Towards in-field sample-preparation and detection: Development of new sample preparation formats using molecularly imprinted polymers for the combination with field-deployable detectors

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I) M. Zarejousheghani, M. Möder, H. Borsdorf, A new strategy for synthesis of an in-tube molecularly imprinted polymer-solid phase microextraction device: Selective off-line extraction of 4-nitrophenol as an example of priority pollutants from environmental water samples, Anal Chim Acta, 798 (2013) 48-55.

II) M. Zarejousheghani, S. Schrader, M. Möder, P. Lorenz, H. Borsdorf, Ion-exchange molecularly imprinted polymer for the extraction of negatively charged acesulfame from wastewater samples, J Chromatogr A, 1411 (2015) 23-33.

III) M. Zarejousheghani, P. Fiedler, M. Möder, H. Borsdorf, Selective mixed-bed solid phase extraction of atrazine herbicide from environmental water samples using molecularly imprinted polymer, Talanta, 129 (2014) 132-138.

IV) M. Zarejousheghani, S. Schrader, M. Möder, M. Schmidt, H. Borsdorf, A new strategy for accelerated extraction of target compounds using molecularly imprinted polymer particles embedded in a paper-based disk, J Mol Recognit, 31 (2018).

V) Zarejousheghani M, Schrader S, Möder M, Mayer T, Borsdorf H, Negative electrospray ionization ion mobility spectrometry combined with paper-based molecular imprinted polymer disks: A novel approach for rapid target screening of trace organic compounds in water samples, Talanta, 190 (2018) 47-54.

Dedication

I am dedicating this thesis to my beloved family.

To my parents who raised me with love.

To my sisters who supported me in my life.

To my lovely wife who has blessed me with the true love.

To my son who is the greatest gift in my life.

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Abstract

Nowadays due to the introduction of diverse and vast amount of chemical compounds into the environment, its protection needs permanent monitoring. This issue is more critical in developing countries where equipped laboratories are also less established. Therefore, development of the cost-effective and field-portable sample-preparation/detection methods is necessary especially for monitoring of environmental water samples. Due to the known matrix complexity and relatively low analytes concentrations in environmental water samples, a sample-preparation method is generally essential before analysis. This is more recommended when field deployable detectors like ion mobility spectrometry (which they suffer generally from lower sensitivities) are used for analysis. Traditional sample-preparation methods preconcentrate successfully the analytes of interest and other constituents which create sample matrix. Therefore, a general preconcentration could intensify the matrix effect and negatively affect the quantitative determination of analytes of interest during final measurements. Selective sorbents could help to concentrate the analytes of interest and mitigate the matrix effect using an optimized washing process after sample loading procedure. Due to inherent advantages of selective molecularly imprinted polymer (MIP) materials which are low-cost and simplicity of their production and also their ability to function in harsh environmental conditions, they were selected to be evaluated during our study. Various traditional extraction formats and new innovative strategies have been developed in which MIP materials have been used to concentrate selectively analytes of interest from environmental water samples. However, development of selective extraction methods which can be used for large volumes of water samples and at high flow-rates is still a great concern. In this study, MIP sorbent materials for 4-nitrophenol, atrazine and acesulfame were synthesized. These chemicals are whether environmental pollutant or anthropogenic marker.

In the first step, solid phase microextraction (SPME) approach was selected due to its known quality advantages and the potential application for in-field sample-preparation. The 4-nitrophenol imprinted polymer was synthesized in an in-tube SPME format. At optimized extraction condition, sample volumes up to 2 mL with sample flow-rate of 0.033 mL min^{-1} were achieved. Despite the interesting results obtained during this study in which high-performance

liquid chromatography with a diode-array detector (HPLC-DAD) was used as detection system, the values obtained for sample volume and flow-rate cannot support the in-field sample-preparation of large volumes of environmental water samples. Therefore, in the next step solid phase extraction (SPE) approach was selected that is known as exhaustive extraction method which can be used for larger sample volumes. MIP for acesulfame, which is a low-calorie artificial sweetener and known anthropogenic marker, was synthesized using a new synthesis strategy. Synthesized MIP particles were used to fill a traditional SPE cartridge. At optimized extraction conditions, 50 mL of water sample could be passed through the molecularly imprinted solid phase extraction (MISPE) cartridge at maximum 0.5 mL min^{-1}. Due to an elevated back-pressure, the obtained flow-rates were low. That happened because I) the synthesized polymer particles showed strong swellable character when they were in contact with different conditioning, washing and elution solvents, II) polymer particles were also in the nano-size ranges and III) they had relatively irregular shape. These are three known general reasons which are responsible for the elevated back-pressure and therefore reduced flow-rate in MISPE applications. In the next step and for atrazine as target molecules, we tried to mitigate the observed back-pressure problem by mixing the synthesized irregular atrazine imprinted polymer with more regular and rigid commercially available ingredients. The new extraction strategy termed mixed-bed SPE was combined with gas chromatography mass spectrometry (GC-MS) for selective determination of atrazine in environmental water samples. In comparison to its relative SPE cartridge, developed method improved the sampling flow-rate, limit of detections (LOD), relative standard deviation (RSD) and column-to-column reproducibility and reduced the required total organic solvent and total sample preparation time. Using developed method and for 10 mL sample, the flow-rate was increased up to 3 mL min^{-1}. However, these values also cannot still satisfactorily support in-field sample-preparation approach. Therefore, inspired from solid phase disk extraction (SPDE) methodology, which has been originally developed for sample-preparation of large volumes of environmental water samples and at high flow-rates, we tried to develop a cost-effective and simple methodology to enable a selective sample-preparation at accelerated sample volumes and flow-rates. To this aim, the same amount of acesulfame imprinted particles which were used already in SPE format, were

dispersed within a commercial filter paper. Using the selective filter paper, the sample volumes were increased up to 500 mL with sample flow-rates of 30 mL min^{-1}. These values could satisfactorily support our aim to use a sample-preparation method for field application. Developed sample-preparation method was combined with liquid chromatography-tandem mass spectrometry (LC-MS/MS). To this stage, we could successfully develop a selective sample-preparation methodology which can be used for relatively large sample-volumes and at relatively high flow-rates. However, the used HPLC/MS-MS detector is a laboratory-based expensive detector which needs experienced personals despite its outstanding performance. Therefore, we developed an electrospray ionization-ion mobility spectrometry detector (ESI-IMS) which is known as a relatively cheaper and potentially field-portable detection system. Finally, the home-made ESI-IMS was optimized for the analyte of interest and combined with developed selective filter-paper extraction method. In summary, during this study selective sample-preparation methods were improved step-by-step and finally used in-filed. Eventually, developed field-applicable sample-preparation method was combined with a potentially field-portable detection system which was designed, constructed and optimized in our group.

