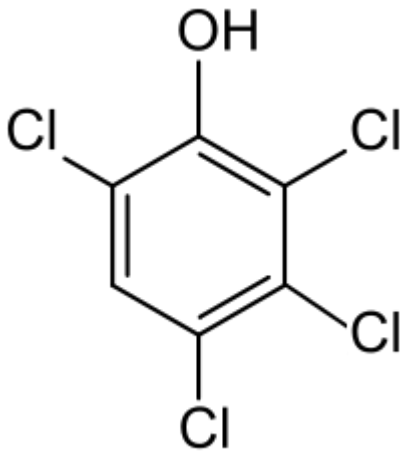

Figure 2. Structure of 2,3,4,6-TeCP.

It has been studied that 2,3,4,6-TeCP has a 96-h LC_{50} value of 1.1mg/l for Poecilla reticulate. A 24-h LC_{50} value for goldfish is 0.75 mg/l. 2,3,4,6-TeCP has also been found in dead fishes. The dose that was found was 75 mg/kg. In 1980s 2,3,4,6-TeCP concentration of the sediment in Lake Ketelmeer was 1.7 µg/kg on dry basis. 2,3,4,6-TeCP decomposes in soil completely in more than 72 days. (Vershueren 2001b.)

2,3,4,6-TeCP bioaccumulation has been studied at an old sawmill in Finland. In the study earthworms were examined at the sawmill that had not been used for 28 years. At the sawmill, soil concentrations of 2,3,4,6-TeCP were 336 $\mu g/g_{soil}$ in dry basis. The concentration of 2,3,4,6-TeCP in earthworms was 430 $\mu g/g_{fat}$ and 1 980 $\mu g/g_{fat}$. The biomagnification of 2,3,4,6-TeCP has not been studied. It has been, however, estimated that tetrachlorophenol is more likely to biomagnify than lower CPs. (ATSDR 1999, 153.)

PCP is a synthetic substance consisting of a benzene ring with five chlorine atoms bonded to it. PCP is solid at room temperature: it melts at 190 °C and boils at 309 °C. Pure PCP is colorless but impurities can alter its color to grey, light blue, beige or pink. PCP's chemical formula is C_6HCl_5O and molecular weight 266.35 g/mol. PCP can exist in both ionized and unionized form. The form is pH dependent: PCP has a *pKa* value of 4.7. In 6.7 pH 99 % of PCP is ionized. (ATSDR 2001, 2, 138-139, 152.) The structure of PCP is presented in figure 3.

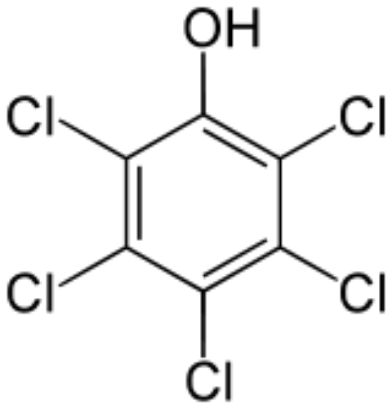

Figure 3. Structure of PCP.

PCP can also exist as sodium pentachlorophenate (Na-PCP). These two substances are highly toxic substances. PCP has been used in fungicides, pesticides and insecticides amongst other uses. Due to its high toxicity, PCP is the most studied chlorophenol. (Ayude et al 2009, 765.)

The effects of PCP have been studied in animal tests. With a high concentration oral exposure, an increased risk of cancer has been noticed. PCP has also negative effects to the fertility. It has been noticed that PCP affects to reproductive organs, leads to miscarriages and decreases the odds for successful pregnancy. These results may not be, however comparable in the case of humans. (ATSDR 2001, 5.) For earthworm a *4-wk* LC_{50} value is 10 - 32 $mg/kg_{wet\ soil}$ and *2-wk* LC_{50} value 32 $mg/kg_{wet\ soil}$. For a large goldfish *96-h* LC_{50} was 0.19 mg/l and for a large fathead minnow 0.20 mg/l. (Vershueren 2001c.)

PCP does not oxidize or hydrolyze easily, but is easily photolyzed and degraded by plants, animals and microorganisms. In acidic environment, PCP can be adsorbed. It has been found out that PCP can bioaccumulate for some levels. PCP released in atmosphere can enter the surface by the means of dry and wet deposition. When in water, PCP usually photolyzes and biotransformates. It is then absorbed by sediments and further metabolized by microbes or

adsorbed. (ATSDR 2001, 145, 152.) The sediments of Lake Ketelmeer contained PCP 8.4 µg/kg on dry basis. In the River Rhine the amount of PCP has declined from 650 ng/l, in 1978, to lower than 50 ng/l in 1988. (Vershueren 2001c.)

2.2 Methods used for chlorophenol detection

Chlorophenols have been mostly analyzed by gas chromatography (GC) or high pressure liquid chromatography (HPLC). In the case of gas chromatography detectors including flame ionization and electron capture have been used. However chlorophenols are difficult to measure directly with gas chromatography and are therefore usually derivatized to less polar form prior to analysis. With GC and sample pretreatment, even detection limits (LODs) of ng/l can be achieved, depending on the detector and the derivatization. Same level of LODs can be achieved with HPLC with same treatment. (ATSDR 1999, 173, 180.)

Llompart et al (2002, 147.) have analyzed chlorophenols with solid phase microextraction – gas chromatography – mass spectrometry (SPME-GC-MS) method and derivatization. With this combination quantification limits (LOQs) lower than 0.1 µg/l were reached. Al-Janabi et al (2011, 106-107) analyzed chlorophenol content of Tigris River and Baghdadian drinking water using HPLC. They enriched the samples first in solid phase extraction (SPE) tubes followed by pH adjustement. With this procedure they reached LODs of 0.007 – 0.012 mg/l.

Morales et al (2012, 1095.) determined chlorophenols with SPME-GC-MS coupling without derivatization. They studied the effect of different parameters on extraction efficiency, including fiber material, extraction temperature and extraction time. The decision limit reached with polydimethylsiloxane-divinylbenzene (PDMS-DVB) fiber was 0.29 – 0.67 µg/l. De Morais et al (2011, 2531.) used SPME-GC-ECD coupling to detect chlorophenols with acetylisation. The reached LODs were lower than 6 ng/l for poly-CPs.

Although low LODs can be achieved with these methods, there are also some drawbacks. GC may require sample extraction and cleanup to achieve proper quantitation and separation. With HPLC, long run times and extraction steps are usually required in analysis. (Lokhnauth & Snow 2005, 5938.) In addition HPLC and GC require high-priced and sophisticated equip-

ment (Ayude et al 2008, 765). Although GC and HPLC provide reliable results at trace levels, it requires time to analyze the samples. However with IMS, the analysis can be completed much faster, in a few seconds. The rapidness is a key advantage, when quantification is not the main goal. (Orzechowska 1997, 3.)

3 SOLID PHASE MICROEXTRACTION (SPME)

SPME was developed in the beginning of the 1990s for a new method for sampling and sample preparation. SPME has wide applicability: it has been used in pharmaceutical, food, forensic and environmental analysis. In environmental analysis SPME is suitable for water, air, sediment and soil samples. Sampling can be performed both on-site and off-site. (Oyang & Pawliszyn 2006a, 1059; Oyang 2012, 251; Arce et al 2008, 144.)

SPME is an adsorption/absorption and desorption technique. Its advantages are simplicity, rapidity, high sensitivity, effectiveness, elimination of the need of solvents and the fact that only a small volume of sample is required. With SPME technique, sample isolation and concentration can be performed in a single sampling step. In addition, SPME sampling has a relatively low costs and it can be automated quite simply. However the main disadvantages of fiber SPME are that the stationary phase is unprotected when it is ejected from the syringe. Also the fused silica fiber is fragile, which can cause problems. In addition, an SPME fiber usually costs 100 – 200 € and only approximately 100 measurements can be carried out with one fiber. (Oyang & Pawliszyn 2006a, 1059; Kataoka 2005, 73, 76.)

Firstly, the basic principle of SPME is introduced in this chapter. It is followed by the description of the structure of the fiber SPME, the most widely used SPME technique. After that, other methods and automation of SPME are reviewed shortly. Then SPME calibration methods are introduced, followed by sampling with HS- and DI-SPME methods. Lastly, SPME sensitivity and performed studies with chlorophenols are reviewed.

3.1 The structure and basic principles of fiber SPME

The principle of SPME is simple. It is based on an equilibration of the analytes in the sample and in the stationary phase of the SPME device. This is achieved in practice by introducing a stationary phase-coated small diameter fiber in contact with a sample matrix. When in contact, analytes from the sample move into the solid phase until equilibrium is achieved. The amount of extracted analytes is dependent on the volumes of the sample and the stationary phase in addition to dependence of the distribution coefficient between those two. To remove

analytes from the solid phase of the SPME to the analyser, thermal desorption is needed. (Arthur & Pawliszyn 1990, 2145; Duan et al, 2011, 1568-1569.)

When sampling with SPME, complete removal of the analytes from the sample is not required, as it is in the case of SPE. (Arthur & Pawliszyn 1990, 2145.) In addition, fibers can be cleaned with solvent after sample extraction, hence it is possible to remove possible sample matrix particles (Kataoka 2005, 67). Wide variety of solid phases has been developed for SPME (Arthur & Pawliszyn 1990, 2145).

Fiber SPME is a sampling technique, which uses a fused-silica fiber with stationary phase coating. The fiber SPME equipment is made up of a fiber holder and a needle. Inside a needle, there is a built-in fiber. This assembly resembles a modified syringe. The fiber holder is adjustable and consists of a plunger, barrel made of stainless steel and a depth gauge with a needle. The stationary phase consists of thin films of polymers, polyacrylate for instance. The fiber can be used multiple times and replaced if necessary. (Kataoka 2005, 66.) The structure of fiber SPME device is illustrated in figure 4.

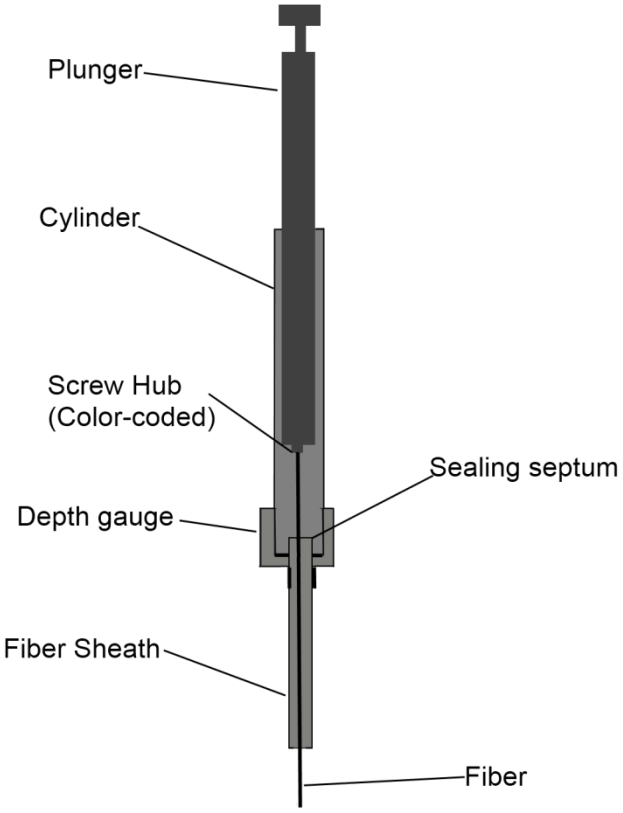

Figure 4. The structure of a fiber SPME device. (Wercinski & Pawliszyn, 2).

The appropriate SPME device is leak tight, so the sample is protected in extraction and desorption. Also the fiber exposure and retraction must be easily performed by a holder. In addition, fiber needle must have an appropriate diameter for puncturing a septum properly and containing the fiber. Fibers also have to be easily replaced in the holder. (Shirey 1999, 59)

There are four different types of fibers: non-bonded, bonded, partially crosslinked or highly crosslinked fibers. This classification of the fibers is based on the immobilization of the solid phases. Non-bonded phases can be used with water soluble organic solvents, although light swelling is possible with nonpolar solvents. Bonded phases can be used with all organic solvents excluding some nonpolar solvents. Partially crosslinked phases can be used with most water soluble organic solvents and also with some nonpolar solvents. Highly crosslinked phases are almost equivalent to partially crosslinked phases. The most important factor, when choosing appropriate fibers, is the affinity of the coating for an analyte. (Kataoka 2005, 66-67.)

Film thickness and polarity are the most usual classification properties of the fibers. The film thickness affects to the extraction time of the analyte. Extraction with the thicker coating is more time consuming than with the thinner coating, but more analyte can be extracted. Usually thicker coating is required for volatile compounds and thinner for semi-volatile compounds (Kataoka 2005, 67.) Thicknesses of the fibers usually vary from 7 µm to 100 µm. The most typical material for nonpolar phases is polydimethylsiloxane (PDMS), while polyacrylate (PA) and carbowax (CW) are used as more polar phases. (Shirey 1999, 62-63.)

Coatings, such as, PDMS, PA, CW, DVB and carboxen (CAR) are generally used in SPME devices. Also new coating materials, such as polypyrrole (PPY), polyphtalazine ether sulfone ketone (PPESK) and polyurethane (PU) have been developed (Duan 2011, 1572.) PDMS fiber can be currently purchased with three film thicknesses: 100 µm, 30 µm and 7 µm. 100 µm PDMS fibers can extract more analytes, but the extraction step takes more time than with thinner thickness. (Shirey 1999, 62-64.)

Unlike most phases, PA is solid at room temperature. As a fiber material PA is durable and rather solvent resistant, although in high temperatures it oxidizes easily. Although PA is a

durable material, it takes time to extract analytes, because of its rigidness. When exposed to temperatures higher than 280 °C PA fiber turns its color to dark brown. This darkening doesn't however affect to the properties of the fiber. If PA fiber however changes its color to black, it should not be used anymore. (Oyang 2012, 252; Shirey 1999, 62-64.)

CW has been used as a phase material in gas chromatography. As an SPME fiber material it however has some weaknesses. CW has a tendency for swelling or dissolving into water. Because of the swelling, it is possible that CW phase could be damaged when the fiber is retracted from the sample. Also CW is easily oxidized in temperatures higher than 220 °C. To avoid the problems caused by swelling, a highly crosslinked carbowax was developed. (Shirey 1999, 64)

Choosing an appropriate fiber for the target analyte is based on fiber's selectivity for the target analyte and target analyte's volatility. However the affinity of the coating for the analyte is the most important factor, because the sample matrix and the coating are competing for analytes. (Wercinski & Pawliszyn 1999, 3.) Also required detection limits have to be taken into account. Physical characteristics of the analytes are its molecular weight, vapor pressure, concentration, boiling point, functional group and polarity. Type of the detector also effects for choosing the appropriate fiber. (Shirey 1999, 68-69.)

For desorbing the analyte from the SPME fiber, thermal desorption is needed. In thermal desorption, the heat is used to increase the volatility of the analyte. When volatility of the analyte increases enough, it can be removed from the solid phase. The desorbed sample can then be lead into analyzer, for example IMS. (Arce et al 2008, 144.)

3.2 Variations of SPME and automation

In addition to fiber SPME various related techniques, such as, in-tube SPME, solid-phase dynamic extraction (SPDE), thin-film microextraction (TFME) and stir-bar sorptive extraction (SBSE) have been developed. In-tube SPME was developed primarily for automation and high-throughput application purposes. It consists of an open tubular capillary, which is used as an SPME device. In-tube SPME enables continuous extraction, desorption and injection using autosampler. In this technique analytes are extracted into capillary which is coated in-

ternally with a stationary phase. The main advantages of an in-tube SPME are that it is simple, easy to automate, rapid, highly sensitive and has low costs. In addition, the need of solvents is eliminated and only a small volume of sample is required. (Kataoka 2005, 66, 68, 82.)

To achieve consistent extraction and desorption times, analyst must be near the sampling area. When done manually, several times a day, sampling with SPME can be a time-consuming task. With autosampling, the precision and productivity are improved. With autosampler, it is possible to mix or heat the sample or automate different methods. This enables the examination of effects caused by changing various parameters. (Penton 1999, 36.)

Rodacy et al (2000, 22, 27.) developed a prototype of an automated fiber SPME-IMS system. In their design, the analysis cycle is similar to the manual analysis, but is automated. Cam system was used to puncture the SPME needle through septa and following steps, exposure and desorption in the IMS inlet, were executed by a direct drive system. The automated system prototype was built from commercial components. The described system was proven adequate for analysis.

However, most of the automated SPME devices are designed to be coupled with a GC or LC devices. For GC automation, fiber and in-tube SPME methods have been proven the most efficient methods. The fiber SPME is the most suitable choice for GC due to its compatibility with GC inlet. For LC there are some difficulties concerning commercial fibers and LC port. For LC, in-tube SPME is used. Complete automation of sampling systems have been managed to build only for fiber and in-tube SPME. With SBSE, semi-automatic systems have been build. (O'Reilly et al 2005, 2010-11, 2019, 2021.)

De Morais et al (2011, 2532.) used a CombiPal autosampler provided by CTC Analytics in their research concerning the applicability of HS-SPME method for CP determination from water samples. However the further analysis was made with GC system instead of IMS. Also Gerstel has an SPME autosampling system for GC/MS coupling.

3.3 Sampling with fiber SPME

When sampling with a fiber SPME device, two techniques can be used. These techniques are headspace SPME (HS-SPME) and direct immersion SPME (DI-SPME). In a HS-SPME technique fiber is exposed to the vapor phase of the sampling vial while in DI-SPME technique the fiber is immersed into a liquid phase. (Kataoka 2005, 67.) These two techniques are discussed in more detail in the following subchapters.

When sampling with SPME, solvents are not normally needed and samples can contain particles (Oyang & Pawliszyn 2008, 185; Kataoka 2005, 67). However, if sampling matrix is wanted to be altered, then the salt can be added to the sample. Also temperature and pH adjustments can be made. (Kataoka 2005, 68.)

3.3.1 Calibration methods

Calibration methods for SPME can be classified as follows: traditional calibration methods, equilibrium extraction method, exhaustive extraction method and diffusion-based calibration (Oyang & Pawliszyn 2008, 194). In this work, only traditional calibration methods are discussed because according to Oyang & Pawliszyn (2008, 185), they are appropriate for laboratory analysis.

External standard method, internal standard method and standard addition methods are traditional calibration methods. All of these methods can be used for quantification. External standard method, which is also known as calibration curve method is the most common used tradition calibration method for on-site sampling. (Oyang & Pawliszyn 2008, 185; Oyang & Pawliszyn 2006b, 693.)