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List of Abbreviations

Abbreviations	Stands for:
AIBN	2,2´-Azobisisobutyronitrile
ATRP	Atom transfer radical polymerization
BPA	Bisphenol A
CNTs	Carbon nanotubes
CRP	Controlled/living Radical Polymerization
CD	corona discharge
DLLME	Dispersive liquid liquid microextraction
DMS	Differential mobility spectrometry
DCGD	Direct current glow discharge
ELISA	Enzyme-linked immunosorbent assay
EGDMA	Ethylene glycol dimethacrylate
ESI	Electrospray ionization
EQCM	Electrochemical quartz crystal microbalance
FRP	Free radical polymerization
FAIMS	High-field asymmetric waveform ion mobility spectrometry
GC-MS	Gas chromatography–mass spectrometry
HF-LPME	Hollow fiber liquid-phase microextraction
HPLC-DAD	High-performance liquid chromatography with diode-array detection
HPLC-MS/MS	High-performance liquid chromatography-tandem mass spectrometry
IF	Imprinting factor
IMS	Ion mobility spectrometry
IOW	Integrated-optical-waveguide
LLE	Liquid-liquid extraction
LPME	Liquid phase microextraction
MIP	Molecularly imprinted polymer
MISPE	Molecularly imprinted solid phase extraction
MAA	Methacrylic acid
MSPD	Matrix solid-phase dispersion
MEPS	Microextraction by packed sorbent
MSPE	Magnetic solid-phase extraction
µ-SPE	Micro-solid-phase extraction
MWCNTs	Multiwall wall carbon nanotubes
NIP	Non-imprinted polymer
4-NP	4-Nitrophenol
NMP	Nitroxide mediated polymerization
NH4OH 25%	Ammonium hydroxide solution (25%)
PT-SPE	Pipette-tip solid-phase extraction
PCR	Polymerase chain reaction
PEEK	Polyetheretherketone

PI	Photoionization
QuEChERS	Quick, Easy, Cheap, Effective, Rugged, and Safe
QCM	Quartz crystal microbalance
RAMs	Restricted access materials
RAFT	Reversible addition fragmentation chain transfer
SDB	Styrene divinylbenzene
SBSE	Stir-bar sorptive extraction
SPE	Solid phase extraction
SDME	Single drop microextraction
SPED	Solid phase extraction disk
SWCNTs	Single wall carbon nanotubes
SELEX	Systematic evolution of ligands by exponential enrichment
SFRP	Stable free radical polymerization
SPME	Solid-phase microextraction
TFME	thin film SPME
TOF	Time-of-flight
VBTA	(Vinylbenzyl)trimethylammonium chloride

1. Introduction

Sampling/sample-preparation are two important steps in most analytical procedures, whereby the sample is collected and treated prior to its analysis in order to pre-concentrate the analytes and remove matrix compounds [1]. Despite the ongoing innovations in the development of analytical instruments, sampling/sample-preparation are still important preliminary steps which take about 60% of total cost and time of the analytical procedures [2, 3]. Depend on the type of sample matrices (e.g. solid, semi-solid, liquid or gas) various methods have been developed. Developed methods for the gas samples have the lowest and for the liquid samples have the highest diversity. For the gas-samples, the most effort has been devoted to develop highly efficient sampling methodologies. Contrarily, numerous sample-preparation methods have been developed for liquid samples which are mostly limited to the laboratory applications. In this study, we aimed to develop novel sample-preparation methods for liquid samples which have high-enough selectivity towards special targets. The methodologies were developed step by step from low to high sample volumes/sample flow-rates to enable them for in-field application as selective sample-preparation methods.

1.1. Sample-preparation techniques for liquid samples

1.1.1. Solvent-based extraction methods

Liquid-liquid extraction (LLE) is the known traditional solvent-based extraction method which has been used in the laboratory- and industrial-scales. According to the principles of "Green Chemistry", the minimization of solvent- and reagent-use is an actual argument for developing extraction methods. Therefore, various miniaturized solvent-based extraction methods termed *liquid phase microextraction* (LPME) have been developed [4]. LPME approach includes different methodologies like *single drop microextraction* (SDME) [5], *hollow fiber liquid-phase microextraction* (HF-LPME) [6] and *dispersive liquid liquid microextraction* (DLLME) [7].

1.1.2. Sorbent-based extraction methods

In these methods, various solid materials are used as extracting agent in different formats. Over time, significant efforts have been devoted to develop various extraction formats and sorbent

materials to improve simplicity, selectivity, sorption capacity and chemical or physical-mechanical stability. *Solid phase extraction* (SPE) is certainly the most popular and widely-used sample preparation technique for liquid samples that can be applied in off-line or online systems, with the advantage of possible automating [1, 4]. *Matrix solid-phase dispersion* (MSPD) [8] and *QuEChERS* [9] are two SPE formats developed for non-liquid samples. In MSPD method, solid, semi-solid or viscose samples are mixed with sorbent materials and the prepared mixture is used to pack a SPE cartridge. QuEChERS (standing for Quick, Easy, Cheap, Effective, Rugged, and Safe) [9] was introduced in 2003 for determination of pesticides in food samples and since then has attracted great attention [10]. In this method, targets were extracted primarily using an organic solvent (e.g. acetonitrile). The organic phase is separated using salting-out effect. Then, small amount of solid sorbent (*dispersed solid phase extraction*; dispersive-SPE) along with anhydrous MgSO4 were dispersed within the organic solvent to remove many polar compounds (as a cleanup step) and residual water. *Membrane protected micro-solid-phase extraction* (μ-SPE) [11] is another SPE format in which solid sorbents are packed within a porous membrane. *Microextraction by packed sorbent* (MEPS) [12] was introduced in 2004 and is simply the miniaturized format of SPE cartridges (the amount of solid sorbent is reduced from about 50-200 mg in SPE to about 2 mg in MEPS). *Solid-phase microextraction* (SPME) as a sorbent based-based extraction method was introduced in in early 1990s by Pawliszyn [13]. Contrary to MEPS, SPME is not the miniaturized version of SPE. It is a non-exhaustive, simple, portable sampling and sample preparation method which uses smaller volumes of solvent and is solvent-free in some cases [14]. Since its introduction, various sorbent materials have been used in different SPME formats like: *SPME fibers*, *thin film SPME* (TFME), *dispersive-SPME* [15] (similar to dispersive-SPE process but for pre-concentration purposes and not just cleanup) and *in-tube SPME* [16]. In late 1990s and during a SPME experiment, it was discovered that the stirring magnet which was coated with Teflon could also adsorb chemical targets. The new extraction technique termed *stir-bar sorptive extraction (SBSE)* [17] could provide higher sensitivity due to higher amount of sorbent phase. *Magnetic solid-phase extraction* (MSPE) [18] is an extraction technique which uses modified-magnetic materials as extracting agent. These materials can be dispersed within the liquid samples and recovered again easily with a permanent magnet. For

low-volume liquid samples, *pipette-tip solid-phase extraction* (PT-SPE) was developed in which µL-ranges pipette-tips are modified with sorbent materials and used as extraction method [19]. In order to employ SPE method for large number of samples in an automated procedure, a new format was introduced and termed *multi-well SPE plates* [20]. However, among all different SPE formats, disposable cartridges are still the most applied format of SPE due to its easy preparation procedure in the laboratories. Nevertheless, using nano-sized sorbents with swellable characteristics or irregularly shape result in an elevated back-pressure. Therefore, high flow-rates through the SPE cartridges are not allowed which is a bottleneck for high throughput analysis as the limited sample volumes for sensitive analysis. Other difficulties are channeling and voiding effects which result in a decreased column-to-column reproducibility. For the fast handling of large volumes of environmental samples, *solid phase extraction disk* (SPED) devices were introduced to the market by 3M [21] in which membranes (PTFE or glass fibre) are uniformly loaded with adsorbent particles [22]. Expected advantages for SPED are high flow rates and large sample volumes, significant reduction of channeling and voiding effects, highly efficient mass transfer and reduced risk of clogging [22]. Despite the much effort which has been devoted to develop new sample preparation methods for liquid samples, it is still a challenge to develop selective, fast, simple and cost-effective methodologies for high-throughput sample preparation of environmental water samples.

1.2. Sorbent materials

Alongside with method developments, numerous new sorbent materials have been introduced and coupled with the extraction methods. The main focus of these efforts has been to increase the binding capacity, to accelerate the binding kinetics and to enhance the selectivity. In general, these materials can be categorized into two types: non-selective and selective sorbents.

1.2.1. Non-selective

The non-selective sorbents group encompasses a large number of materials from classical to new-developed sorbents. Silica-based sorbents functionalized with C8, C18, amines or carbonyl

groups and also polymeric sorbents, such as Oasis HLB, Strata-X, XAD-2, XAD-4 and SDB (Styrene divinylbenzene) are some of the used traditional sorbents [23].

Nanomaterials [24] have been introduced as a new class of sorbent materials. Among different types of nanomaterials, metallic and carbon nanomaterials are the most versatile which attracted great attention. Fe_3O_4 is the known magnetic nanoparticle which can be easily synthesized and uniformly coated with silica layer. The prepared Fe_3O_4@SiO_2 can be further modified with different extra sorbent layers [25]. Fullerene C_{60} was the first nanomaterial from carbon family which was used in SPE. Later, carbon nanotubes (CNTs) including single wall carbon nanotubes (SWCNTs) and multiwall wall carbon nanotubes (MWCNTs) were discovered and attracted more attention due to their high surface-to-volume ratios and their stability towards different chemical, mechanical and thermal conditions. A new emerging type of carbon nanomaterial is graphene was introduced by Novoselov et al. in 2004 [26] and since then is implemented extensively for different purposes including sample preparation.

1.2.2. Selective

1.2.2.1. Immunosorbent

Immunosorbents function is based on the capability of antibody to bind to a specific target termed antigen. Antibody is a Y-shaped protein and is produced by immune system. The most known application of immunosorbent is in *enzyme-linked immunosorbent assay* (ELISA) as a diagnostic test. In ELISA, the antibody support the selective attachment to a special target and then enzyme catalyzes a reaction to produce generally a color change as the colorimetric signal. ELISA has been used for detection and determination of specific targets in complex samples like blood and urine. There are two ELISA methods termed I) indirect detection or II) direct detection. In the indirect approach, the antibody, which is produced by an immune system in response to the presence of an antigen, is detected (Figure 1-1). In the indirect method, first the pure antigen is coated on a surface (note: between each step, the surface must be washed to remove not bonded molecules). Then, the sample which is supposed to have the specific antibody, produced against the targeted antigen, is added to the modified surface. In the case of antigen presence (the target of interest) in the sample and therefore availability of related

produced antibody, this antibody will attach specifically to the surface via specific antibody-antigen interaction. After that, an enzyme-linked antibody is added that contains an antibody and an enzyme which are covalently bonded. This new antibody is designed to be able to bind specifically to the former antibody which is attached to the surface. In the case of successful successive attachments, enzyme would be available on the surface which can catalyze a reaction to produce a colorful product as signal. It is worthy to note again that during these stepwise additions, the presence of specific antibody produced by immune system causes to have finally the enzyme on the surface.

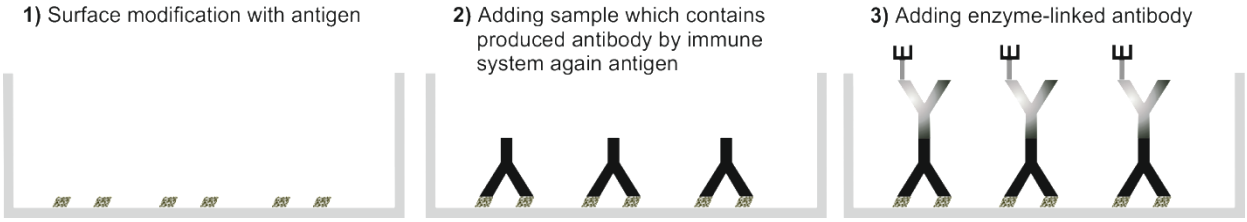


Figure 1-1: ELISA method using indirect approach.

Using direct approach, the antigen in sample is detected directly (sandwich ELISA, Figure 1-2). In this method and for the first step, the pure antibody which is produced for targeted antigen is bonded to a surface (note: between each step, the surface must be washed to remove not bonded molecules). Then, the sample which is supposed to have this specific antigen is added to the modified surface. After that, an enzyme-linked antibody is added which its antibody section is also designed to bind specifically to the targeted antigen. Again, during these stepwise additions, the presence of targeted antigen in the sample causes to have enzyme on the surface (antigen is sandwiched between two antibody). When the enzymes are available on the surface, adding the specific molecule in final step causes the color change which shows the presence of antigen in the sample. Antibodies can be used also as immuno-capture and following determination with modern analytical instruments like LC-MS/MS [27]. Despite the high selectivity of immunosorbent assays towards specific targets, it is difficult to produce desired antibodies. They are expensive and sensitive to the environmental conditions which restrict their comprehensive application for a broad range of molecules.

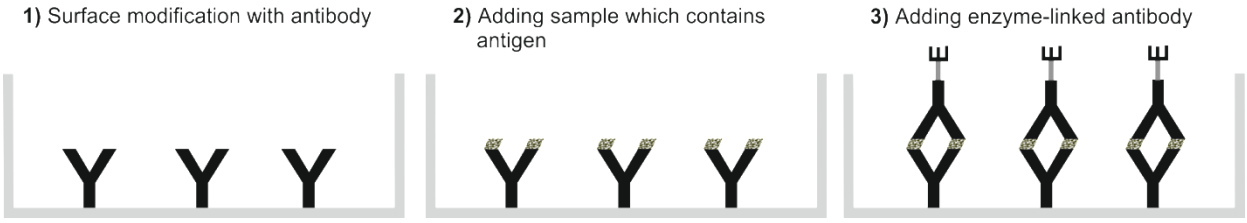


Figure 1-2: ELISA method using direct approach (Sandwich ELISA).