The external standard method is generally used for SPME calibration. In the external standard method, standard solution series are prepared. Based on these solutions and peak responses, the calibration curve is calculated. In this curve, the dependence between analyte concentration and peak responses can be seen. After completing the analyzing the standard solutions, real samples are analyzed in the same conditions. The analyte concentration in samples is then

calculated with the calibration curve equation. Benefit of this method is that the extensive sample preparation is not needed. However, the key is that the sampling procedure must remain constant through the analyses of both, samples and standard solutions. Also in the case of matrix effects, blank matrix samples have to be run. (Oyang & Pawliszyn 2008, 185.)

In the standard addition method, known quantity of the analyte of the interest is added to the sample. This matrix, containing both sample and the addition, is then analyzed. The plot is then drawn and the plot of the response is extrapolated to zero. The extrapolation gives the concentration of the untreated sample. This target analyte concentration can be then calculated on the basis of the plot slope and the extracted analyte. Standard addition method is usually used when the amount of samples is small and sample composition is complex and unknown. With this method sample matrix effects can be compensated. However in this method intensive preparation of samples is required. Especially for a large amount of samples this can laborious and takes a lot of time. (Oyang & Pawliszyn 2008, 186.)

In the internal standard calibration, compound, which differs from analytes and can be easily determined, is added to the samples and calibration solutions. This additive compound should have similar equilibrium properties than the analytes of the interest have. In the internal standard method, calibration solutions with different concentrations of the analyte with constant concentration of the internal standard are prepared. The plot is drawn on the basis of the ratio between analyte and calibration solution peak areas. Benefits of the internal standard method are that it compensates the matrix effect and losses in the sample preparation step. It can also alleviate problems concerning irreproducibility of the parameters. However in the case of complex samples, finding appropriate standards can be challenging. (Oyang & Pawliszyn 2008, 186.)

3.3.2 HS-SPME

HS-SPME is a sampling technique, where the fiber is exposed to the vapour phase of the sample. With this technique it is possible to extract analytes from solid, liquid or gaseous samples. (Kataoka 2005, 68.) HS-SPME technique is normally used because the fiber lifetime

is longer with this technique and matrix effects are reduced (Morais et al 2011, 2532). HS-SPME sampling assembly is illustrated in figure 5.

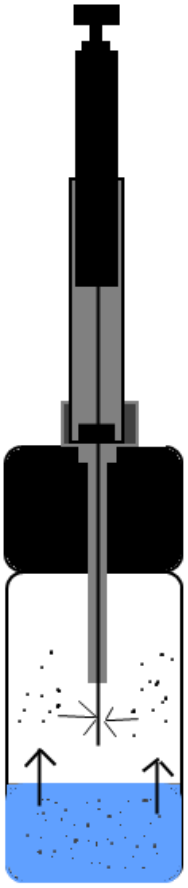

Figure 5. HS-SPME sampling assembly (Kataoka 2005, 67).

At the equilibrium state of a HS-SPME sampling, the concentration can be expressed as

$$c_0 V_0 = c_h V_h + c_s V_s + c_f V_f \quad (1)$$

in which, c_0 = the concentration of the original sample [mg/l]

V_0 = the volume of the original sample [l, ml]

c_h = the concentration of the analyte in the headspace [mg/l]

V_h = the volume of the headspace [l, ml]

c_s = concentration of the analyte in the sample phase at the equilibrium [mg/l]

V_s = volume of the sample at the equilibrium [l, ml]

c_f = concentration of the sample in the fiber [mg/l]

V_f = volume of the fiber [l, ml]
(Penton 1999, 40.)

Furthermore the concentration of the analyte in the fiber at the equilibrium state can be derived from the previous equation.

$$c_f = \frac{c_0 V_s K_{fs}}{V_f K_{fs} + V_h K_{hs} + V_s} \quad (2)$$

in which, K_{fs} = partition coefficient between the fiber and aqueous phase [-]
K_{hs} = partition coefficient between the headspace and aqueous phase [-]

(Penton 1999, 40.)

The concentration in the vial headspace without the fiber at the equilibrium state can be expressed based on the equation 1 by neglecting the last term. The concentration c_h can then be solved as follows.

$$c_h = \frac{c_0 V_0 - c_s V_s}{V_h} \quad (3)$$

HS-SPME is more applicable to use than DI-SPME if the analyte is volatile. Also in some cases use of an HS-SPME technique leads to a lower background noise levels. (Kataoka 2005, 68.) However detection limits are not dependent of the SPME technique if other variables are kept constant (Wercinski & Pawliszyn 1999, 8).

Prior to the main extraction stage the fiber is conditioned if it is not used before. If the fiber is used before, it is thermally cleaned prior to analysis. With cleaning, possible contaminants are removed and the interference is therefore minimized. (Kataoka 2005, 67.) Cleaning and conditioning can be made for example in GC injection port in the appropriate temperature and time (Sigma-Aldrich 1999). After cleaning the fiber, sample is moved to the vial and closed with a septum cap. The vial is heated to the desired temperature and incubated. After the incubation, extraction can be done by piercing the septum with the SPME needle and ejecting the fiber into the headspace of the sample. The fiber is exposed to the vapor phase for deter-

mined time and is then retracted and taken out of the vial. After extraction the fiber is placed into thermal desorption unit which is coupled to the analyzer. (Wercinski & Pawliszyn 1999, 2-3.) One possible assembly for desorption is presented in figure 6.

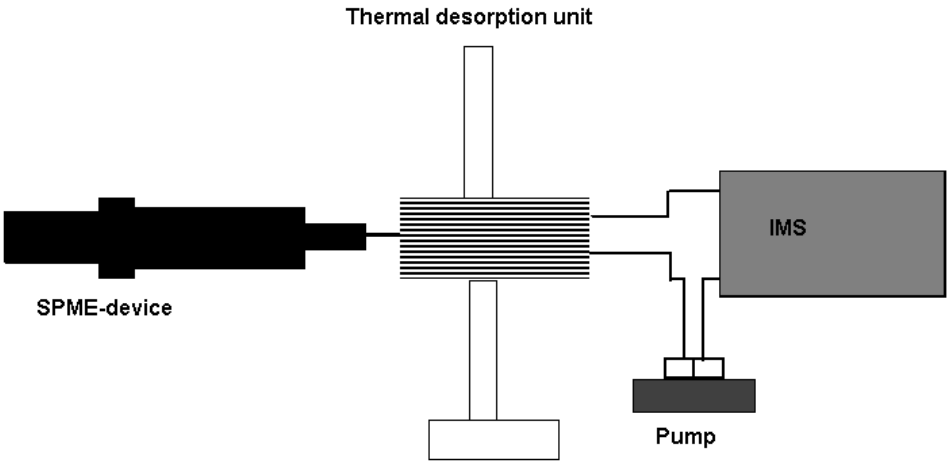

Figure 6. A coupling assembly for SPME-IMS system (Arce et al 2008, 145).

3.3.4 DI-SPME

As the name suggests, in DI-SPME the fiber is immersed into the sample phase. Unlike HS-SPME, DI-SPME is only suitable for liquid samples. (Kataoka 2005, 68.) When performing SPME sampling, the detection limits and sensitivities are not dependent on the technique. Therefore, when choosing appropriate extraction technique, the main focus should be kept in the possible interfering contaminants of the matrix. These contaminants can be for example grease or oil. (Wercinski & Pawliszyn 1999, 7, 8.) The DI-SPME extraction assembly is illustrated in figure 7.

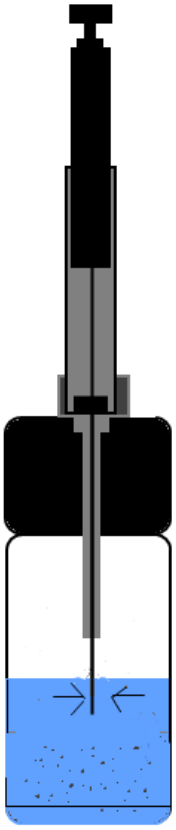

Figure 7. DI-SPME sampling assembly (Kataoka 2005, 67).

When sampling with DI-SPME technique the procedure is similar to one with the HS-SPME. However instead of exposing the fiber to vapor phase, fiber is brought into contact with the liquid phase of the sample. In addition, stirring can be also applied to the sample increasing the equilibration rate. (Kataoka 2005, 67-68.) DI-SPME cannot be applied to samples with too high or low pH values, due to fact that extreme pH values can damage the fiber. In addition, if sample matrix contains a great amount of non-volatiles, DI-SPME should not be applied. (Shirey 1999, 46.)

In the case of DI-SPME, the volume of headspace is neglected. Therefore the equilibrium concentration can be expressed as

$$c_0 V_0 = c_s V_s + c_f V_f \quad (4)$$

As can be seen, the equilibrium can be calculated with the same equation as in HS-SPME, but since the headspace volume, V_h, is zero, the entire term can be neglected. The fiber concentration can be then expressed in the equilibrium state as:

$$c_f = \frac{c_0 V_s K_{fs}}{V_f K_{fs} + V_s} \quad (5)$$

3.4 SPME sensitivity and optimization for chlorophenols

There are several parameters that affect to the sensitivity of the SPME: coating volume, distribution constant K_{fs} between fiber and the sample, extraction variables, such as temperature, stirring and possible salt addition (Wercinski & Pawliszyn 1999, 8-9; Penton 1999, 39). The SPME coating volume affects directly to the amount of an analyte that is possible to absorb. This can be done by either increasing the fiber thickness or the fiber length. As mentioned earlier, thicker coating enables larger analyte absorption but also extends the extraction time. However fiber volume can be increased by increasing the fiber length. If fastest possible extraction time is desired, it can be achieved with a thinner fiber. However this also decreases the sensitivity. (Wercinski & Pawliszyn 1999, 8.)

Other way to improve SPME sensitivity is to increase the K_{fs} value between the fiber and the analyte. The easiest way to achieve this is to select the most suitable fiber coating for the desired analyte. K_{fs} values can also be increased by optimizing the extraction temperature. When temperature is increased, also distribution coefficients increase while distribution constants decrease. These factors lead to a faster equilibrium. Increasing sensitivity by temperature increase can be applied for components with high boiling point and also for high molecular weight. However this method should not be applied for components with low boiling points. For latter, the temperature increase decreases sensitivity. In the case of HS-SPME, the extraction time shortens with temperature increase because the analyte concentration in the vapor phase is increased. (Wercinski & Pawliszyn 1999, 8-9; Penton 43.)

The salt addition can be used to force polar compounds into the vapor phase from the liquid phase. Extraction time can be shortened by stirring the sample, because the equilibrium is

reached faster. Stirring is suitable for low volatile compounds. Other parameters that affect to the response and extraction time are phase ratio and vial size. (Penton 1999, 41-44.)

According to Shirey (1999, 79) PA fiber is more suitable for polar compounds like 2,4,6-TCP than PDMS fiber. However PDMS fiber is more suitable for PCP, which is relatively nonpolar.

Morales et al (2012, 1095, 1100.) have performed an experiment where different fiber parameters were optimized for chlorophenols without derivatization. According to the experiment, it can be said that extraction time of 35 minutes for 2,4,6-TCP with PA fiber is sufficient. For 2,3,4,6-TeCP extraction is stable between 35 and 50 minutes with PA fiber. Morales et al (2012, 1106.) came to the conclusion that best of the studied fibers (PA, PDMS and PDMS/DVB) for chlorophenols was PDMS/DVB fiber. The selection was based on the figures of merit.

De Morais et al (2011, 2531-2532.) made experiments with chlorophenols using 100 µm PDMS fiber, 65 µm PDMS/DVB fiber, 75 µm CAR/PDMS fiber and 60 µm PEG fibers. They studied fiber type, salt addition, derivatization, extraction temperature and time in addition to desorption temperature. PDMS/DVB fiber was found to be most suitable for detecting chlorophenols with acetylation and salt addition.

Llompart et al (2002) studied acetylation derivatization of SPME for chlorophenols. 100 µm PDMS, 65 µm PDMS–DVB, 85 µm PA, 74 µm CAR/PDMS and 65 µm CW–DVB fibers were used. In the study salt was added to the derivatized sample. Salt was used to add the ionic strength of the solution. This increases the partition coefficients by making organic compounds less soluble. Salt addition led from 4 to 17 times higher responses. Conclusions were made that PA and CW/PDMS fiber were least efficient for extraction. 85 µm CAR/PDMS was found to be the most suitable for lower chlorophenols and PDMS for higher chlorophenols. It was also found out that best extraction parameters for CAR/PDMS were 30 minutes in 100 °C and for PDMS 30 minutes in 60 °C.

In studies performed by Djozan & Bahar (2003, 639-640.), stirring speed improved the extraction performance. They performed tests with phenol and 4-chlorophenol. It was also found out that NaCl addition of more than 8 g to the 25 ml of sample does not improve extraction

efficiency anymore. PH value has an effect to the extraction efficiency. When pH is decreased, efficiency is increased. However after the pH decreases below 7, efficiency remain constant. On the other hand, Hongtao et al (2010, 566) found out in their experiments that the 15 % NaCl was the most efficient concentration with silmylmethyl derivatization.

Moeder et al (2000, 101) studied biologically active substances with SPME/GC/MS. Chlorophenols were also included in the study as a comparison. In the study 65 µm PDMS/DVB fiber, 100 µm PDMS fiber and 85 µm fiber were used. It was found out that PA fiber had the best efficiencies for 2,4,6-TCP and PCP, while PDMS/DVB fiber had the best efficiency for 2,3,4,6-TeCP.

4 ION MOBILITY SPECTROMETRY (IMS)

The theory of IMS is based on ion formation and behavior in the gases at the ambient temperatures. It was developed in between the 1890s and 1910. (Borsdorf & Eiceman 2006, 324.) However it took approximately sixty years for IMS to become a modern analytical technique in 1970. It was not until 1990s when IMS devices were truly commercialized due to advances in technology. (Marquez-Sillero et al 2011, 677.) IMS has been traditionally used for detecting explosives, drugs and chemical warfare agents. It has also been used for quality control purposes in pharmaceutical sector and has now intensively expanded in food, clinical and environmental analysis purposes. (Armenta et al 2011, 114, 122.)

In environmental analysis IMS has been used for analyzing contaminants in aqueous solutions, detecting hazardous vapors in air and determining VOCs and semivolatiles in soil samples amongst others. Majority of environmental samples are liquid and quite complex. These samples may require pretreatment, such as cleanup. (Armenta et al 2011, 119.) This can, however, be avoided by using HS-SPME, which was discussed earlier in the text.

IMS is a rapid and sensitive analysis method and it can be used by unskilled workers. IMS also has low costs, good flexibility and portability. (Armenta et al 2011, 114, 119). IMS system does operate in ambient conditions and has low power consumption and weight (Marquez-Sillero et al 2011, 677-678).

However IMS has some challenges to overcome. Complexity of environmental matrices and low detection limits required for many pollutants are the main limitations in addition to the effects of humidity. (Marquez-Sillero et al 2011, 677-678.) In addition 60 % of IMS devices are custom-built and not commercialized. In case of some analytes, strong signal suppression can occur. (Armenta et al 2011, 115, 119.)

In this chapter theory in which IMS is based is discussed. After the basic principles of IMS the structure of traditional IMS device is introduced. Also the SPME-IMS coupling is discussed, since it is the coupling used in the experimental part of this work. Other IMS methods, such as differential mobility spectrometry (DMS) and high-field asymmetric waveform ion

mobility spectrometry (FAIMS) are not dealt with in this work because they are already discussed elsewhere.

4.1 Basic principles of IMS

In ion mobility spectrometry ions are formed by an ionization source under atmospheric pressure. These product ions, formed by reactant ions, move in an electrical field upstream of a carrier gas flow. Ions collide with each other and the gas molecules and accelerate in the electrical field. These accelerations and collisions of a particulate ion lead to an average velocity, called drift velocity, on a particulate length. Ions, which have different masses, have different drift velocities. These velocity differences lead to a separation of ions. (Stach & Baumbach 2002, 1.)

Ions move through voltage gradient in carrier gas and hit the detector (Borsdorf 2011, 474). When ions hit the detector a small current is caused. The current is converted to voltage and amplified. These voltage differences are then plotted as a function of the drift time. The plot is known as a mobility spectrum. (Borsdorf & Eiceman 2006, 346.) A mobility of a specific ion is always constant in specific conditions. Based on these "signatures" different substances can be identified. (Sun & Ong 2005, 113.)

Parameters, such as temperature, moisture and pressure affect to the ion mobility. Hence the comparison of the mobility measured in different conditions is impossible. Therefore the ion mobility can be normalized regarding to pressure and temperature. The normalized ion mobility is known as reduced mobility. Reduced mobility enables the comparison between measurements made in different conditions. However the normalization cannot fully compensate the possible alterations in ion identity caused by the environment. (Borsdorf & Eiceman 2006, 346.)

$$K = \frac{v_d}{E} \quad (6)$$

in which, K = ion mobility [cm^2/Vs]
v_d = drift velocity [cm/s]
E = electric field gradient [V/cm]
(Marquez-Sillero et al 2011, 679).

$$K_0 = K \frac{T_0}{T} \frac{p}{p_0} \quad (7)$$

in which, K_0 = reduced mobility [cm^2/Vs]
p = pressure [Pa]
p_0 = standard pressure [Pa]
T = temperature [K]
T_0 = standard temperature [K]
(Marquez-Sillero et al 2011, 680).

To calibrate the mobility scale of IMS device, a reference compound method has been developed. Reference compound method is based on a measurement of a reference compound, which reduced mobility is known. Based on its drift time obtained from the measurement, reduced mobility of the reference compound is then calculated. When using positive polarity of IMS, compounds such as 2,6-di-*tert*-butyl pyridine or dimethyl methylphosphonate can be used. In negative mode, compounds such as methyl salicylate (MSA) or trinitrotoluene (TNT) can be used. After measuring the reference compound, desired analytes are analyzed. (Kaur-Atwal et al 2009, 2-3.) The reduced mobility can be then calculated as follows:

$$K_{0u} = K_{0ref} \frac{t_{dref}}{t_{du}} \quad (8)$$

in which, K_{0u} = reduced mobility of the analyte [cm^2/Vs]
K_{0ref} = reduced mobility of the reference compound, literature value [cm^2/Vs]
t_{dref} = drift time of the reference compound, measured [ms]
t_{du} = drift time of the analyte [ms]
(Kaur-Atwal et al 2009, 2-3).

Modern IMS analyzers are based on these principles, as were IMS analyzers used over 40 years ago. However improvements are made regarding engineering and technology. The conventional method is also known as a time-of-flight method. (Borsdorf & Eiceman 2006, 324.) This method is described in the following text.