1.2.2.2. Aptamer

Aptamers as the artificial alternative for antibodies were introduced in 1990 [28]. They are made of DNA and RNA (oligonucleotides) using SELEX method (standing for: *systematic evolution of ligands by exponential enrichment*). In this method, the target is incubated in a randomized pool of oligonucleotides. The bonded oligonucleotides to the target were separated and amplified using *polymerase-chain-reaction* (PCR) method. Aptamers have been used for different analytical and diagnostic purposes. Due to their high selectivity, aptamers have been used as selective sorbent materials in different sample preparation techniques like SPE, SPME and microfluidic sample preparation methods [29]. To this end, aptamer must be connected to the surface of a suitable solid support using a carefully selected linker-molecule [29]. Selection of suitable support and linker-molecule can strongly affect the final selectivity of aptamer-modified substrate. Despite the high selectivity of aptamers, their production needs relatively complicated procedure and expensive instruments. Besides, they are also sensitive to harsh environmental condition which restricts their comprehensive application.

1.2.2.3. Restricted access materials

Restricted access materials (RAMs) are simply the materials which their accessibility by macromolecules like proteins are restricted using an outer hydrophilic shell acted as a physical or chemical barrier (Figure 1-3) [30]. Therefore, in a complex sample like serum and plasma which contain many protein biomolecules, RAMs are ideal adsorbents for molecules with lower molecular weights like drugs. Many efforts have been devoted to develop new RAMs which were implemented for sample preparation purposes [31]. However these materials are ideal sorbents for a group of low weight chemical molecules in biological matrices with the aid of macromolecules exclusion.

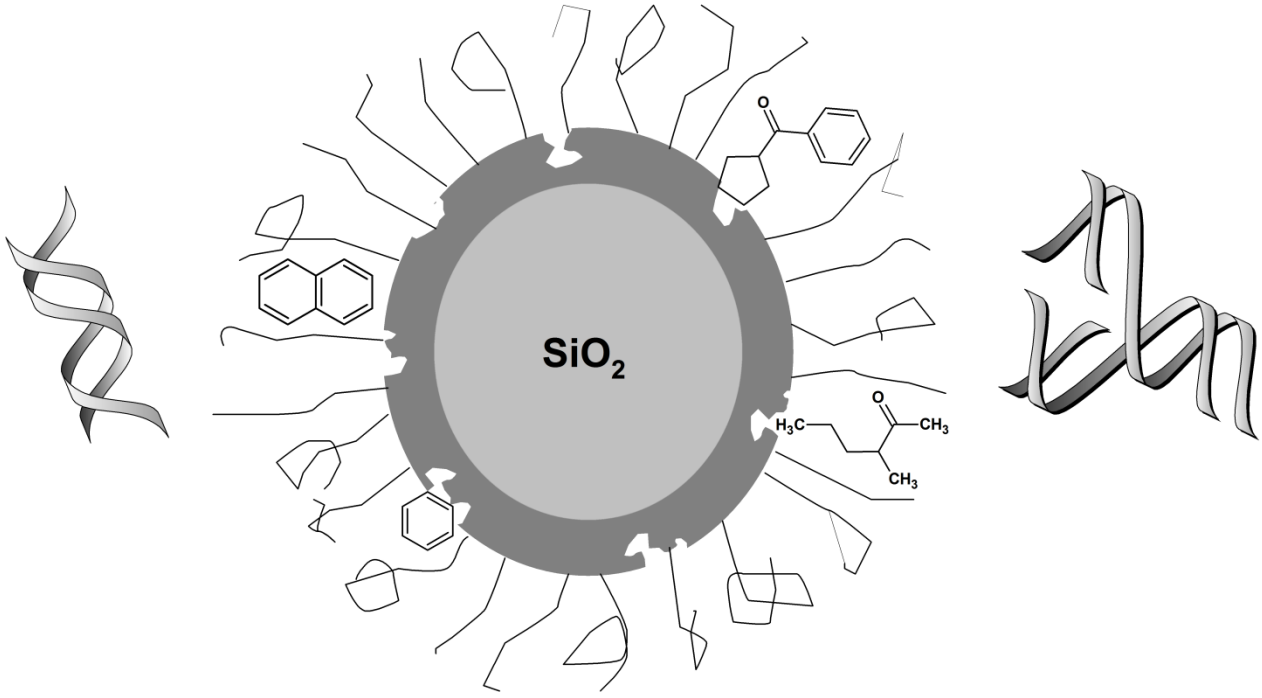


Figure 1-3: Structure and separation mechanism of restricted access materials (RAMs).

1.2.2.4. Molecularly imprinted polymer

Molecularly imprinted polymer (MIP) materials are artificial selective receptors which imitate the behavior of antibodies. The first positive results for MIPs were obtained by Wulff's group using covalent approach and published in early 1970s by [32]. In 1981, Mosbach introduced a new strategy to produce MIP materials termed non-covalent approach which is still the most common synthesis method [33]. MIP materials are highly cross-linked organic/inorganic co-polymers in which the cavities (recognition sites) are imprinted for special targets (templates). Before polymerization, the template must form a complex with selected monomers through covalent (Figure 1-4), semi-covalent (which is a hybrid of covalent imprinting and non-covalent imprinting approaches; Figure 1-5) or noncovalent (Figure 1-6) interactions in the presence of a solvent (porogen).

The complexed monomers are stabilized around the template using a cross-linker agent in a polymerization process. The imprinted cavities then remain within the polymer matrix even after removal of the template. These cavities therefore have a complementary size, shape and spatial position of the functional groups towards the template. Increasing the rigidity of the polymer increases its selectivity performance; nevertheless in a highly cross-linked polymer, the

recognition capability could decrease due to either kinetic limitations or incomplete template removal.

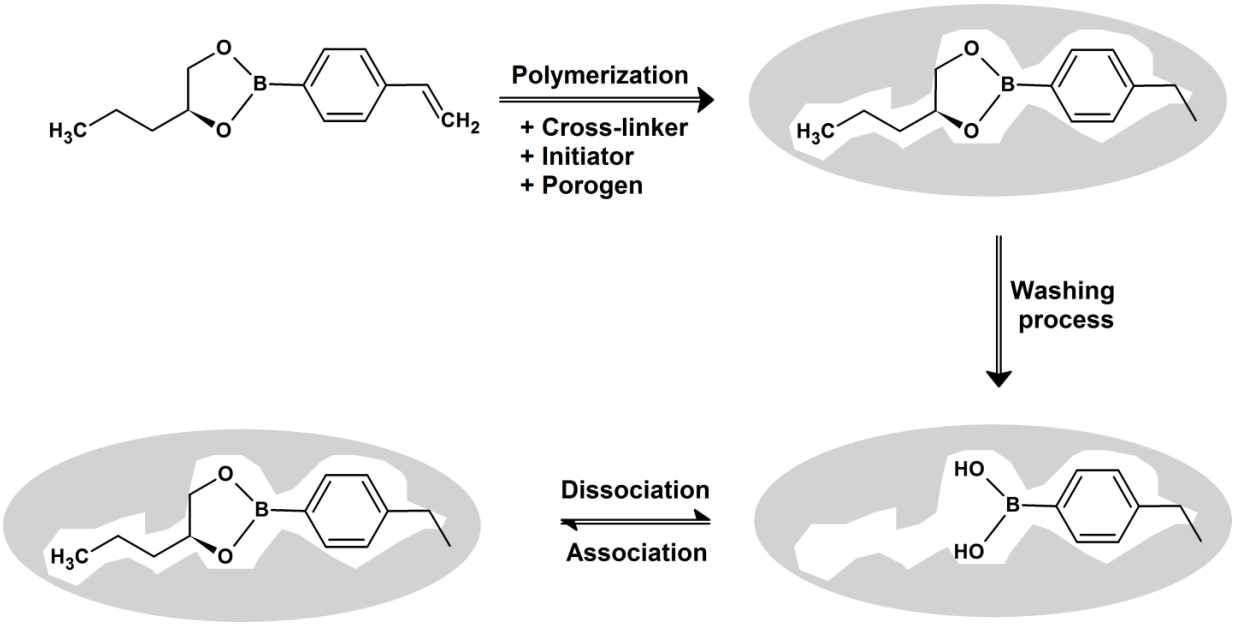


Figure 1-4: Imprinting the polymer matrix using covalent approach. Polymerization can be carried out e.g. using a boronic acid ester as a covalantly bonded template-monomer. The covalant bond in these esters can be fast and easy formed and dissociated. (1,2-Pentanediol as target template)

In the covalent approach, monomers are connected to the template molecules via covalent bonds. After polymerization, these bonds are cleaved whereupon the recognition cavities will be remained within the polymer matrix. In the recognition process, the same covalent bonds must be regenerated to trap selectively the template molecules. Despite the higher selectivity of such a system, finding the reversible covalent bonds restrict this approach to specified templates and functional monomers.

In the non-covalent approach, other intermolecular interactions (hydrogen bonds, electrostatic or ionic interaction, π-π interaction, and hydrophobic effects) are responsible to arrange the functional monomers around the template molecules. Therefore, a larger group of functional monomers can be implemented in this approach which makes the total engineering polymer design much easier when it is compared with covalent approach. In summary, despite the fact that the covalent approach provides more selective polymers, they can be used for a specified group of templates and monomers in a restricted polymerization condition and also the

adsorption process take more time. In the semi-covalent strategy [34], the recognition cavities are produced for a template (or the analog) which is covalently bonded to a functional monomer. After the cleavage of the covalent bond, during the recognition process the template molecule will react with monomer via non-covalent interaction. However and due to the simplicity and versatility of non-covalent approach, nowadays it is the most common used engineering method to design imprinted polymers.

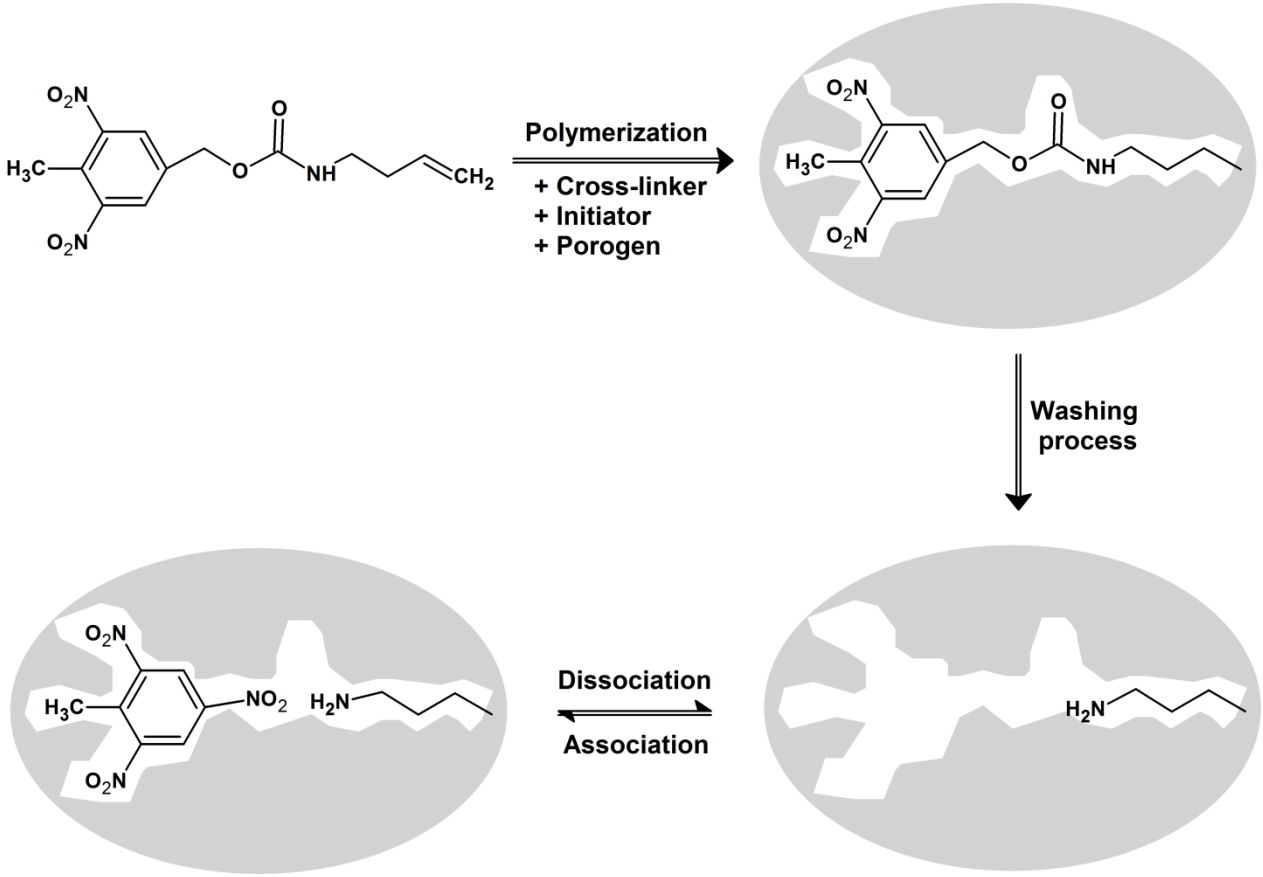


Figure 1-5: Imprinting the polymer matrix using semi-covalent approach. The polymerization is carried out for covalently bonded template-monomer. After bond-cleavage, the imprinted cavities are used for rebinding a template molecule via non-covalent interactions (trinitrotoluene as target template)

In non-covalent approach, selection of porogen is critical which direct the selectivity and porosity of final synthesized polymers. An important point is that the porogen does not disturb the non-covalent interactions between template and functional monomers during template-monomer complex preparation.