In IMS the ions are formed by ionization in both positive and negative polarity. The detection, however is performed only in negative or positive mode. Chemicals with high proton affinity are more likely to be ionized by positive-ion reactions. On the other hand chemicals with high phase acidity are more likely to be ionized by negative-ion reactions. The ionization source does not ionize the sample directly. Firstly, reactant ions are formed by ionization of air molecules. Reactant ions then ionize sample molecules and product ions are formed. Positive reactant ions are usually $H^+(H_2O)_n$ and negative reactant ions $O_2^-(H_2O)_n$. In the case of positive reactant ions, the molecules (M) of the sample are ionized to product ion (protonated monomer, $MH^+(H_2O)_{n-x}$) and also water (xH_2O) cleavage occurs in this reaction. Negative reactant ions ($O_2^-(H_2O)_n$) form product ions ($MO_2^-(H_2O)_{n-x}$) and water (xH_2O) with reaction of the sample molecules (M). (Eiceman & Karpas 2005, 5-7, 91.)

$$M + H^+(H_2O)_n \rightarrow MH^+(H_2O)_{n-x} + xH_2O \quad (9)$$

$$M + O_2^-(H_2O)_n \rightarrow MO_2^-(H_2O)_{n-x} + xH_2O \quad (10)$$

(Eiceman & Karpas 2005, 6-7.)

However, if the analyte concentration is high enough, also additional reactions will occur. In these cases analyte reacts with a protonated monomer, producing a proton bound dimer and water. If the analyte concentration is even higher, proton-bound trimers, and tetramers may occur. Occurrence of these reactions also depends on temperature, moisture and properties of analyte. (Eiceman 2002, 260.) When proton-bound dimers and higher components occur, reactant ion peak intensity decreases along with protonated monomer. However a new peak indicating the presence of protonated dimer appears to the right side of monomer peak. Dimers and trimers can also occur in negative polarity. Unlike proton transfer reactions in the positive mode, in the negative mode ions are formed by adduct formation, charge transfer or proton abstraction reactions. (Eiceman & Karpas 2005, 89-90.)

$$M + MH^+(H_2O)_n \rightarrow M_2H^+(H_2O)_{n-x} + xH_2O \quad (11)$$

(Eiceman & Karpas 2005, 6).

To produce ions, an ionization source is required. The ionization can be based on radioactive ionization, electrospray ionization, photoionization or flame ionization amongst other methods. Currently the radioactive ion sources are most widely used in IMS devices. (Guharay et al 2008, 1460.) Radioactive sources are widely used because they produce reactant ions steadily. They do not require much power and are light and easy to use. (Borsdorf & Eiceman 2006, 329.) However radioactive ion sources are not desirable in portable IMS devices because of certain restrictions in legislation. The use of radioactive source requires special licensing procedures and permits. In addition the transportability is limited and certain tests, which add costs, have to be performed. (Borsdorf & Eiceman 2006, 333.)

4.2 Structure of conventional IMS

Conventional IMS equipment consists of a sample introduction system such as SPME, a drift tube, an aperture grid and a detector. The drift tube includes an ionization source and a gate grid, which is also known as ion shutter. Detector, usually a Faraday plate, amplifies the signal and transfers it into the data unit. (Borsdorf et al 2011, 473; Stach & Baumbach 2002, 2.)

In conventional IMS, sample molecules are brought into ionization region with a carrier gas. Sample molecules are then ionized by the reactant ions. When an electric field gradient is applied, ions tend to move towards the detector due to their electrical charge. In conventional IMS, electric field gradient is continuously on. Different sample ions move with different average speed. The average speed is dictated by the properties of ions, including molecular weight and shape. Unionized sample molecules are carried out of the drift tube by drift gas. (Borsdorf et al 2011, 474; Borsdorf & Eiceman 2006, 343-344; Stach & Baumbach 2002, 2.)

However to obtain an appropriate spectrum, ions have to be released in small swarms in a specific interval instead of continuous flow. For this purpose the shutter grid is used. The shutter grid is opened at the specific intervals, usually with 20 – 30 ms for 100 – 300 µs, and

ion swarms then move towards detector and collide with the plate. The signal is then transferred to the spectrum. (Borsdorf et al 2011, 474; Borsdorf & Eiceman 2006, 343-344; Stach & Baumbach 2002, 2.) The structure of the conventional IMS device is presented in figure 8.

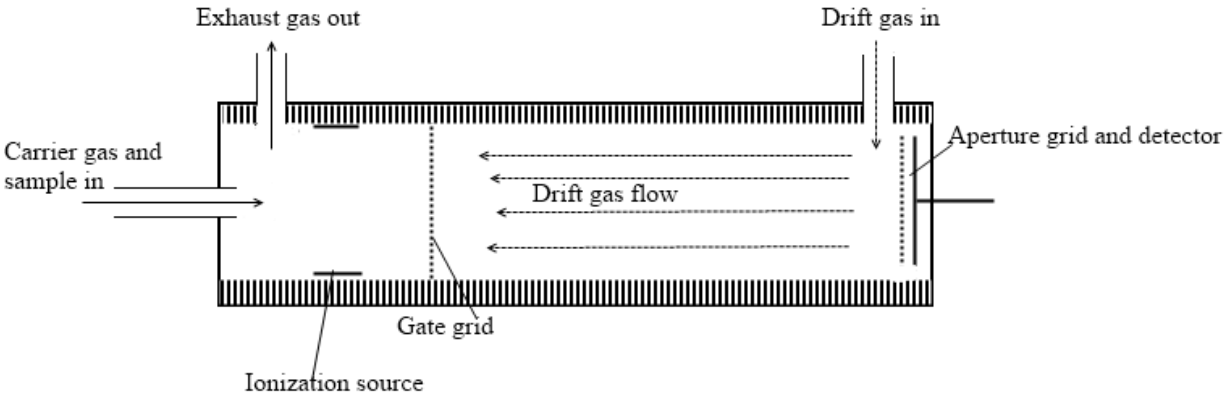

Figure 8. The structure of the drift tube (Sun & Ong 2005, 116; Stach & Baumbach 2002, 3).

In the following chapter the structure and the basis of operation of the drift tube, detector and aperture grid are discussed in more detail. Also an SPME-IMS coupling is introduced.

4.2.1 Drift tube

Drift tube is a central component of an IMS device. The formation and the characterization of ions take place in there. Drift tube is connected to other components, which enable the functioning of the drift tube. These other components include power sources, heaters, gas flow providers and electronic devices, such as signal processing devices, data units and ion shutter. (Eiceman & Karpas 2005, 119.) In this chapter the structure and operating principles of drift tube are discussed. The main focus is at traditional drift tubes with uni- and bidirectional flows and linear electric field. Also the principle of ion shutter is discussed in this chapter.

The drift tube can be divided into different regions: ion source and reaction region, drift region and detector region. In an ion source and reaction region, ions are formed, and in a drift region a separation of ions takes place. In a detector region ions are detected. (Eiceman & Karpas 2005, 144.) The areas of a drift tube are presented in figure 9.

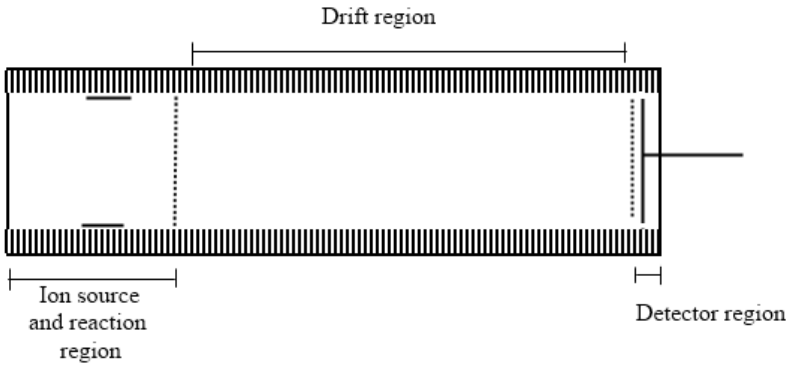

Figure 9. Regions of a drift tube (Eiceman & Karpas 2005, 120).

Sample molecules are introduced into a drift tube in gaseous form. The sample vapors are delivered into a reaction region with a carrier gas, in easiest case purified air. (Eiceman & Karpas 2005, 120-121.) Drift tubes can be categorized as a uni- and bidirectional drift tubes, based on the directions of the carrier gas and drift gas flows (Stach & Baumbach 2002, 3). In the case of a bidirectional flow system the carrier gas containing the sample enters the drift tube in the tube end opposite of the detector. The sample molecules are then ionized and injected to the detector. In this design the exhaust gas outflow occurs near the ion shutter. If the carrier gas flow rate is too high, some sample neutrals may be carried to the drift region and signal may be distorted. (Borsdorf & Eiceman 2006, 327.) Structures of bi- and unidirectional drift tubes are presented in figure 10.

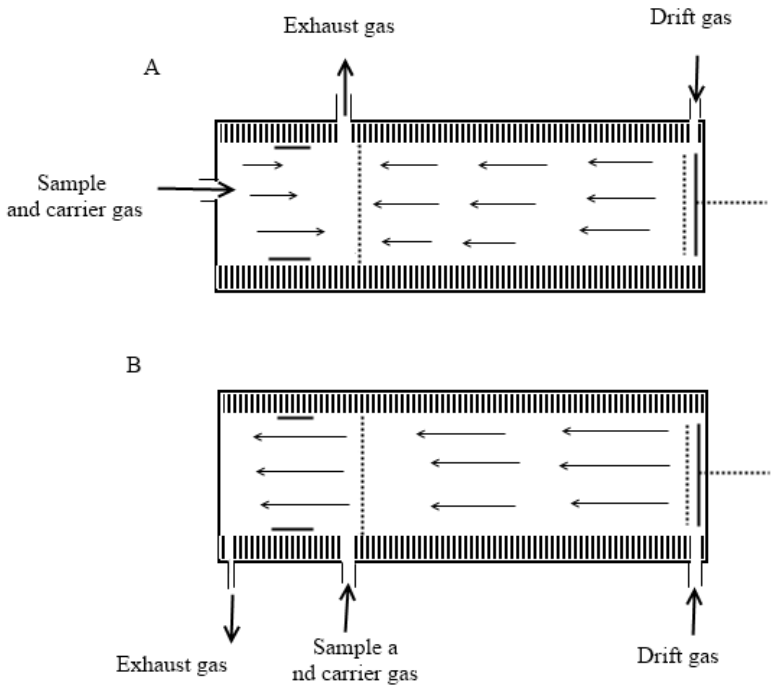

Figure 10. Flow arrangements for drift tube,
A = bidirectional, B = unidirectional flow system. (Borsdorf & Eiceman 2006, 327).

In a unidirectional drift tube, carrier gas, containing the sample, is introduced into the drift gas flow near the ionization region. The sample molecules are the ionized and injected to the detector via the ion shutter while unionized molecules are flushed away with the exhaust gas. (Borsdorf & Eiceman 2006, 327-328.)

As mentioned earlier, ions are forced to move in a drift tube by an electric field gradient. In the case of a positively reserved ion, the drift happens from a higher voltage to lower voltage and in the case of negatively reserved ions vice versa. Ions of no interest are flushed away from the drift tube with the exhaust gas flow. (Sun & Ong 2005, 115, 120.) The ion flow to the detector is controlled by the part known as ion shutter. Ion shutter consists of wires which are placed parallel and coplanar. Wires are isolated both mechanically and electrically and placed on a support that is not conductive. (Eiceman & Karpas 2005, 151.) When an electrical field, vertical to the drift tube field, is applied to the wires, ions are prevented from moving to the detector. When the field is removed, ions can enter the drift region and the detector. (Sun & Ong 2005, 120.)

Although ion shutter is crucial for the operation of IMS, it is also the limiter of the IMS performance. This is because the pulse width has a major effect to the spectrum resolution and peaks. Also in cases where only 0.1 % of ions are sampled, the duty cycle is low and signal losses occur. However, in normal case 1 % of ions are sampled. (Borsdorf & Eiceman 2006, 344.)

The structure of commercial drift tubes consists of focusing rings made of metal or ceramic material. These rings are stacked alternating electrodes and insulators. Electrodes are connected by a resistor chain. Drift tubes described above are expensive and laborious to construct. (Armenta et al 2011, 115.)

The drift tube is typically from 5 cm to 20 cm having an inner diameter of 1 – 2 cm. The typical electric field is between 200 and 400 V/cm requiring a voltage supply of 1 – 8 kV. Ion shutter commonly inject ions for a 50 – 300 µs with maximum drift time usually 20 - 30 ms. With these parameters, it can be said that ion shutter is opened 30 times per second. Drift gas flow rates are typically 200 – 700 ml/min. (Borsdorf & Eiceman 2006, 344-345.) Nitrogen or air is usually used as a drift gas (Hill et al 1990, 1202 A). Nitrogen or air can also be used as a carrier gas (Baumbach 2005, 1061).

4.2.2 Detector and aperture grid

A Faraday plate is the most widely used and simple detector that is used in a conventional drift tube. A Faraday plate is a circular metal plate in which ions collide and then annihilate. When ions collide to the plate, a small current, 10 – 100 pA, is developed. The induced current is then transmitted to the amplifier, where it is amplified and converted to voltage of 1 – 10 V. (Eiceman & Karpas 2005, 164.)

Usually the detector assembly also contains an aperture grid. Aperture grid is a metal mesh or consists of wires which are assembled parallel to each other. (Borsdorf & Eiceman 2006, 346.) Aperture grid is placed in front of the detector, in approximately 0.5 – 2 mm distance. The aperture grid has 30 – 90 V higher potential compared to the detector and the electrical field between grid and the detector is 300 – 600 V/cm. Aperture grid works as a suppressor of

induced current, caused by the ion swarm flow through the drift region. (Eiceman & Karpas 2005, 164; Borsdorf & Eiceman 2006, 346.) The suppression is achieved by coupling the detector capacitively with the arriving ions (Stach & Baumbach 2002, 3). The potential difference over the aperture grid and the detector affects to the spectrum peak shape and the ion intensity. The maximum sensitivity is achieved if both detector and aperture grid are same size. However this could lead to the signal broadening and weakened resolution. (Eiceman & Karpas 2005, 164.)

The detector signal is analogous but is nowadays digitized and usually averaged. After that the acquired data is storaged. The data handling and storage is done by computers. Current computers are efficient enough to perform required data processing to the spectra. The signal averaging is a process where the data from specific amount of spectra is averaged. With averaging, improved signal-to-noise ratios are achieved. If spectra are acquired with 5 ms interval, then averaging of 400 spectra is performed in approximately 2 seconds. (Eiceman & Karpas 2005, 165.)

Usually the device manufacturer offers software for data processing. Data processing software can be, however, written by a skillful person to meet the needs. With the appropriate software, the spectra are normally plotted. The most used plot is the signal intensity as a function of drift time. Also plots of analyte concentration as a function of time or pseudo 3-D plots are used. In pseudo 3-D plots, different scans are arranged to the order of appearance, providing a 3-dimensional plot. (Eiceman & Karpas 2005, 165-168.) As an example, the spectrum of 2,4,6-TCP (signal intensity as a function of drift time) is presented in the figure 11.

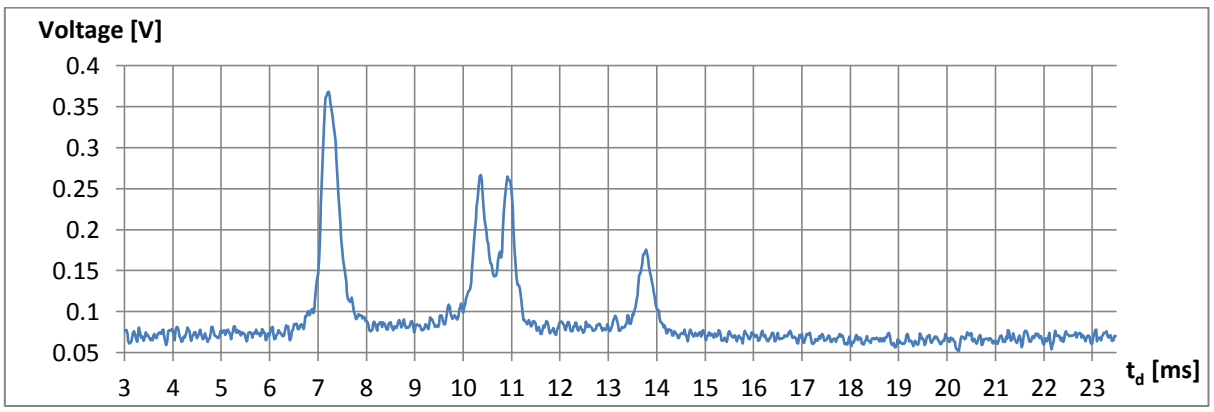

Figure 11. The spectrum of 2,4,6-TCP as a function of time.

4.2.3 SPME-IMS

SPME sampling combined with IMS device has been used for various compounds. These compounds include CWAs, heroin, cocaine, diazepam and explosives, to name a few. SPME-IMS cannot, however, be applied to the analysis of non-volatiles and thermally label analytes. To combine SPME with IMS, a thermal desorption is required to move analytes from the SPME fiber to the IMS. (Arce et al 2008, 144.)

Orzechowska et al (1997, 3.) coupled SPME device to IMS by drilling hole to the O-ring of IMS. The hole was used as an inlet in which SPME fiber was inserted and then exposed. The sample was then desorbed by IMS heat source. Membrane was used to prevent the SPME fiber contamination from desorber plate. Lokhnauth & Snow (2005, 5939.) also used a similar approach by interfacing the SPME directly to IMS. In their approach fiber was placed in the center of sampling region on a desorption tray and ejected. Then the tray was introduced to the IMS injection port. In the injection port, fiber was brought in contact with heated IMS inlet. After desorption, analytes were transferred to the drift tube by a carrier gas.

In addition, thermal desorption can also be executed by a thermal desorption unit. This can be done in practice for example with a quartz-glass tube with heating tape wrapped around the tube. Such a tube had a length of 9 cm, having an inner diameter of 5 mm. The tube was heated at 200 °C. When the sample was desorbed from the fiber, sampling pumps were used to introduce analytes to the IMS. (Arce et al 2008, 144-145.)

Also other type of desorber, called transfer-line desorber, has been developed for SPME-IMS. The transfer-line desorber consists of a stainless steel tube, which has been coated with a silico-steel. The temperature is controlled by a custom made temperature controller. The purpose of assembly described is to desorb and transfer the sample rapidly to the IMS. (Arce et al 2008, 145.) According to Arce (2008, 145) SPME-IMS systems do not have standardized coupling configuration.

THE EXPERIMENTAL PART

The experimental part of this work focuses on the analyzation process of chlorophenols from water samples. At first certain parameters are optimized to increase the SPME yield. After the optimization, calibration curves and K_0 values for target analytes are determined. Also certain validation parameters, such as LOD, repeatability and sensitivity are calculated.