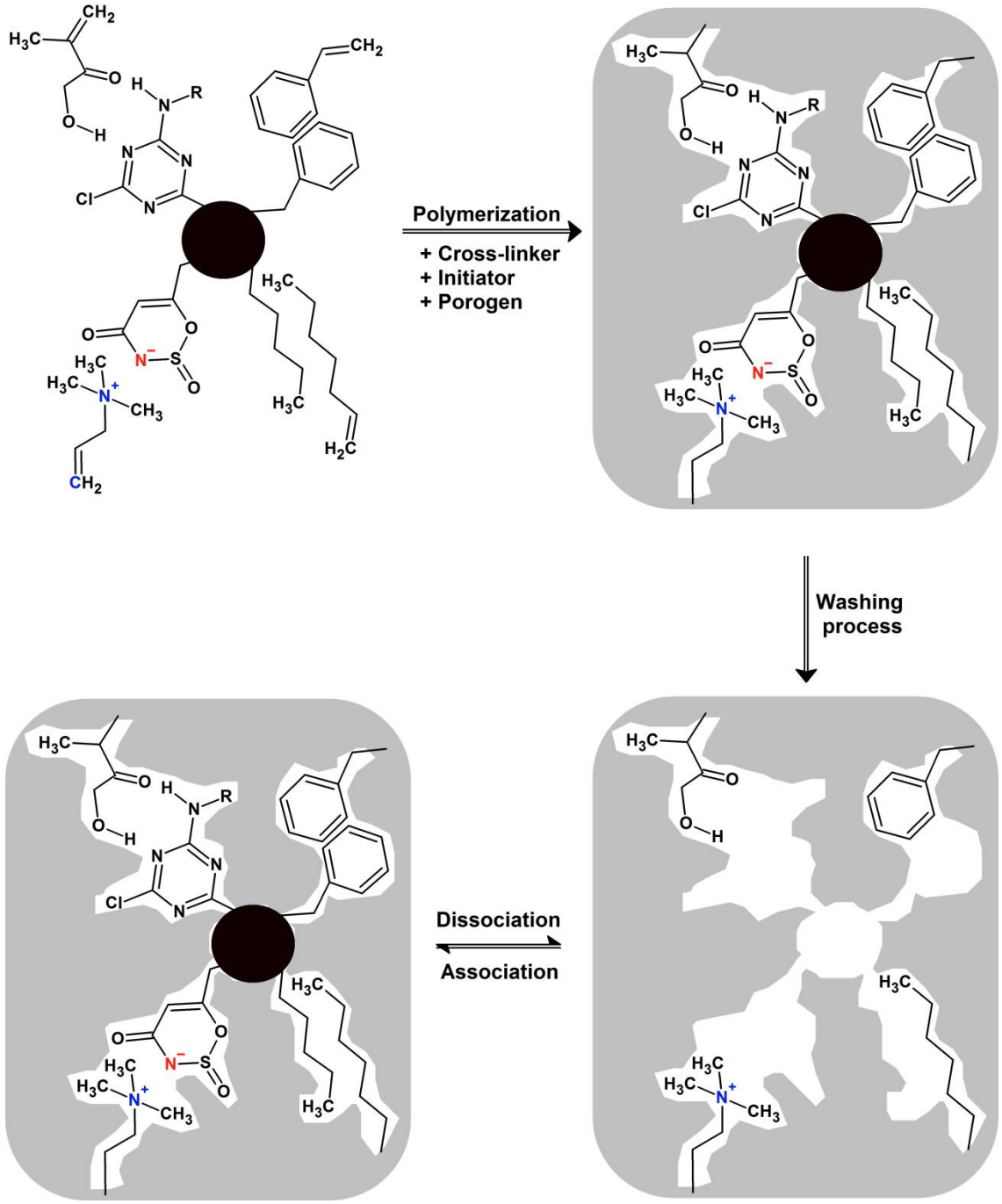


Figure 1-6: Imprinting the polymer matrix using non-covalent approach. The polymerization is carried out for non-covalently bonded template-monomers. Later, the imprinted cavities are used for rebinding the template molecule using the same non-covalent interactions.

Since the hydrogen-bond interaction is the most used non-covalant intermolecular interaction, water is not a suitable porogen. Therefore, a novel synthesis strategy termed *coordination imprinted polymer* (CIP) [35] was introduced. In this approach, dative covalent bond plays an

important role. A cation produces a complex with template or functional monomer via dative bonds. After that, this complex will be used as the template for further polymerization. Due to higher stability of this type of interaction even in water as porogen, this methodology could provide more selectivity for the targets in water samples.

The selectivity feature of synthesized polymers to the targets has not been evaluated using standardized methods. Generally, synthesized MIPs and non-imprinted polymers (NIPs; for NIP synthesis, the polymerization mixture is prepared exactly the same like MIP materials but without template molecule) are compared regarding their thermodynamic adsorption capacities (µg target/mg polymer) and this ratio is expressed as an imprinting factor (IF) constant. The selectivity of recognition sites or selectivity factor compares IFs of the template and its chemical analog.

Since the introduction of MIP technology using bulk polymerization method, various other methods like: precipitation, emulsion and suspension polymerization have been developed to synthesize polymers with spherical particles.

In traditional bulk polymerization method, template molecule and functional monomers are dissolved in low volume of porogen to produce template-monomer complex (template/monomer/porogen; ≈ 1 mmol/4 mmol/2-5 mL). However, the bulky synthesized polymers are rigid and must be grounded with pestle and mortar. Obtained irregular paticles with heterogeneous sizes must be sieved to obtain particles with intended sizes (e.g. ≈ ≥30 µm for SPE). By increasing the porogen volume, synthesized polymers (or even oligomers) will be precipitated when they reach a threshold level due to the low concentration of polymerization precursors and lower solubility of synthesized polymers or oligomers (precipitation polymerization method). However, lower precursor concentrations on the other hand can cause a weaker interaction between template and functional monomers. Using the emulsion strategy, organic polymerization mixture in droplet format is stabilized within a water phase using an emulsifier (e.g. surfactants). In suspension polymerization, a mechanical agitation is implemented to stabilize the organic droplets during polymerization process.

As mentioned before, the polymer particles resulting from these methods, using free radical polymerization (FRP), were mostly heterogeneous. Therefore, new synthesis methods termed

Controlled/living Radical Polymerization (CRP), were developed which produce more homogenous particles and form polymer networks under more favorable thermodynamic conditions [36]. CRP includes four different methods as follows: 1) *iniferter-induced ''living'' radical Polymerization*, 2) *nitroxide mediated polymerization* (NMP) (Iniferters and NMP are generally referred to as "*stable free radical polymerization*" (SFRP) techniques), 3) *atom transfer radical polymerization* (ATRP) and 4) *reversible addition fragmentation chain transfer* (RAFT). Among these four synthesis methods, NMP is the least common and RAFT is the most recently developed and versatile CRP technique for synthesizing imprinted polymers.

MIP materials, synthesized using CRP method in comparison to FRP, are supposed to have lower polydispersity index and lower surface area. However, they could have more recognition properties like binding capacity, binding affinity, imprinting factor and binding kinetics. CRP methods offer the ability to control the molecular weight of synthesized polymers and also the possibility of extending a polymeric chain (e.g. hydrophilic chain to make it water compatible) with consecutive blocks on the surface of synthesized polymer.

Another interesting synthesis approach is imprinting the porous silica-based organic-inorganic (SOI) hybrid materials for specified targets. In this strategy, organosilane monomers and crosslinkers are used in the co-codensation of SOI materials. Creation of recognition sites within the crosslinked metal-oxide network needs careful selection of sol-gel precursors and their ratios [37].

By using aggregated ionic surfactants as supramolecular templates in the co-condensation reaction, meso-porous SOI can be made instead of micro-porous SOI (pore diameters 2-15 nm instead of less than 2nm). These meso-porous materials can have different structures resulting from the hexagonal, cubic or lamellar conformation of the surfactant aggregation at different concentrations and temperatures. The addition of template molecules to the reactants at carefully selected ratios allows selective sites to be imprinted at the surfaces or within the framework to form molecular imprinted meso-porous SOI materials (Figure 1-7)

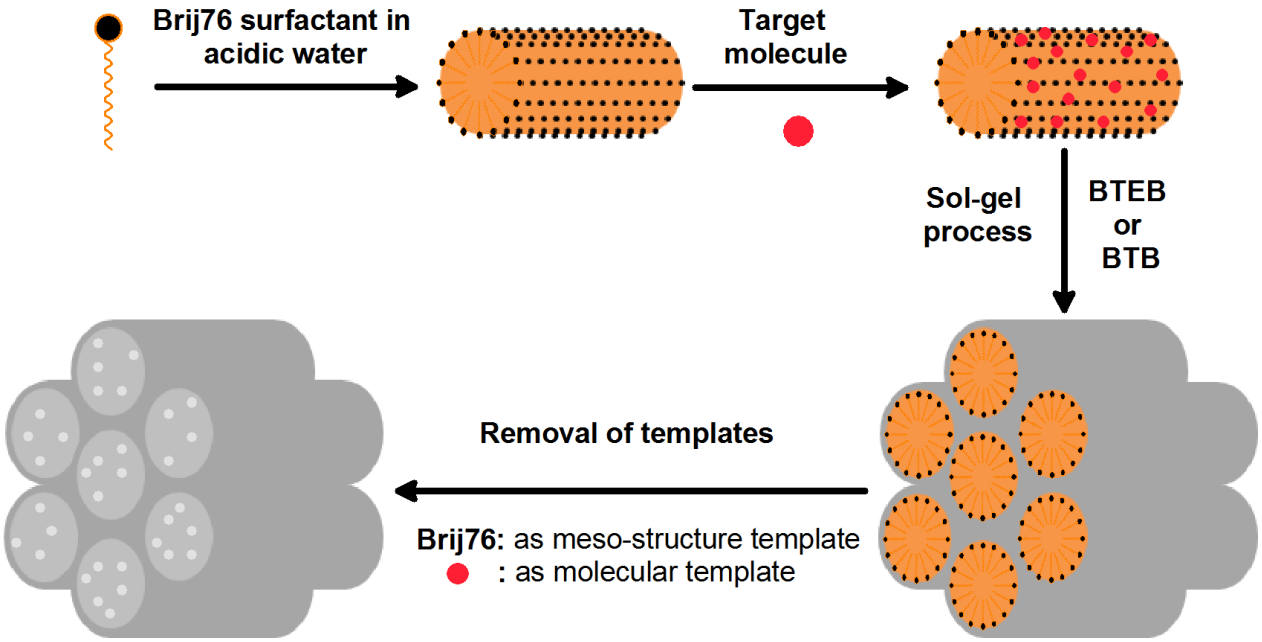


Figure 1-7: Imprinting on the wall-surface of a meso-porous silica-based organic-inorganic material. Brij76: Polyalkylen glycol ether; BTEB: Bis(trimethoxysilylethyl)benzene; BTB: 1,4-Bis(triethoxysilyl)benzene.

Imprinted polymers can be coated on previously modified MWCNTs [38] (Figure 1-8A) or spherical nanoparticles (e.g. silica nanoparticles [39] Figure 1-8B).

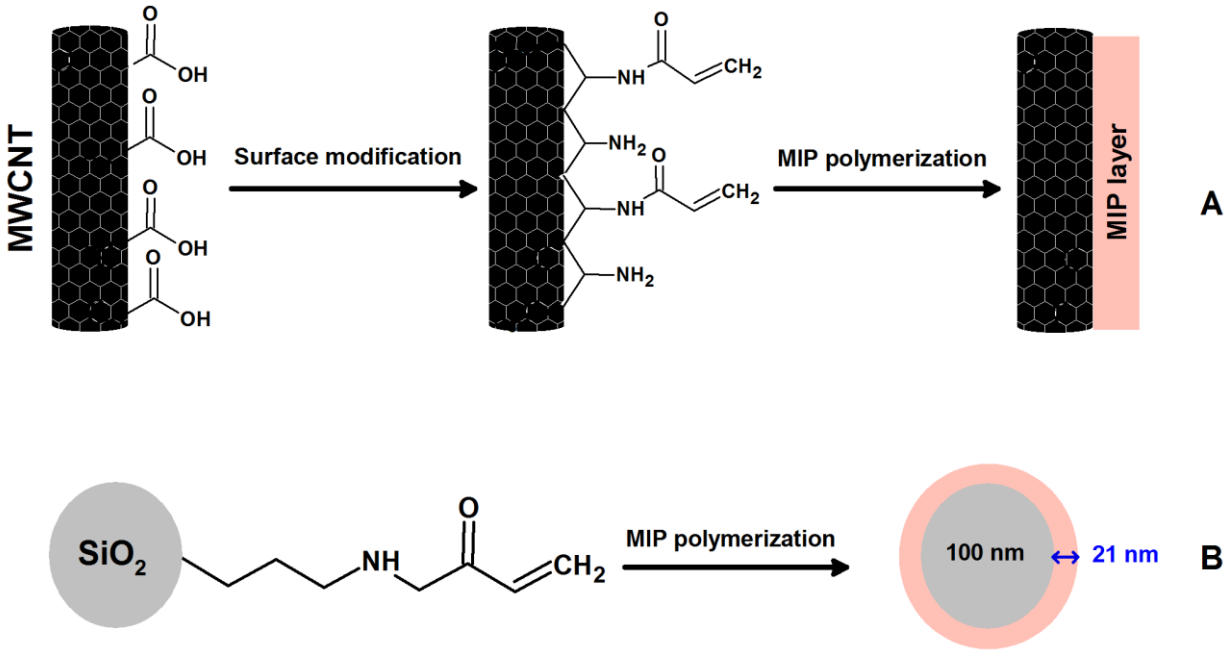


Figure 1-8: Surface modifications of nanoparticles using molecularly imprinted polymers.