After the determination of calibration curves and validation parameters, three actual water samples are analyzed. Each chapter also includes a discussion of error sources. Results obtained, excluding calibration curves are presented in chapter 7.

5 OPTIMIZATION OF PARAMETERS

Parameters to be optimized were extraction time, fiber depth, extraction temperature, and the type of fiber. In addition, the effect of NaCl addition was studied. Samples were stirred in every optimization step with magnetic stir bars. Optimization was performed for 2,4,6-TCP, 2,3,4,6-TeCP and PCP each, since they are the analytes of interest. However, in the case of PCP, optimization was not performed due to problems related to the persistence of analytical signal. The optimization was performed to ensure the optimal extraction conditions with the actual sample.

The IMS device used was a commercial Ni-IMS provided by GAS. The IMS used had a 300 MBq ^{63}Ni as a beta radiation source. Nitrogen was used as a drift gas. The carrier gas used was compressed air that was purified by molecular sieves and activated carbon filters. Samples were extracted with a manual SPME fiber assembly, provided by Supelco. 100 µm PDMS and 85 µm PA fiber were used. Also 75 µm CAR-PDMS fiber was tested in 2,3,4,6-TeCP fiber optimization. For the sample desorption a custom made brass chamber was used.

Before every test the IMS was baked in 90 °C for a half an hour to clean the device from possible contaminants. After the baking IMS drift tube temperature was set to 70 °C and was incubated for half an hour. The voltage of the IMS device was set in -3.5 kV. During the analysis, drift gas flows and carrier gas flows were set to Q_1 = 190 ml/min and Q_{drift} = 225 - 235 ml/min resulting to an exhaust gas flow Q_{out} = 440 - 450 ml/min. Q_{out} value was measured with a flow meter and it was noticed that the exhaust gas outflow was higher than the total gas inflow. The higher outflow may be caused by the thermal expansion of the gases or the inaccuracy of the flow meter. The brass chamber assembly consisted of a brass chamber and a thermal heater. The brass chamber temperature was controlled by a microcircuit equipped with a thermocouple. The brass chamber temperature was kept in 250 °C. The carrier gas entered the brass chamber from the bottom and exited the chamber on the top. The carrier gas containing the sample was forwarded to the IMS device.

Parameters were optimized for 2,4,6-TCP using a concentration of 10 mg/l. Firstly the stock solution containing 1 000 mg/l 2,4,6-TCP in EtOH was prepared. The stock solution was used for making lower concentration dilutions. The stock solution was diluted with pH adjusted

ultra clear water (UCW). The pH was adjusted with 0.5 M HCl to 2.2 pH. For parameter optimization of 2,4,6-TCP two 100 µm PDMS fibers were conditioned for half an hour in 250 °C.

To optimize parameters for 2,3,4,6-TeCP, same analyte concentration, 10 mg/l was used. However, a 101.8 mg/l stock solution was prepared in EtOH, since the amount of 2,3,4,6-TeCP purchased from Sigma Aldrich was not sufficient enough for 1 000 mg/l concentration. The procedure in dilution preparation was otherwise same as with 2,4,6-TCP. The same PDMS fibers were used as with 2,4,6-TCP until the extraction time determination. For the determination of extraction time and following optimization, new PDMS fibers were conditioned.

For PCP parameter optimization, stock solution with 1005.2 mg/l concentration of PCP was prepared. As with other chlorophenols, also PCP was diluted into EtOH. The stock solution was then used to prepare solution with concentration of 10 mg/l. The dilution was again made with pH adjusted water, having a pH value of 2.2. However it was later noticed during the analysis that PCP gave a long lasting signal. Therefore the determination of optimal parameters for PCP was passed.

For sample extraction, a water bath was arranged. 10 ml of sample was put into 22 ml vial provided by Supelco. The each sample was stirred with a magnetic rod. Vials were placed in a rack which was put into a water bath. The bath was heated by a hot plate stirrer and the bath temperature was observed with an analogous thermometer. The temperature was then adjusted manually according to the thermometer reading. In the beginning of an every optimization of a desired parameter MSA was used as a reference compound to adjust the mobility scale. The measurements were carried out in a negative mode of IMS. The measurements were repeated three times with every step of parameter optimization. SPME fibers were cleaned in the brass chamber in 250 °C temperature after sampling. The brass chamber assembly is presented in figure 12 and the thermal bath is presented in figure 13.

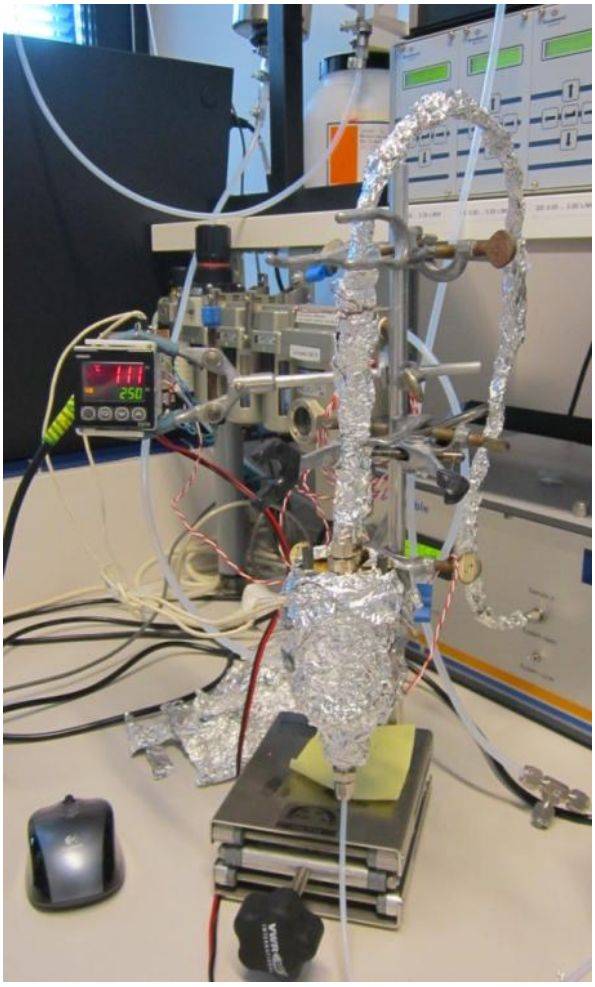

Figure 12. The brass chamber assembly.

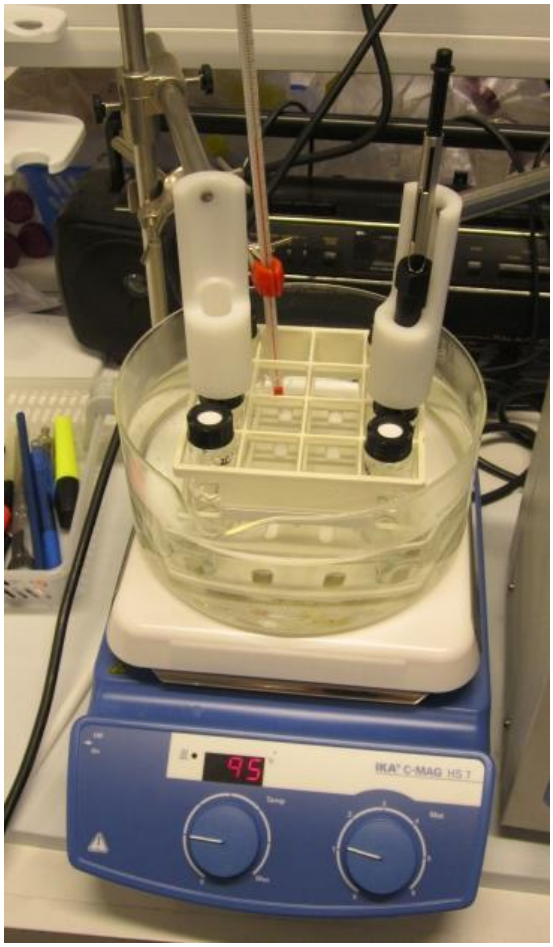

Figure 13. The water bath with vials, SPME instrument and thermometer.

The data was analyzed with a custom made program, Integrator 01, which calculated the peak area by integrating. The mean values of the peak areas for every step of a parameter optimization were then calculated. For example the analyte peak areas for 30 min extraction were first added together and the average was calculated. Then the curve of peak areas was plotted as a function of an observed parameter, for example time in this case. From the curve, the most suitable value was chosen.

In the next subchapters certain optimization parameters are studied. Although the results for 2,4,6-TCP and 2,3,4,6-TeCP are presented in same chapters, the analysis of all parameters were first ran with 2,4,6-TCP. After completion of all measurements with 2,4,6-TCP, measurements with 2,3,4,6-TeCP were started.

5.1 Extraction time

To find out the best possible extraction time for 2,4,6-TCP and 2,3,4,6-TeCP, 5 min, 10 min, 15 min, 20 min, 25 min and 30 min extraction times were observed. As mentioned earlier, target analyte concentration of 10 mg/l was used for optimization. 2,4,6-TCP solution was diluted with a pH controlled UCW, which pH was adjusted to 2.212. The vials were placed in a thermal bath of 60 °C. The extraction was performed with two PDMS fibers and vials with a 3.5 cm fiber depth. The extraction was performed three times with each extraction time. Same procedure and parameters were used in the case of 2,3,4,6-TeCP, excluding the fact that dilution UCW had a 2.208 pH and the fact that also 35 and 40 min extraction times were studied. The extraction time optimization for 2,4,6-TCP and 2,3,4,6-TeCP was performed in different days.

The curve was then plotted in the basis of analyzed data. The response curve for 2,4,6-TCP is presented on the figure 14 and the response curve of 2,3,4,6-TeCP in figure 15.

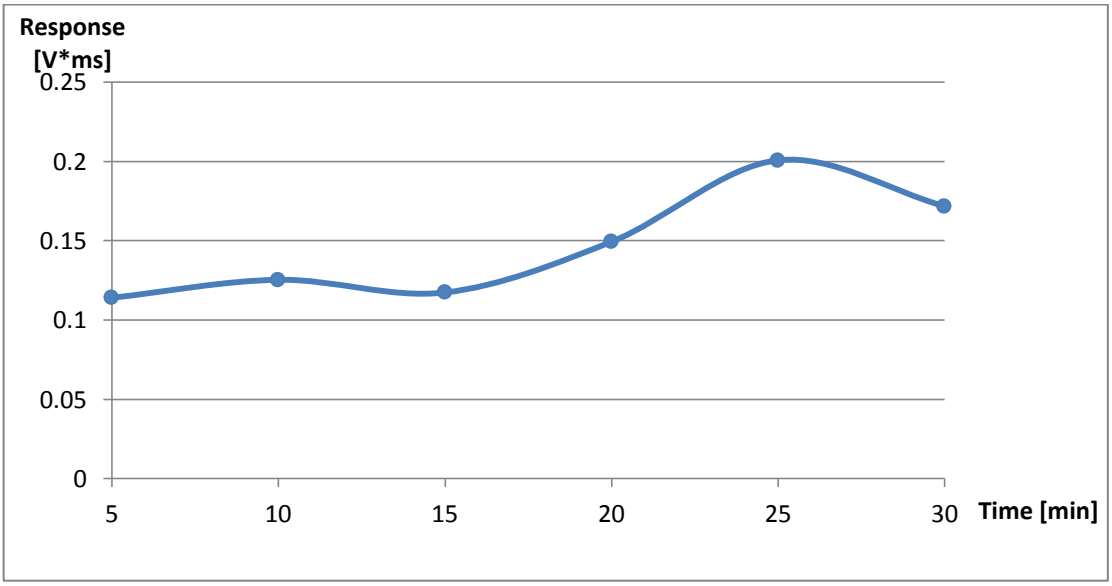

Figure 14. The effect of extraction time to 2,4,6-TCP.

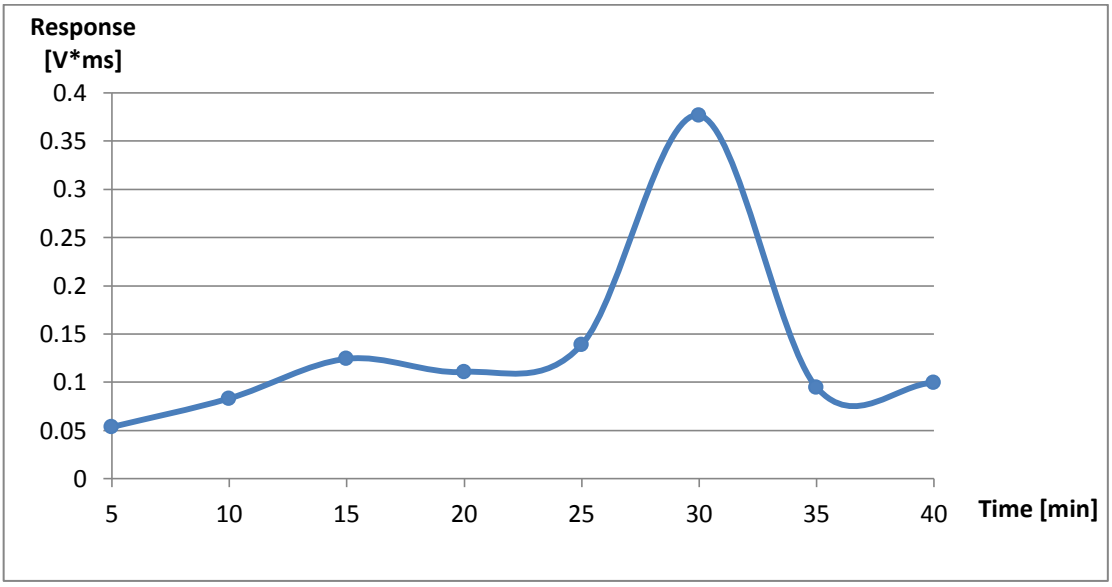

Figure 15. The effect of extraction time to 2,3,4,6-TeCP.

On the basis of the figure 14, the extraction time of 20 minutes was chosen to be used in later analysis for 2,4,6-TCP since it gave a good response and fitted best for the analysis cycle. However, it was noticed that with a longer extraction time analytical signal lasted longer.

As can be seen from the figure 15, 30 minute extraction time is the most optimal for 2,3,4,6-TeCP. However in the rest optimization steps, 15 minute extraction time was used for 2,3,4,6-TeCP, since it yielded in good responses and sped up the analysis cycle.

5.2 Fiber depth

In the optimization of fiber depth, following depths were used: 1.5 cm, 2.5 cm, 3.5 cm and 4 cm. The minimum depth of 1.5 cm was chosen since with lower fiber depths the needle did not penetrate the septum properly. Also the maximum depth was dictated by the height of liquid phase in the vial. With greater depths the fiber could have been brought in contact with the sample liquid. Depths mentioned were used for both analytes of interest. However the extraction time for 2,4,6-TCP was 20 minutes and for 2,3,4,6-TeCP 15 minutes. The extraction temperature was kept at 60 °C.

The measurements were performed with a two 100 µm PDMS fibers and vials. The data was analyzed in a same manner as in previous chapter. This time the curve was plotted as a func-

tion of fiber depth. The curve for 2,4,6-TCP is presented in figure 16 and for 2,3,4,6-TeCP in figure 17.

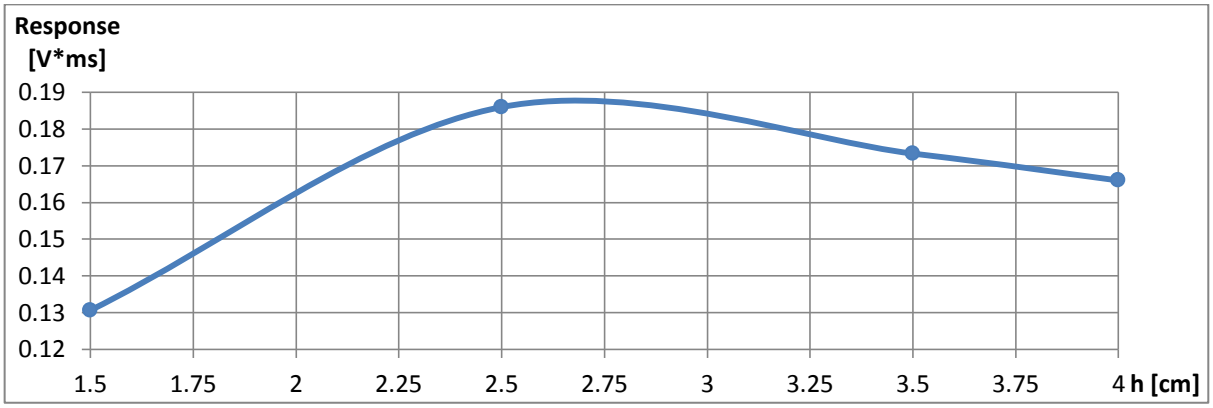

Figure 16. The effect of fiber depth to 2,4,6-TCP.

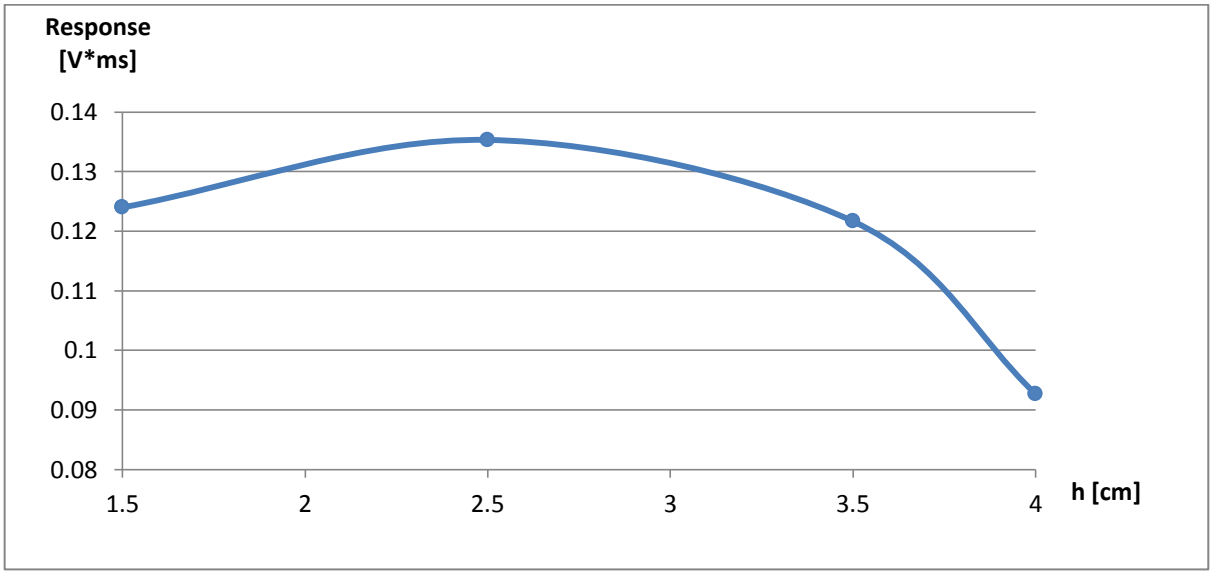

Figure 17. The effect of fiber depth to 2,3,4,6-TeCP.

Based on the figure 16, the fiber depth of 2.5 cm was chosen to be used in latter optimization of 2,4,6-TCP. It was observed that the depth of 1.5 cm gave a noisy analytical signal and the signal improved with 2.5 and 3.5 cm depths. The greater fiber depths yielded longer lasting analytical signals.

As can be seen from the figure 17, 2.5 cm seems to be the most suitable fiber depth for 2,3,4,6-TeCP. With low depths EtOH gave a disturbing signal at first, although it weakened after a few scans.

The changes in measuring environment and possible sources of errors were also considered. One variable is the evaporation of the water from thermal bath and the changes in bath temperature. Also changes in the vial positions could have an effect to the SPME fiber extraction process. In addition adjusting the needle depth manually can cause slight variations to the actual depth of the fiber. It was also noticed that during the measurements electrical noise was observed. The electrical noise had an effect to IMS signal. This may affect to the background correction of Integrator 01 program. Inaccurate background correction may lead to errors in calculated peak responses.

5.3 Extraction temperature and salt addition

The optimization of extraction temperatures was performed with 2.5 cm fiber depth and 20 minutes extraction time for 2,4,6-TCP. The tested temperatures were 40 °C, 50 °C and 60 °C. For 2,3,4,6-TeCP extraction time was kept in 15 minutes, fiber depth at 3.5 cm and tested temperatures were 40 °C, 50 °C, 60 °C and 70 °C. In the case of 2,3,4,6-TeCP the earlier optimized parameters were not used, since they were not analyzed until the end of parameter optimization.

The data obtained from the measurements was then analyzed and plotted in curves. The response curve for 2,4,6-TCP is presented in figure 18 and for 2,3,4,6-TeCP in figure 19.

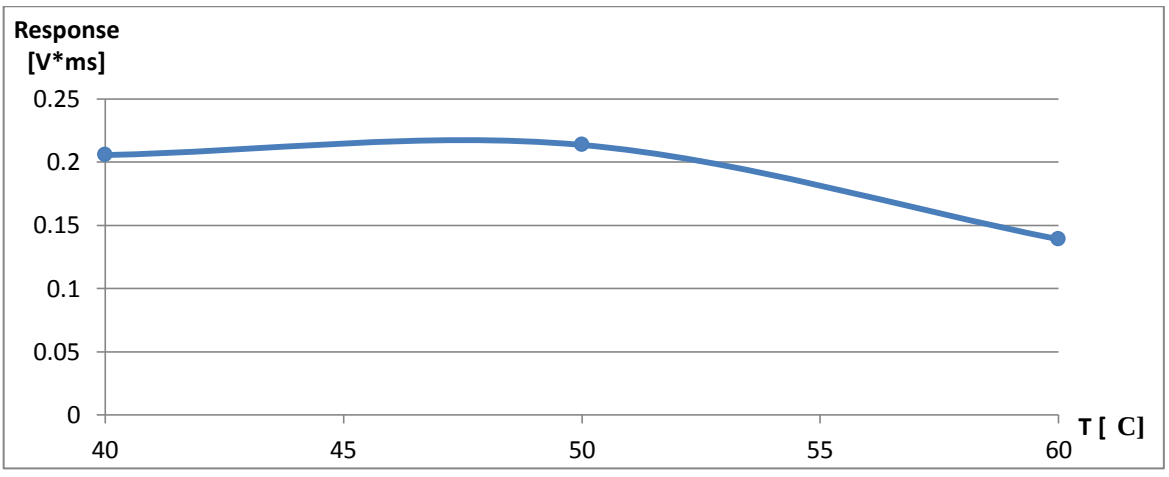

Figure 18. The effect of extraction temperature to 2,4,6-TCP.

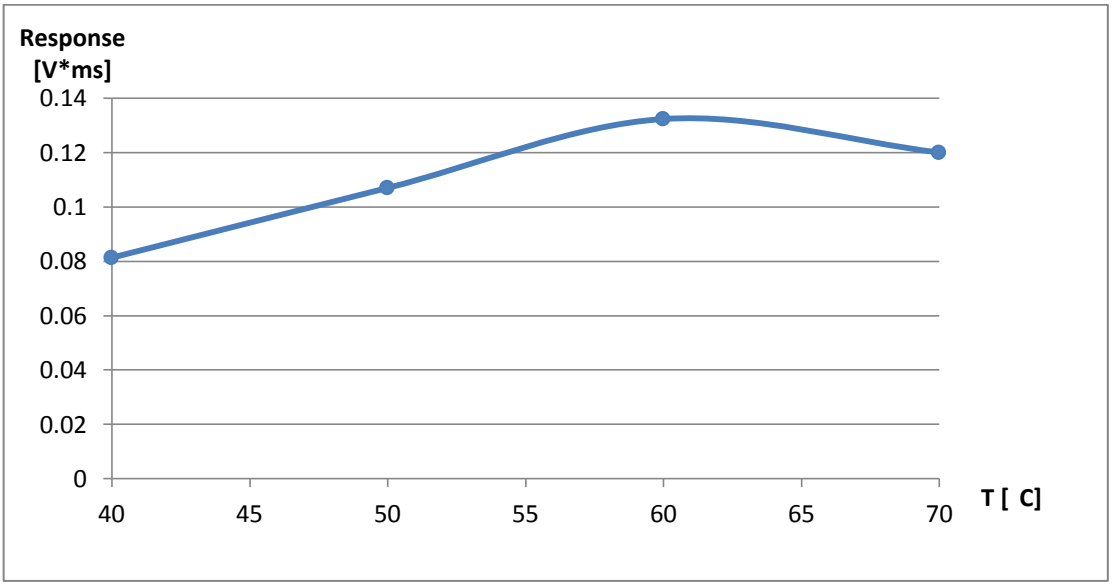

Figure 19. The effect of extraction temperature to 2,3,4,6-TeCP.

It can be seen from the figure 18 that the best extraction temperature for 2,4,6-TCP is 50 °C. However, with 60 °C the analytical signal was most persistent.

The most suitable extraction temperature for 2,3,4,6-TeCP was 60 °C. In 70 °C increased responses for EtOH were detected. The optimization of the extraction temperature was particularly challenging because the temperature of the thermal bath can easily alternate. When the target temperature was reached, the water bath was left to incubate for 5 - 10 minutes. After incubation extraction was started. The temperature varied within ± 2 °C of the target temperature.

The effect of salt addition was also studied. This was done in the same day than the temperature optimization. For 2,4,6-TCP, 100 g/l of NaCl was added to the vials and were left to incubate and mix for an hour in 60 °C. After the incubation, extraction was performed. For 2,3,4,6-TeCP the same procedure was used, except the fact that extraction temperature was set to 70 °C.

The results of NaCl addition for 2,4,6-TCP are presented in figure 20 and for 2,3,4,6-TeCP in figure 21. It can be seen from the figure 20 that salt addition improved the extraction efficiency of 2,4,6-TCP for 42 % and was therefore used in the rest of the optimization procedure of

2,4,6-TCP. As can be seen from the figure 21, salt addition improved the responses of 2,3,4,6-TeCP for 10 %.

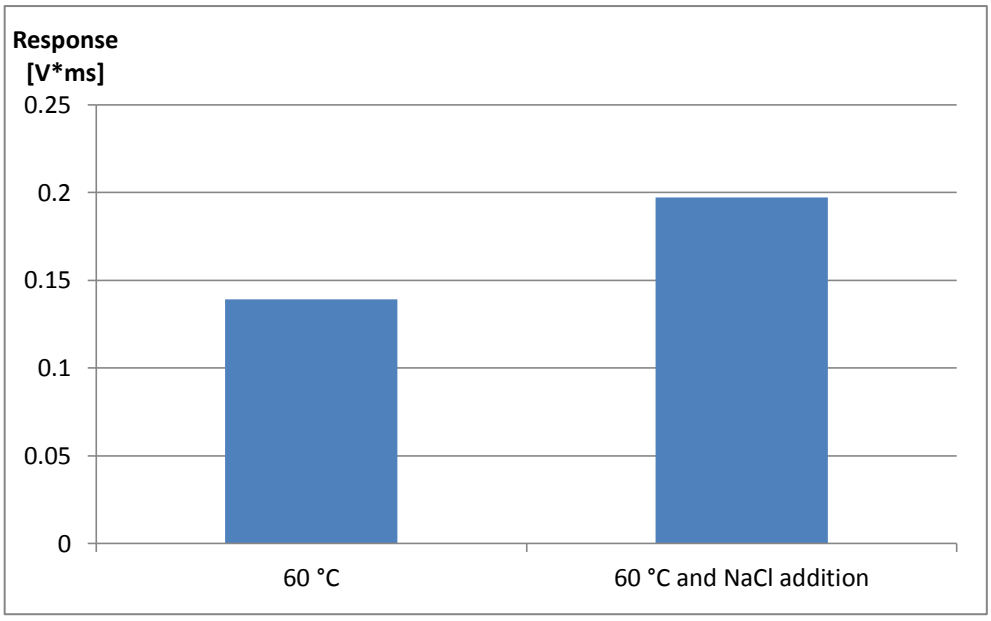

Figure 20. The effect of NaCl addition to 2,4,6-TCP response.

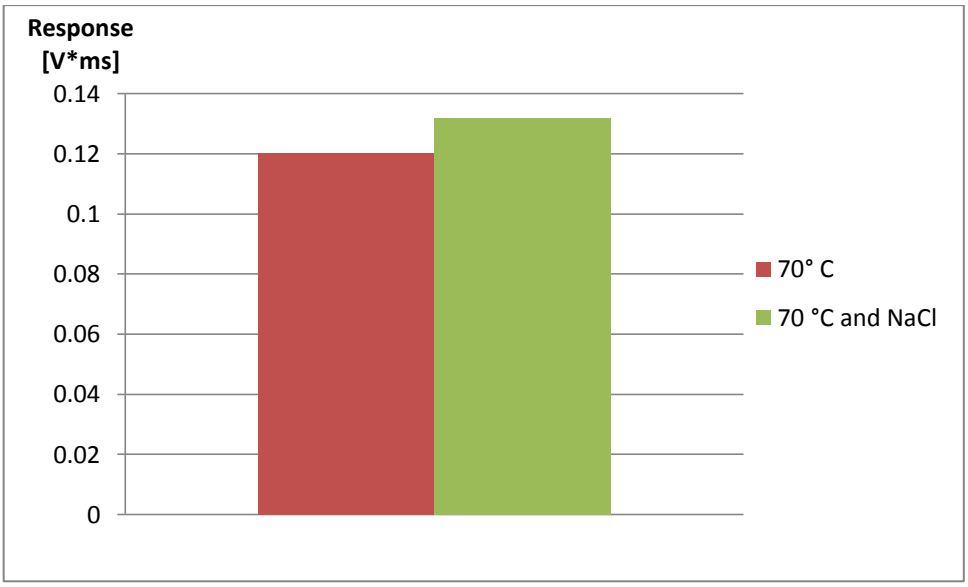

Figure 21. The effect of NaCl addition to 2,3,4,6-TeCP response.

5.4 Type of fiber

Effects of the fiber type to the extraction efficiency were observed for both 2,4,6-TCP and 2,3,4,6-TeCP. In the optimization of fiber type, earlier observed NaCl addition was used. The

observed fibers were 85 µm PA and 100 µm PDMS for 2,4,6-TCP. The extraction temperature was set to 60 °C and the fiber depth was 2.5 cm and extraction time 20 minutes. Responses for PA and PDMS fibers are presented in figure 22.

To choose the most appropriate type of fiber for 2,3,4,6-TeCP, three different fibers were tested: 100 µm PDMS, 85 µm PA and 65 µm CAR-PDMS fibers. The efficiency of fibers was studied after the test series of fiber depth. The extraction was performed with each fiber at 3.5 cm depth and in 60 °C extraction temperature. The responses of different fiber materials with 10 mg/l concentration of 2,3,4,6-TeCP are presented in the figure 23.

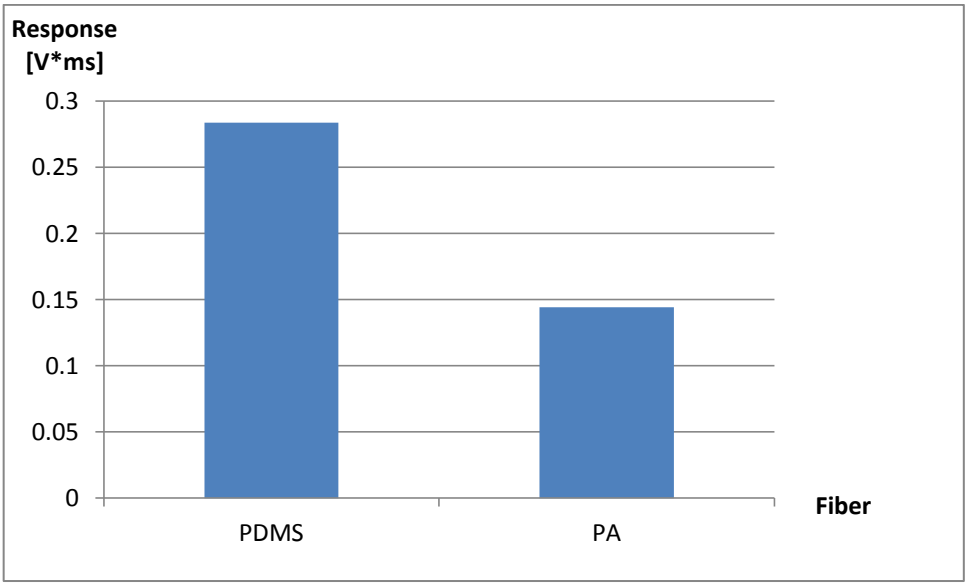

Figure 22. The effect of fiber type to 2,4,6-TCP.

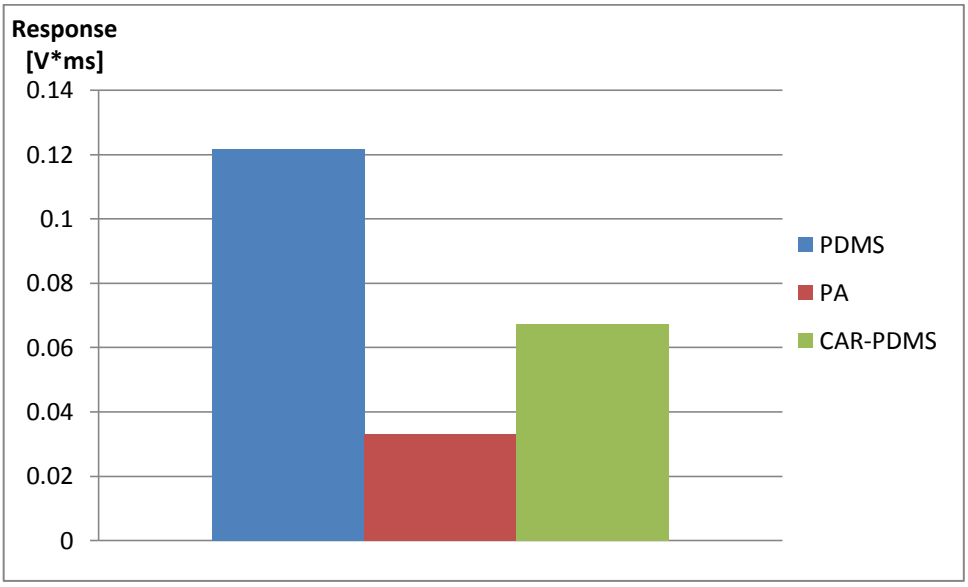

Figure 23. The effect of fiber type to 2,3,4,6-TeCP.

It can be seen from the figure 22 that PDMS fiber yielded better results for 2,4,6-TCP. PA fiber, however gave a longer lasting but more interferenced signal and poorer response. There were also differences between PA fibers, while other PA fiber gave a weak signal, the other signal was strong. However, the stronger signal was not as intense as any signal obtained by PDMS.

It can be said in the basis of figure 23 that the use of PDMS fiber yielded in the best results for 2,3,4,6-TeCP. The CAR-PDMS fiber also yielded good results whereas PA fiber responses were quite poor. With PA fiber extraction, ethanol disturbed the signal more than with the other fibers.

6 ANALYZATION AND METHOD VALIDATION

In this chapter, calibration curves for each chlorophenol of interest are determined. Calibration curves are needed, since the analyte concentration determination in real water samples is performed with the aid of calibration curves. After the determination of calibration curves, three real water samples are analyzed.

To determine the suitability of SPME-IMS system for chlorophenols, certain validation parameters should be determined. In this work, validation parameters to be determined are LOD, repeatability, sensitivity and extraction recovery. The determination of validation parameters is presented before analyzing actual water samples. However, the actual water samples were analyzed before determining the validation parameters and moving to the new facility.

6.1. Determination of the calibration curves

The determination of the parameters to be applied with the concentration series of 2,4,6-TCP, 2,3,4,6-TeCP and PCP was based on the obtained data. The parameters which were used in calibration curve determination were: 20 minute extraction, 3 cm fiber depth, 55 °C extraction temperature and PDMS fiber. The drift tube temperature was kept at 70 °C and the desorption temperature at 250 °C. The voltage was set to -3.5 kV.

20 minute extraction was chosen because of the optimization of a time usage. In addition, although signals were not as intense as possible, they were more persistent. The persistence of signals was also the reason for the fiber depth of a 3 cm, since 2.5 cm gave the most intense response and with 3.5 cm depth the signal was more persistent. Therefore 3 cm was chosen for the fiber depth. The extraction temperature was chosen to be 55 °C, since it was linearly between the optimal extraction temperatures of 2,4,6-TCP and 2,3,4,6-TeCP.

NaCl addition in 100 g/l concentration was used since it improved analyte responses and all vials were stirred. Also new PDMS fibers were conditioned to determine the calibration curves as accurately as possible. PDMS fibers were conditioned with the same procedure as earlier. When determining calibration curves, also reduced mobilities were calculated. In the

following chapters spectra for target chlorophenols are presented in 5 mg/l concentration. With this concentration, differences in peak intensities can be observed.

6.1.1 2,4,6-TCP

Concentrations of 0, 0.25, 0.5, 1, 2.5, 5 and 10 mg/l were used. Also 15 mg/l concentration was prepared but the response was so intense that RIN peak disappeared. Therefore 15 mg/l concentration was excluded from 2,4,6-TCP calibration. The pH of dilution water used was 2.205. The dilutions are presented in the table 1. 10 mg/l concentration solution was used also for preparing solutions with concentrations less than 5 mg/l.

Table 1. The dilutions of concentration series.

The final concentration [mg/l]	Added stock solution [ml]	Stock solution concentration [mg/l]	Volume of solution [ml]
15	0.375	1000	25
10	0.5	1000	50
5	0.125	1000	25
2.5	6.25	10	25
1	2.5	10	25
0.5	1.25	10	25
0	0	-	25

The spectrum of 2,4,6-TCP in 5 mg/l concentration is presented in figure 24. Calibration curves for concentrations of 0 – 10 mg/l and 0 – 1 mg/l of 2,4,6-TCP are presented in figures 25 and 26.

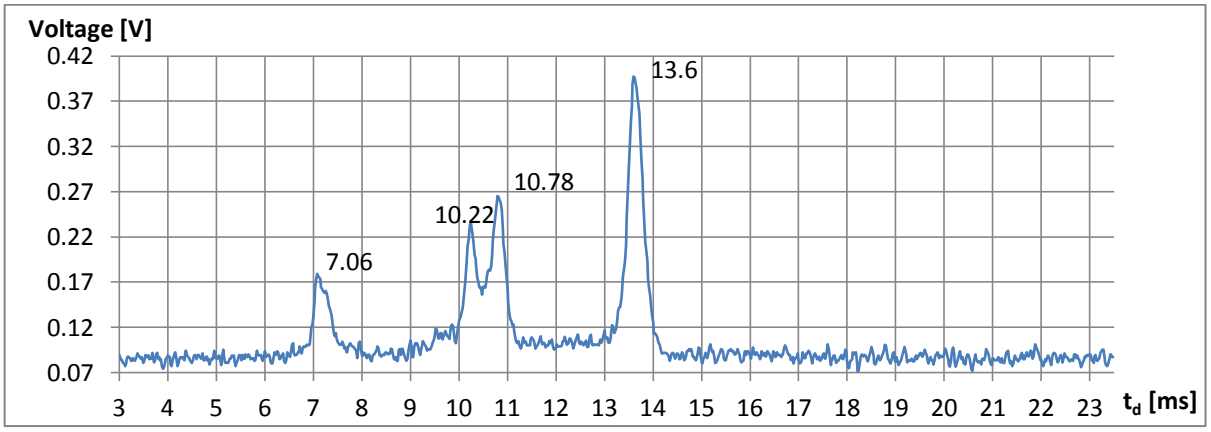

Figure 24. Spectrum of 2,4,6-TCP in 5 mg/l concentration.

It can be seen in from the figure 24 that four peaks are present in 5 mg/l concentration. The one in the left is a RIN response and two following peaks are monomer responses. The peak in the right is a response for dimer. Reduced mobilities were calculated for 2,4,6-TCP with equation 8 and MSA was used as a reference compound. Reduced mobilities were for RIN: 2.30 cm^2/Vs, for monomers: 1.60 and 1.51 cm^2/Vs and for dimer 1.20 cm^2/Vs.

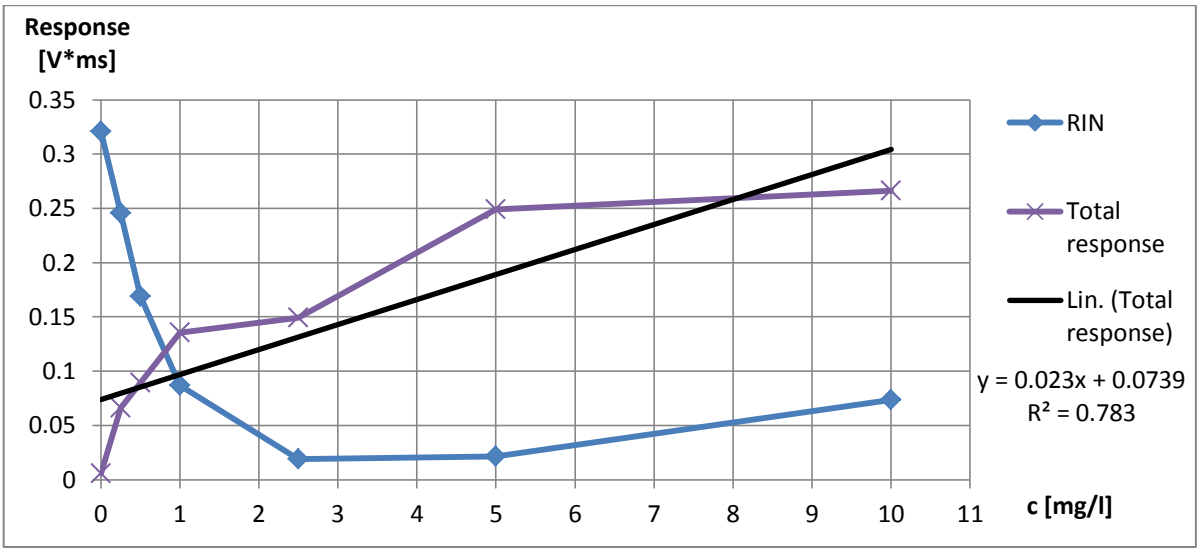

Figure 25. 2,4,6-TCP calibration curve for concentrations of 0 – 10 mg/l.

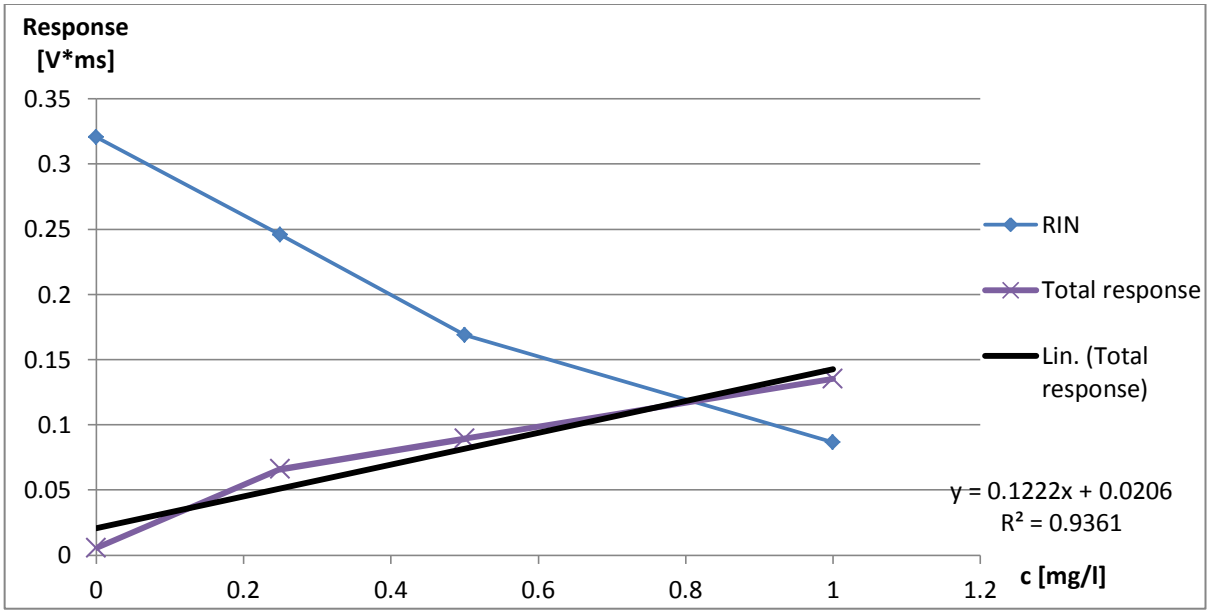

Figure 26. 2,4,6-TCP calibration curve for concentrations of 0 – 1 mg/l.

Responses for RIN and the total responses of monomer and dimer together are presented in the calibration curves. The trendline, presented as a black line in the picture, is based on the total response. As it can be seen from the figure 25, the total response is not very linear. Therefore, to obtain the better linearity of the trendline, new figure was drawn for the concentrations of 0 – 1 mg/l. This improved linearity.

The possible sources of error in the calibration curve determination were slight changes of voltage in the IMS and the changes in nitrogen flow. In the data analyzation some errors may be encountered by the background noise removal in the Integrator 01 program. Also, possible inaccuracy of pipettes used in making dilutions and the possible differences in PDMS fibers may cause errors. Also the fact, that the calibration curve was determined in two days, may cause between-a-day variation.

6.1.2 2,3,4,6-TeCP

In 2,3,4,6-TeCP calibration curve determination, concentrations from 0 – 15 mg/l were used, since 2,3,4,6-TeCP gave lower responses. The UCW used for dilutions had the pH value adjusted to 2.2. The dilutions are presented in table 2.

Table 2. The dilutions for concentration series.

The final concentration of the solution [mg/l]	Concentration of stock solution [mg/l]	Stock solution added [ml]	Volume of solution [ml]
14.9646	101.8	2.94	20
10.01712	101.8	4.92	50
5.01365	101.8	0.985	20
2.50428	10.01712	5	20
1.001712	10.01712	2	20
0.500856	10.01712	1	20
0.250428	10.01712	0.5	20
0	10.01712	0	20

The spectrum for 5 mg/l 2,3,4,6-TeCP concentration is presented in figure 27. The calibration curves for 2,3,4,6-TeCP are presented in figures 28 and 29.

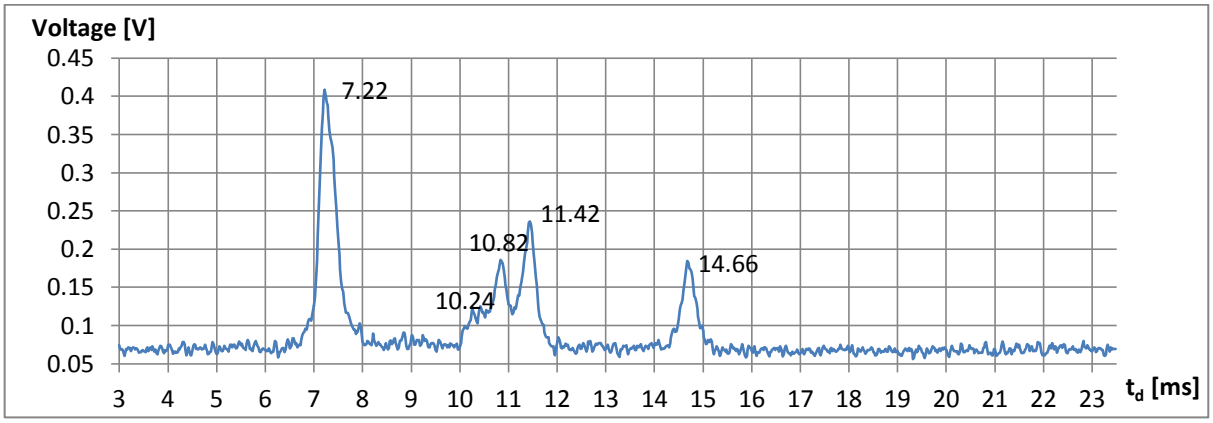

Figure 27. Spectrum of 2,3,4,6-TeCP in 5 mg/l concentration.

From figure 27, five response peaks can be noticed. The peak in 7.22 ms is RIN peak and peaks in 10.24 – 11.42 ms range are monomer responses. The peak in 14.66 ms is a response for dimer. As for 2,4,6-TCP reduced mobilities were calculated in a same manner with 2,3,4,6-TeCP. Reduced mobilites were for RIN: 2.30 cm^2/Vs, for monomers: 1.61, 1.53 and 1.46 cm^2/Vs and for dimer: 1.13 cm^2/Vs.

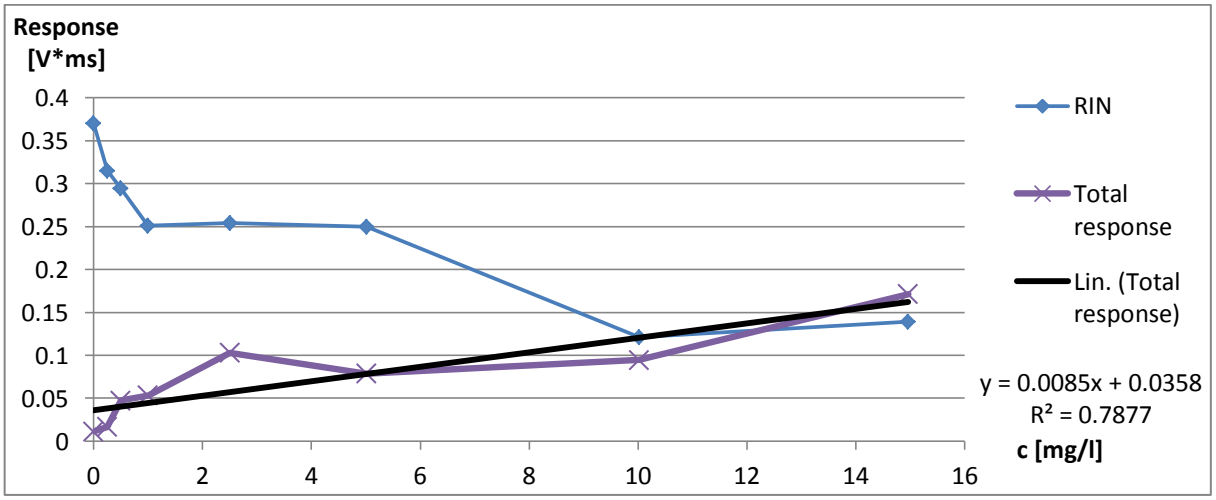

Figure 28. Calibration curve for 2,3,4,6-TeCP in concentration 0 – 15 mg/l.

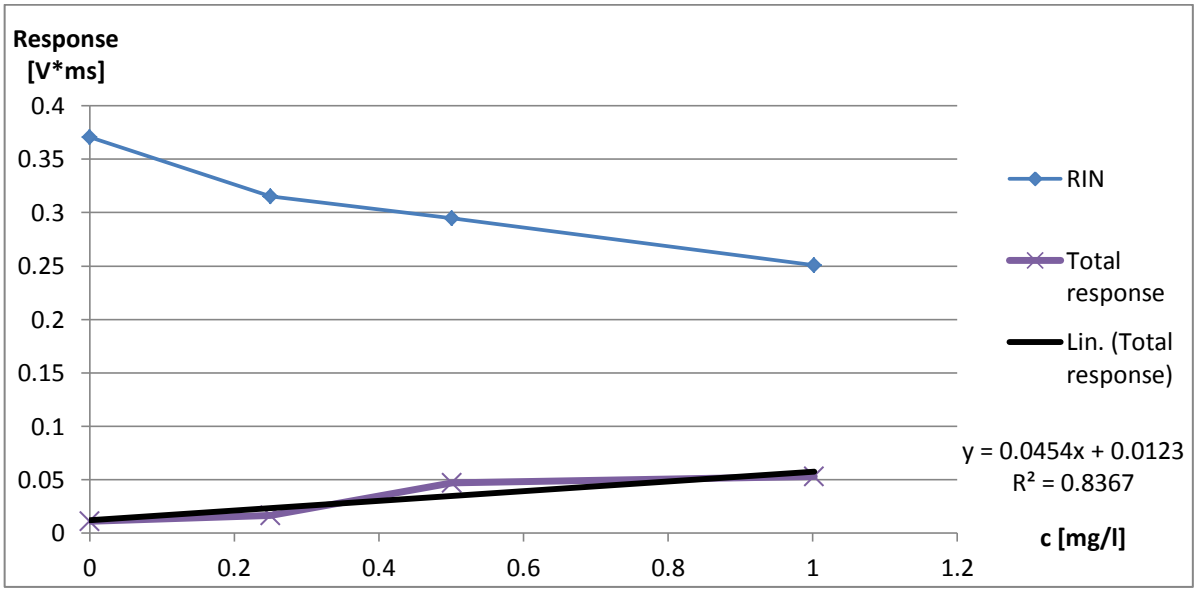

Figure 29. Calibration curve for 2,3,4,6-TeCP in concentration 0 – 1 mg/l.

For some reason 2,3,4,6-TeCP did not produce as linear responses as 2,4,6-TCP. The best responses were obtained with concentrations of 15 and 2.5 mg/l. As can be seen from the figures 28 and 29, the linearity of the calibration curve is not as good as it was with the 2,4,6-TCP. However, in the range of 0 – 1 mg/l, linearity of the trendline is sufficient. The trendline is drawn for total response.

These unexpected errors may be caused by the higher background noise, which was present in measurements. Background noise levels varied remarkably during the tests and could have caused the anomalities observed. Also two automated pipettes were used. These two pipettes may have had effect on pipetted volumes. In addition, the fact that measurements were performed in two different days can cause variation in results.

6.1.3 PCP

The calibration curve for PCP was produced in a same manner as earlier. The dilutions are presented in the table 3. The pH of UCW was 2.202.

Table 3. Dilutions for concentration series.

The final concentration of the solution [mg/l]	Concentration of stock solution [mg/l]	Stock solution added [ml]	Volume of solution [ml]
15.078	1005.2	0.3	20
10.052	1005.2	0.5	50
5.026	1005.2	0.1	20
2.502948	10.052	4.98	20
1.0052	10.052	2	20
0.5026	10.052	1	20
0.2513	10.052	0.5	20
0	10.052	0	20

Analyzation of PCP yielded in lower responses than 2,4,6-TCP and 2,3,4,6-TeCP. The signal for PCP was not obtained until the concentration of 2.5 mg/l. This could, however be related to the higher boiling point of PCP, which can lead to lower analyte concentration in the vial headspace. Although the signal for PCP was lower and took more time to be detected, the response remained longer. Therefore the desorption chamber had to be flushed after every measurement with carrier gas flow of 0.5 l/min for 0.5 – 1.5 hours, depending on the measured concentration.

The response also grew more intense after removal of the fiber from desorption chamber. Therefore, in the calculation of the calibration curve, the mean value of responses for both with and without fiber was used. In the case of PCP, the response for 10 mg/l was the highest. This can be caused by the possible fiber carryover or the fact that fiber could have started to worn out. The spectrum for PCP in 5 mg/l concentration is presented in figure 30. Calibration curves for PCP are presented in figures 31 and 32.

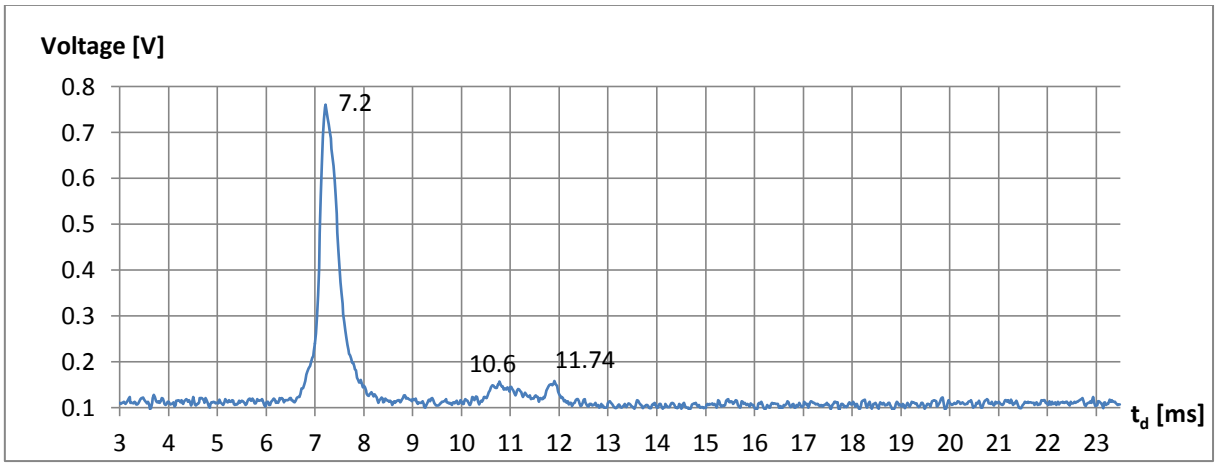

Figure 30. Spectrum of PCP in 5 mg/l concentration.

As figure 30 shows, only three peaks are present in 5 mg/l concentration: RIN peak at left and monomer peaks at right. However with higher concentrations, also dimer peak is present. Reduced mobilities were calculated also for PCP. Following values were obtained for RIN: 2.31 cm^2/Vs, for monomers: 1.55 and 1.39 cm^2/Vs and for dimer: 1.07 cm^2/Vs.

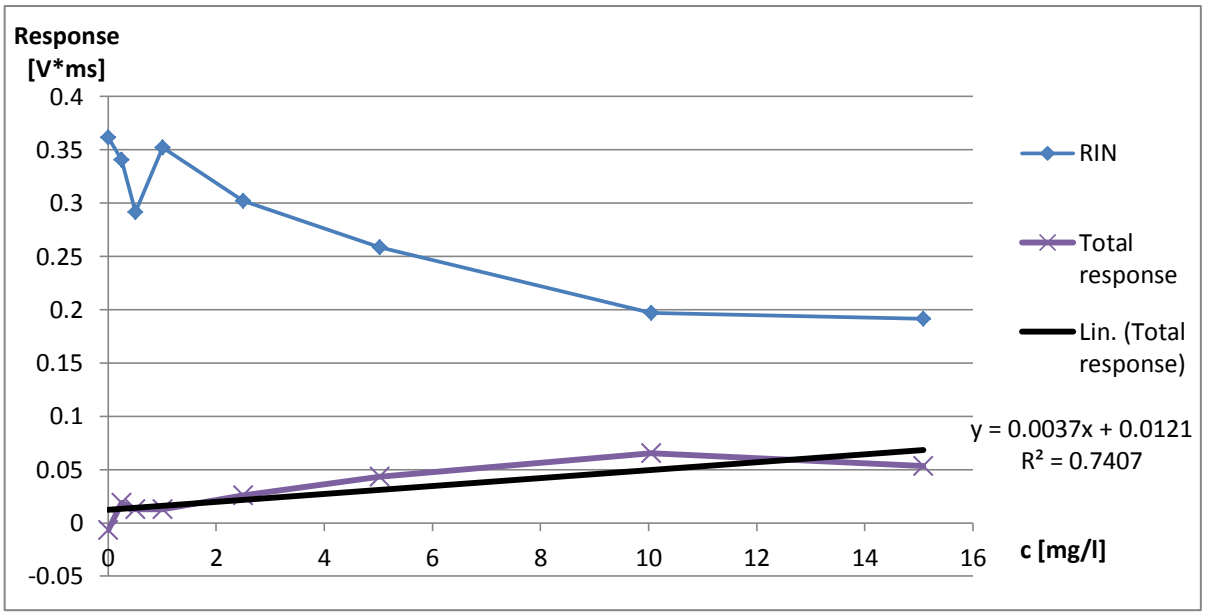

Figure 31. Calibration curve for PCP in concentration 0 – 15 mg/l.

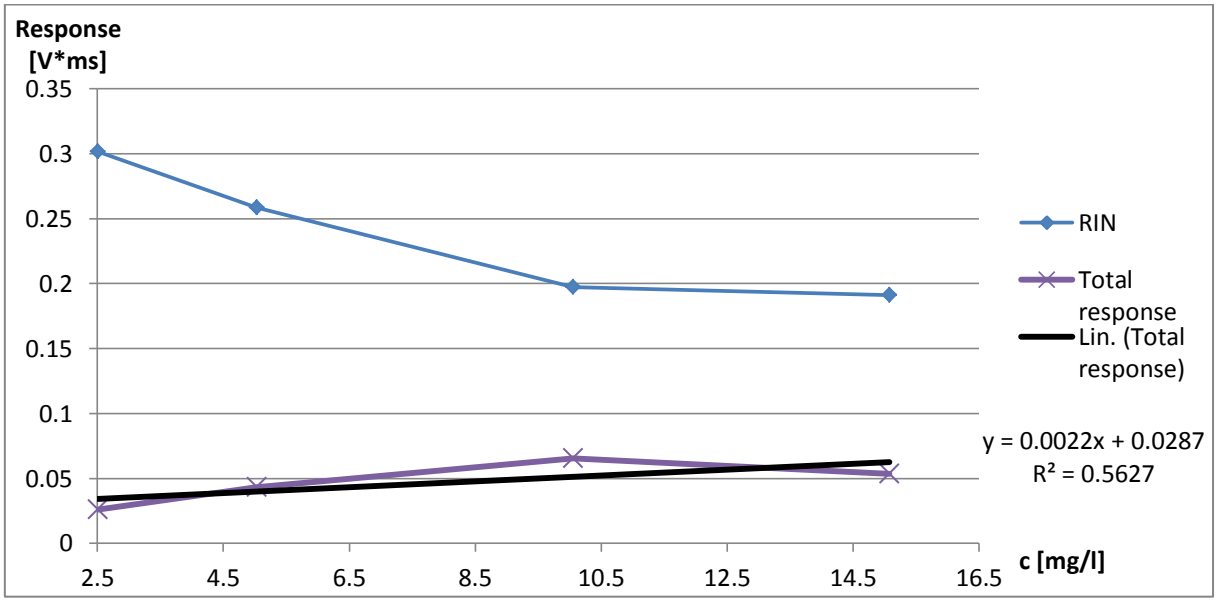

Figure 32. Calibration curve for PCP in concentration 2.5 – 15 mg/l.

As can be seen from the figure 31, a small response can be seen in the concentration of 0.25 mg/l. This is however caused by the inaccurate background correction caused by the Integrator 01. Since no appropriate response was not detected until 2.5 mg/l, the representative calibration curve can be plotted only for concentrations 2.5 – 15 mg/l. From the figure 32 can be seen that in the concentrations of 2.5 – 15 mg/l the linearity is quite poor. As earlier, the trendline is again drawn for total response.

As mentioned earlier, flaws in background correction were the most likely source of error in these measurements. Also possible inaccuracy of automated pipettes may be a source of error. Responses for PCP were measured within various days due to the signal persistency. Since measurements were divided for various days, there may be between-a-day variations present in responses.

6.2 Determination of validation parameters

LOD values were calculated for each chlorophenol of interest. LODs were calculated for the desired concentration as follows:

$$LOD = 3 * r_{\mathrm{bn}} * \frac{c}{r_{\mathrm{peak}}} \quad (12)$$

in which, LOD = detection limit [mg/l]
r_{bn} = response for the noise above the background signal [V*ms]
c = observed concentration for LOD calculation [mg/l]
r_{peak} = response of the peak [V*ms]
(Kanu et al 2006, 54).

LODs were calculated for each chlorophenol in a 5 mg/l analyte concentration. Sensitivity was obtained from the slope of the calibration curves, which were presented earlier in figures 26, 29 and 32. Repeatability can be determined from the standard deviation of repeated results made in same concentration. If proportional values are desired, the standard deviation is divided by the mean value of repetitions.

The extraction recovery *R* was determined by using pH adjusted lake water from Pankalampi, Mikkeli. According to MIKES (2005, 33) the extraction recovery can be calculated by comparing the new results to the results obtained earlier with a concentration selected for observation.

$$R = \frac{r_1}{r_2} \tag{13}$$

in which, R = extraction recovery
r_1 = response for an observed concentration in the new matrix
r_2 = response for an observed concentration in the reference matrix

The recovery determination was based on 2,4,6-TCP concentration series. The series was prepared in the same way as earlier. The initial pH of Pankalampi water was 6.567 and it was adjusted to 2.205 with 0.5 M HCl. Dilutions were made with same concentrations as earlier with 2,4,6-TCP, although 15 mg/l concentration was left out because of the RIN peak disappearance caused by the high response. Dilutions are presented in table 4.

Table 4. Dilutions made in Pankalampi water.

The final concentration of the solution [mg/l]	Concentration of stock solution [mg/l]	Stock solution added [ml]	Volume of solution [ml]
10	1000	1	100
5	10	25	50
2.5	10	12.5	50
1	10	5	50
0.5	10	2.5	50
0.25	10	1.25	50
0	10	0	50

10 ml of dilutions were pipetted in 3 vials per one concentration. As earlier, magnetic stir bars and 100 g/l of NaCl was added into each vial. The rest of the parameters were same as earlier.

During the measurements, IMS obtained rather high background noise and disturbance from an unknown source. Reason for the elevated levels of the background noise may be caused by the moving to the new facility. An example of these disturbances is presented in figure 33

Despite the disturbances in signal, it seemed that there were no differences in matrix effects between the UCW and Pankalampi water. However it was found out visually during the measurements that RIN peak disappeared in 10 mg/l concentration instead of 15 mg/l as earlier. Therefore the calibration curve presented in figure 35 is plotted for 0 – 5 mg/l concentrations.

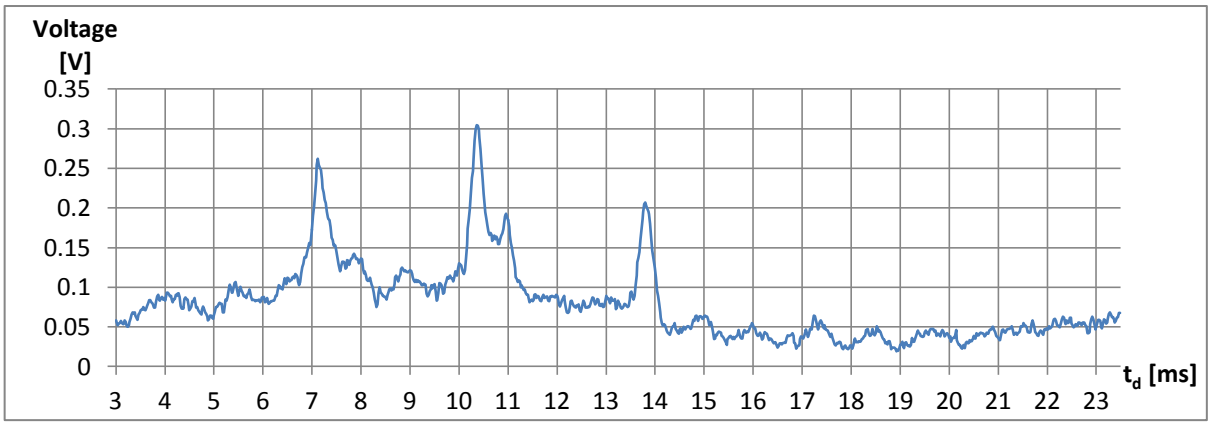

Figure 33. Disturbance observed during the measurements.

This occurrence can be caused by the fact that although the measurement setup was the same, the installation was not identical in the new facility. Also the accuracy of pipettes used may have caused the disappearance of RIN in lower concentrations. It may also be possible that 2,4,6-TCP can give a better response in real matrix.

Obtained data was analyzed using Integrator 01 program. The calibration curve based on the responses for 0 – 1 mg/l is presented in figure 34 and for 0 – 5 mg/l in figure 35.

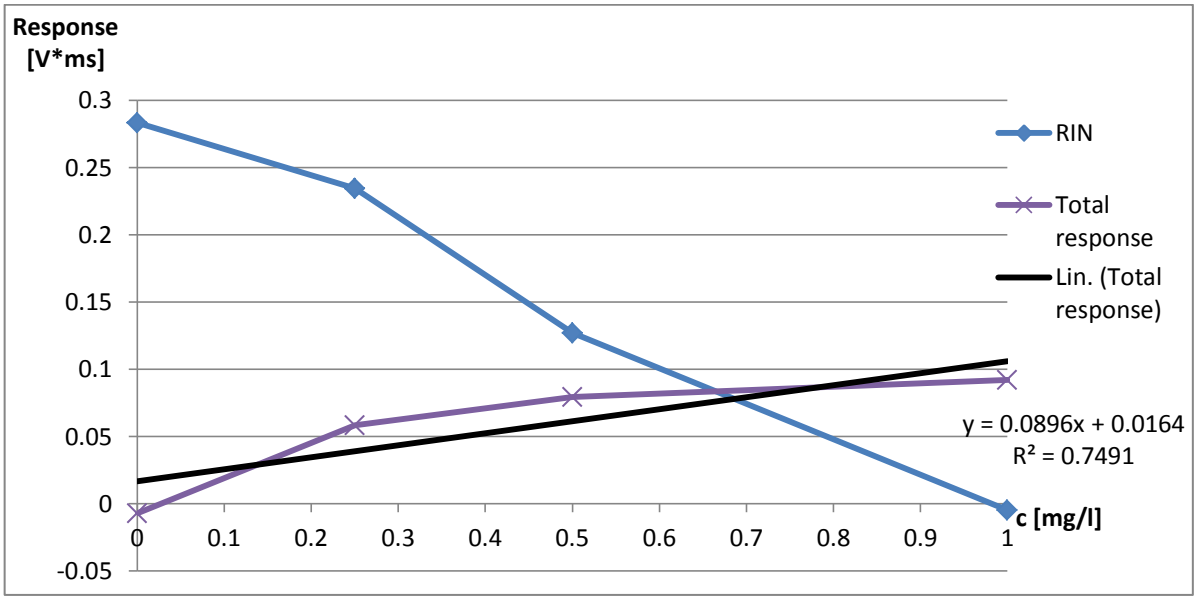

Figure 34. Calibration curve for 0 – 1 mg/l in Pankalampi water.

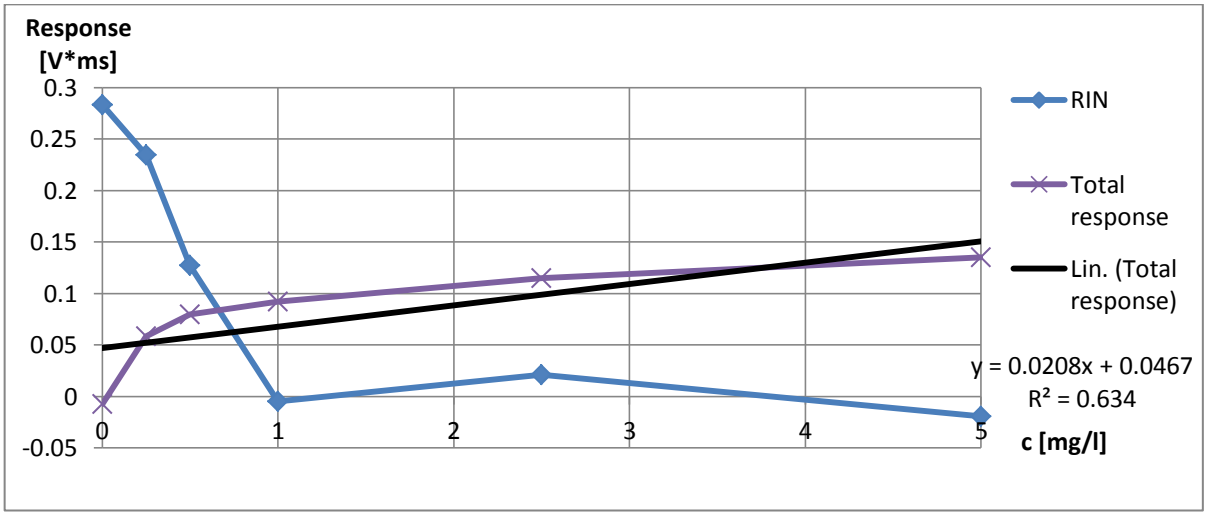

Figure 35. Calibration curve for 0 – 5 mg/l prepared in Pankalampi water.

Calibration curve based on the water of Lake Pankalampi seem to be consistent. However during the calculation of responses with Integrator 01, problems were encountered. Firstly the high background noise, an example is presented in figure 33, complicated the response determination due to problems in background correction. For instance, in some measurements, peaks that were easily visually detectable, such as RIN peak in 0.25 mg/l concentration, yielded in negative responses. Likewise, in some cases monomer responses yielded lower responses than dimer, although monomer peak intensities were higher. The above mentioned also applied vice versa in some cases. However the calculation of total response (monomer and

dimer responses added together) compensated these distortions. The concentrations of 0.25 and 0.5 mg/l were the most representative due to lack of the background noise.

The repeatability was determined by measuring 2,4,6-TCP in concentration of 2.5 mg/l for ten times. The solution was placed into ten vials with magnetic stir bars and NaCl addition. The measurement was then performed in the same way as earlier. The UCW which pH was adjusted to 2.205 was used to make dilutions. Responses were then calculated with Integrator 01 program from the obtained data. The 2.5 mg/l concentration gave a rather high response visually, but due to high background noise, calculated responses were poorer than expected.

6.3 Analysis of actual water samples

To test the applicability of the analysis method in practice, three real water samples were obtained from Ramboll Finland. All of these samples contained chlorophenols in different concentrations. In this text the samples are referred as “sample 1”, “sample 2” and “sample 3”. Like earlier, also pH value of these samples was adjusted with 0.5 M HCl to 2.2. The exact pH values of the adjusted samples were:

-sample 1: 2.212
-sample 2: 2.210
-sample 3: 2.203.

To obtain best extraction efficiencies, two new PDMS fibers were conditioned prior to analysis. Fibers were conditioned for half an hour in 250 °C. 10 ml of each sample was then pipetted into 22 ml vials. Magnetic stir bars and NaCl was added into these vials in 100 g/l concentration. The samples were put into the water bath, adjusted to 55 °C and were left to incubate for more than an hour.

The IMS device was baked in 90 °C for a half an hour to thermally remove possible interfering substances from the device. After baking, it was adjusted to 70 °C and was left to incubate for half an hour. The brass chamber temperature was set to 250 °C. The sampling was done

with two PDMS fibers in HS-SPME. The fiber depth was kept at 3 cm. The temperature of the thermal bath was 55 ± 1 °C throughout the analysis.

At first, as with earlier analyses, MSA was measured with PDMS fibers, since it was used as a reference compound. The samples were analyzed in the following order: sample 1, sample 2 and sample 3. Based on information received from Ramboll Finland, it was assumed that sample 3 had the highest concentration of chlorophenols and was therefore left last to be analyzed.

During the analysis, it seemed that sample 1 gave a slight response. However this can be caused by the background noise. Sample 2 did not yield in any kind of response. The third sample, sample 3, produced two signals that could have been interpreted as signals of 2,4,6-TCP and 2,3,4,6-TeCP. These spectra are presented in figures 36 and 37.

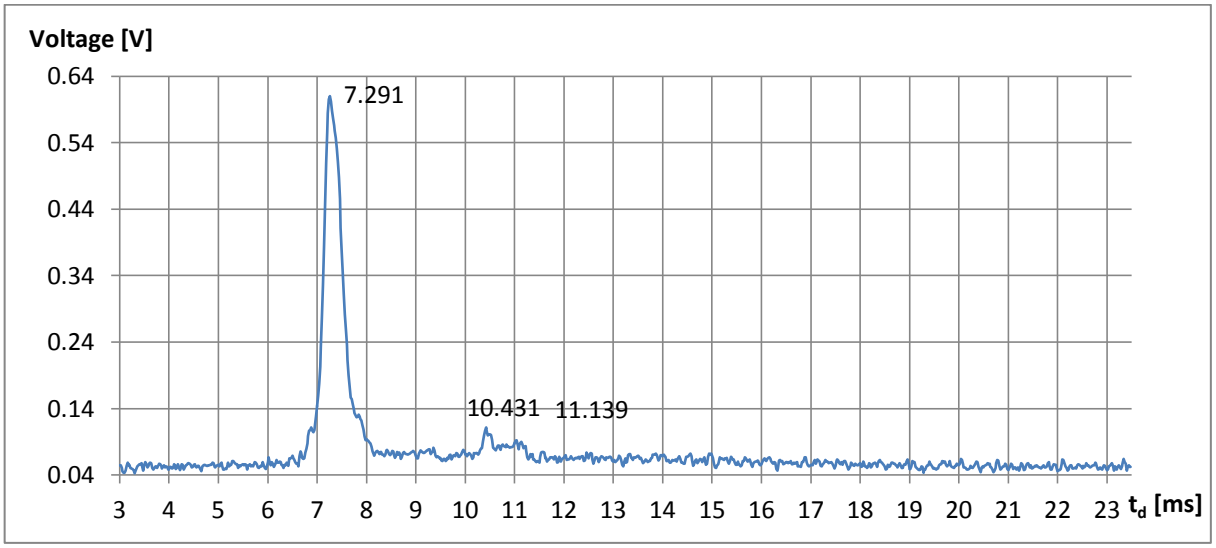

Figure 36. Spectrum of 2,4,6-TCP obtained from sample 3.

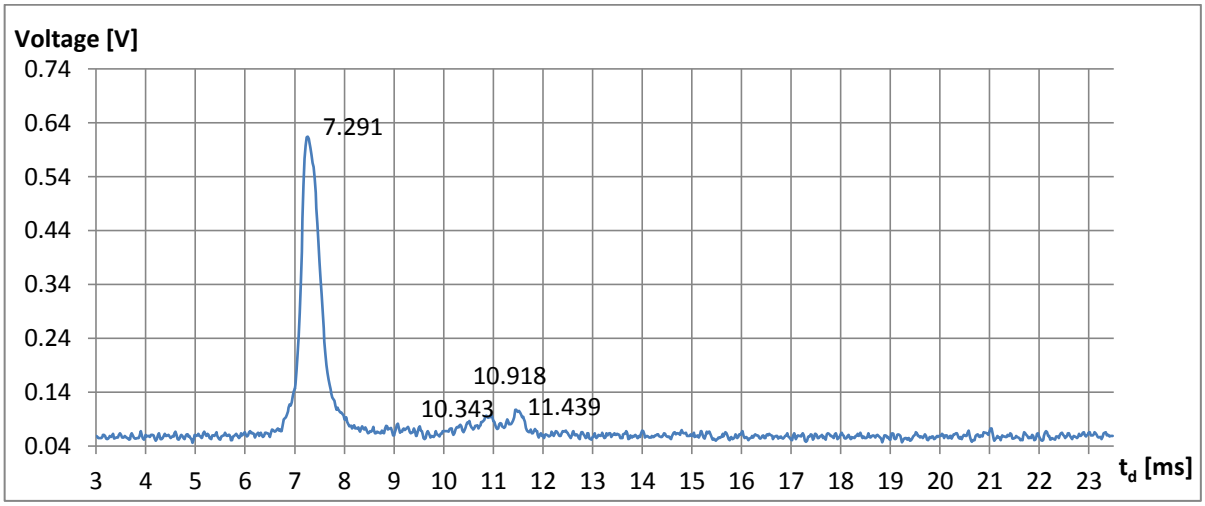

Figure 37. Spectrum of 2,3,4,6-TeCP obtained from sample 3.

The compounds obtained from the spectra were identified as 2,4,6-TCP and 2,3,4,6-TeCP based on their reduced mobilities and times of appearance. 2,4,6-TCP was verified with reduced mobilities of 1.60 and 1.50 cm^2/Vs and 2,3,4,6-TeCP with reduced mobilities of 1.62, 1.53 and 1.45 cm^2/Vs.

It was noticed earlier in the determination of the calibration curves that the signal from 2,4,6-TCP appears almost immediately after thermal desorption and disappears rapidly. It was also noticed that it took longer for the signal of 2,3,4,6-TeCP to appear. The signal was also detectable for a longer time than 2,4,6-TCP. Practically in the analysis of real samples, 2,4,6-TCP appeared rapidly and 2,3,4,6-TeCP appeared after 2,4,6-TCP had disappeared.

The responses for 2,4,6-TCP and 2,3,4,6-TeCP were analyzed with the Integrator 01 program. Using equation 16, 2,4,6-TCP and 2,3,4,6-TeCP concentrations were calculated based on their calibration curve trendline equations presented in figures 26 and 29. Results are presented and discussed more thoroughly in chapter 7. Since no analyte signals of interest detected from samples 1 and 2, data they provided was not analyzed.

The target analyte concentration was calculated as follows:

$$y = ax + b \tag{14}$$

$$x = \frac{y-b}{a} \tag{15}$$

$$c = \frac{y-b}{a} \tag{16}$$

in which $c = x$ = the analyte concentration [mg/l]
$y = r$ = response of the analyte [V*ms]
b = the y intercept of the calibration curve [V*ms]
a = slope of the calibration curve

In addition to HS-SPME extraction with pH adjustment and NaCl addition, also DI-SPME was tested with an unmodified sample 3. The sample 3 was stored in a dark and cold place, at 4 °C. When the sample was taken for pipetting into vials, it was noticed that brown sediment was formed. The container bottle was therefore mixed. After the mixing, 10 ml of sample 3 was placed in two 22 ml vials. Also these vials were stirred. Vials were then placed into thermal bath with a temperature of 55 °C. All the parameters, including fiber depth were kept same as earlier.

The extraction was performed with three different fibers; PDMS, PA and CAR-PDMS. All of the fibers used in DI extraction tests were previously used, which may result in some errors. The CAR-PDMS fiber seemed to give the visually best responses for 2,4,6-TCP and 2,3,4,6-TeCP. The PDMS fiber gave responses for 2,4,6-TCP. PA fiber did not give a response for any chlorophenols of interest. Although responses were obtained, it was clear that they were not as intense as with HS-SPME method, NaCl addition and pH adjustment. There was also much background noise present, causing inaccuracy in results. Because of the inaccuracy, calculations of recoveries between HS and DI methods cannot be performed.

An unknown peak also appeared in all of the DI tests. The peak was considered as the matrix effect and appeared strongly only with DI method. The peak appeared with the highest intensity with the first PA fiber extraction. The drift time, t_d of this unknown peak was 8.927 ms and K_0 1.88 cm^2/Vs.

7 RESULTS AND DISCUSSION

The reduced mobilities for each chlorophenol are presented in figure 38. The K_0 values are presented as a function of number of chlorine atoms present in molecule. In figure 38, number 3 represents 2,4,6-TCP, number 4 2,3,4,6-TeCP and number 5 PCP.

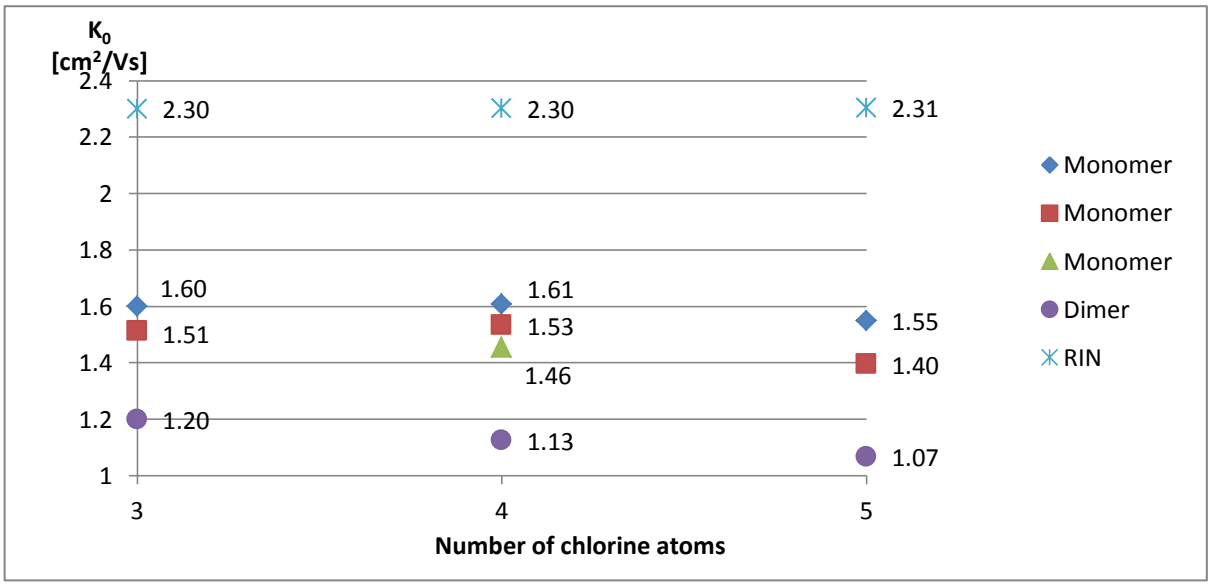

Figure 38. Reduced mobilities presented as a function of number of chlorine atoms.

Following detection limits were obtained:

-2,4,6-TCP:	0.33 mg/l
-2,3,4,6-TeCP:	0.63 mg/l
-PCP:	1.63 mg/l

Obtained detection limits seem to be rational. Slight uncertainty may be accompanied to PCP, since during the determination of calibration curves, 2.5 mg/l concentration was the minimum for obtaining a detectable signal. However, 1.63 mg/l PCP concentration is between the values 1 and 2.5 mg/l concentrations, which were used. Therefore it is possible that LOD for PCP is accurate.

Sensitivity for 2,4,6-TCP was 0.1222, for 2,3,4,6-TeCP 0.0454 and for PCP 0.0022. With 0.25 mg/l concentration of 2,4,6-TCP the recovery was 88,4 % and with 0.5 mg/l 88,8 %.

The repeatability of the measurements was 12.1 %. The repeatability studies carried out in the new facility may not be as reliable as they could have been, if carried in the old facility. In the old facility, the measurements made with IMS negative mode did not yield in as noisy spectra as in the new facility. Therefore it can be stated that if the repeatability studies were carried out in the old facility, repeatability could have been better and more accurate.

In the analysis of actual water samples, samples 1 and 2 did not give any responses for target analytes. However, sample 3 gave responses. The calculated concentration of 2,4,6-TCP was 0.0387 mg/l and concentration of 2,3,4,6-TeCP was 0.4486 mg/l in the sample 3. Actual concentrations of the water samples and concentrations obtained by SPME-IMS are presented in table 5.

Table 5. The verified concentrations and concentrations obtained by IMS.

	Verified concentrations			IMS		
	Target analyte concentration [µg/l]			Target analyte concentration [µg/l]		
Sample #	2,4,6-TCP	2,3,4,6-TeCP	PCP	2,4,6-TCP	2,3,4,6-TeCP	PCP
1	24	210	19	-	-	-
2	0,1	0,86	0,08	-	-	-
3	75	470	71	38,7	448,6	-

As can be seen from the table 5, verified and measured concentrations of 2,3,4,6-TeCP do not differ remarkably. The difference is only 4.6 %. However, in the case of 2,4,6-TCP the difference is 48.4 %. The difference for 2,4,6-TCP is remarkable. The big difference could be explained by the electrical noise that affected to the background correction and also the fact that the lowest chlorophenol concentration used was 0.25 mg/l. The 0.25 mg/l concentration is much greater than 0.075 mg/l. It can be therefore said that by determining 2,4,6-TCP calibration curve with lower concentrations, more precise results could have been achieved.

The major problem of the measurements was caused by the high background noise. Because of the high background noise, responses were not as representable and accurate as they could have been. In addition, if the IMS device used would have reached higher drift tube temperatures, detection of target chlorophenols could have been achieved in lower concentrations. In

the experiments performed, all types of SPME fibers were not tested due to lack of their availability and the time reserved for these studies.

Better results may have also been achieved, if more thorough optimization of parameters could have been performed or multivariate analysis used. However the subject of this study is worth of more research, especially concentrating to the coupling of SPME desorption and IMS device. Also the drift tube temperatures and SPME extraction temperatures may play a significant role in achieving detection in lower concentrations. After more thorough studying of SPME-IMS method development and automation, this technique may be applied to detection of chlorophenols in µg/l concentrations. In addition, results may have been improved, if the transfer lines between desorption chamber and IMS device were shorter and insulated and heated thoroughly.

Based on the studies performed in this work, it can be said that SPME-IMS systems is a fast and practically easy method for detecting chlorophenols in water samples. Prior to analysis, only pH adjustment of the sample is required. After the adjustment, sample has to be placed into the vial with a magnetic stir bar and NaCl in 100 g/l concentration. Due to these quick preparations, analysis of the sample does not require much time and intensive work. In best cases, results of the analysis can be obtained in a same day as sample comes in the laboratory. Also when sampling with HS-SPME, sample matrix does not have an effect to the results

However, with the equipment used in this work, chlorophenols were detected in rather high concentrations, in best cases 0.25 mg/l concentrations. In addition, lowest LODs calculated were with 2,4,6-TCP 0.33 mg/l. The current legislation in Finland restricts the total chlorophenol content of drinking water to 10 µg/l. With the method used, the detection limits are too high for limit surveillance. Currently the equipment used may be applied to robust estimation of the concentrations near possible scenes of accident.

8 SUMMARY

Chlorophenols have been used for many purposes, including pesticides, wood preservatives, glue manufacturing purposes and intermediaries for producing other compounds. They have been classified as possible human carcinogens. In Finland chlorophenols were used until 1980s as wood preservatives in timber industry.

Traditionally chlorophenols have been analyzed using GC-MS or HPLC. With these methods low LODs can be achieved. However these methods may require extraction steps and long run times in analysis. These drawbacks can be avoided by using SPME-IMS coupling. SPME was developed in the beginning of the 1990s for a new method for sampling and sample preparation. In environmental analysis SPME is suitable for water, air, sediment and soil samples. With SPME, sample isolation and concentration can be performed in a single step.

IMS is a rapid and sensitive analysis method which can be used by unskilled workers. IMS also has low costs, good flexibility and portability. IMS system does operate in ambient conditions and has low power consumption and weight. In environmental analysis IMS has been used for analyzing contaminants in aqueous solutions, detecting hazardous vapors in air and determining VOCs and semivolatiles in soil samples, to name a few.

IMS is based on detecting the drift times of ionized sample molecules. Traditionally sample molecules are ionized by a radioactive source and detected by a Faraday plate. Based on the drift times, a spectrum is drawn as a function of drift time and voltage. Based on ion drift times, ion mobilities are calculated. To obtain comparable data, reduced mobilities are calculated to achieve comparable results regardless of the measurement conditions.

SPME-IMS has not been widely used, especially in the analysis of chlorophenols. Therefore its suitability was studied in the experimental part of this work. Studied chlorophenols were 2,4,6-TCP, 2,3,4,6-TeCP and PCP. First in the experimental part, certain parameters were optimized to achieve lowest possible detectable concentrations. The effects of extraction time and temperature, fiber depth and material and salt addition were studied. After the optimization, calibration curves were determined. Calibration curves were determined for each chlorophenols with same parameters: 3 cm fiber depth, 55 °C extraction temperature and 20 min extraction time with PDMS fiber. NaCl addition and magnetic stir bars were used.

After determining the calibration curves, certain validation parameters were determined: LOD, sensitivity, repeatability and extraction recovery. After determination of these parameters, three actual water samples containing chlorophenols were analyzed.

It was calculated that the recovery of SPME-IMS was nearly 89 % and repeatability 12.1 %. With SPME-IMS coupling following LODs and sensitivities were obtained:

-2,4,6-TCP:	0.33 mg/l	0.1222
-2,3,4,6-TeCP:	0.63 mg/l	0.0454
-PCP:	1.63 mg/l	0.0022

With actual water samples, only sample 3 gave responses. Based on reduced mobilities, compound responses were identified as 2,4,6-TCP and 2,3,4,6-TeCP. The following concentrations were calculated, verified concentrations are presented in brackets as a comparison:

-2,4,6-TCP:	0.0387 mg/l	(0.075 mg/l)
-2,3,4,6-TeCP:	0.449 mg/l	(0.470 mg/l)
-PCP:	- mg/l	(0.071 mg/l)

The SPME-IMS system tested in this work is suitable for robust estimation of water chlorophenol content. Lower detection limits could have been achieved, if more thorough testing of extraction parameters could have been carried out. Also with higher drift tube temperatures lower concentrations could have been detected. It also became clear that high background noise disturbed measurement and had a great effect to the accuracy of the results.

SPME-IMS is however, a promising technique for determining chlorophenols and other pollutants from water samples. With automation and further method development lower detection limits and better accuracy can be achieved.

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