In comparison to the other selective sorbent materials including immunosorbents, aptamers and restricted access materials, the advantageous qualities of MIP materials (e.g. low-cost, easy engineering, simplicity of production, potential reusability, physical/chemical stability and their applicability for a wide range of targets) make their applications remarkably widespread. Artificial selective MIP materials have been used considerably to modify sensor transducers or in combination with sampling/sample-preparation methods.

1.3. Application of MIP to modify sensor substrates

In general, for the preparation of the modified sensors, sensor substrates must be uniformly and reproducibly coated with MIP-nanolayers. These organic/inorganic layers should also adhere to the surface of the sensor substrate. The response time of modified sensor can be reduced by decreasing the MIP layer-thickness. The modified sensors must ideally be highly selective, sensitive, robust, inexpensive and have the ability to be miniaturized. To date, many different sensor substrates have been modified using various innovative modification methodologies (e.g. quartz-crystal-microbalance (QCM) [40], electrochemical sensors [41], electrochemical quartz crystal microbalance (EQCM) [42], fluorescence [43] and chemiluminescence [44] sensors, surface plasmon resonance and localized surface plasmon resonance sensors [45], surface-enhanced Raman scattering [46], colorimetric sensors [47] and integrated-optical-waveguide (IOW) [37]).

1.4. Application of MIP in sample-preparation techniques

Due to inherent advantages of MIP materials including simplicity of production and their physical/chemical stability, they have been used as selective sorbents in various sample preparation formats. *Molecularly imprinted solid phase extraction* (MISPE) is a SPE cartridge which is filled with MIP materials. So far, MISPE is the most used technical application of MIPs. There are many published manuscripts in which MIPs are synthesized for different templates and then implemented in SPE format for the selective extraction of target molecules from liquid samples [48]. The most important reason for this reputation is that the traditional SPE cartridges including their selective types, MISPE cartridges, can be easily prepared in the laboratory. However, laboratory-made SPE cartridges which are filled by the sorbents with

irregular shape and/or nano-size ranges and/or swellable characteristic, indicate often an elevated back-pressure which results in a limited sample flow-rates and/or sample volumes. Table 1-1 summarizes the experimental conditions for developed MISPEs which used for selective extraction of different targets from water samples. As can be seen, the optimized flow-rates for the used sample-volumes are whether not reported (N.R.) or are very low.

Table 1-1: Molecularly imprinted polymers used in MISPE format for water samples.

Sample volume (mL)	Flow rate (ml min^{-1})	Used MIP (mg)	Target molecules	Reference
10	N.R.	100	Amino acids	[49]
10	N.R.	400	Bisphenol A	[50]
20	0.5	200	Triazine herbicides	[51]
30	3	500	Gastrodin	[52]
~4	N.R.	200	Melamine; Cyromazine; Triamterene	[53]
100	≤4	200	Sulfaguanidine	[54]
200	5	200	Benzophenones	[55]
25/100	2	100	Estrogenic compounds	[56]
10	0.5	100	Fluoroquilones	[57]
100	1	400	Triazines	[58]
15	N.R.	250	Glyphosate	[59]
5	0.5	100	Carbamate pesticides	[60]
5	N.R.	100	Bisphenol A	[61]
25	N.R.	30	Estrogens	[62]
250	2	200	Endocrine-disrupting compounds	[63]
5	N.R.	15	Acidic pharmaceuticals	[64]
75	N.R.	25	Amphetamine; Methylamphetamine	[65]
100	N.R.	100	Nitrophenols	[66]
10	0.5	500	Tetrabromobisphenol A	[67]
35	1	100	Acid dyes	[68]
250	2.5	N.R.	β-Lactam antibiotics	[69]
50	0.5	100	Phenolic compounds	[70]
100	2.5	150	Fluoroquinolones	[71]
40	0.5	200	Malachite green	[72]
100	1	200	Indomethacin	[73]
25	1	25	β-blockers	[74]
100	6.5	200	Triazines	[75]
10/20	1	100/200	benzo[a]pyrene	[76]
20/50/100	1	50/250	Chlorotriazine pesticides	[77]

N.R.: Not Reported

Therefore, using interesting strategies, different substrates have been used in combination with imprinted polymers to produce selective membranes and filters. They were used for different purposes including dynamic (membrane and filter) and static extraction (Table 1-2).

Table 1-2: Molecularly imprinted polymers used in membrane format.

substrate	Preparation procedure	Reference
Bacterial cellulose nanofibers	Immersing substrate into a dilute pre-polymerization mixture	[78]
Cellulose	In situ polymerization of MIP Layer on the activated surface of cellulose fibers in paper	[79]
Commercial filter and cellulose acetate as the agglutinant	Soaking the filter paper into polymerization mixture	[80]
Cellulose paper	In situ polymerization of MIP Layer on the activated surface of cellulose fibers in paper.	[81]
Cellulose paper	Immersing substrate into a pre-polymerization mixture	[82]
Cellulose	N-methylmorpholine-N-oxide (NMMO) dissolves cellulose	[83]
Commercial filter and cellulose acetate as the agglutinant	Immersing substrate into a pre-polymerization mixture	[84]
Commercial filter and cellulose acetate as the agglutinant	Immersing substrate into a pre-polymerization mixture	[85]
Cellulose	N-methylmorpholine-N-oxide (NMMO) dissolves cellulose	[86]
Bacterial cellulose	In situ polymerization of MIP Layer on the activated surface of cellulose fibers in paper	[87]
Microporous polypropylene fiber membrane	In situ polymerization	[88]
Microfiltration polyvinylidene fluoride	A thin MIP layer was created on the surface of substrate	[89]
High-density polyethylene (HDPE) membranes	A thin MIP layer was created on the surface of substrate	[90]
24-well glass fiber membrane filter plates	MIPs were synthesized in multi-well glass fiber membrane filter plates	[91]
Commercial polyethylene (PE) frits	A small amount of MIP has been synthesized within the pores of the substrate	[92]
Polysulfone (PSF)	MIP powder was put into the dissolved PSF and was cast on a glass pane.	[93]
Nylon-6 membranes	Immersing substrate into a pre-polymerization mixture	[94]

Poly(vinyl chloride)- membrane	MIP was incorporated in a plasticized poly(vinyl chloride)-membrane by casting method	[95]
PTFE membranes	Immersing substrate into a pre-polymerization mixture	[96]
Polyvinylidene fluoride microfiltration membranes	A thin MIP layer was created on the surface of substrate	[97]
Commercial porous polypropylene membranes	Immersing substrate into a pre-polymerization mixture	[98]
Porous polypropylene membranes	Immersing substrate into a pre-polymerization mixture	[99]
Poly(acrylonitrile-co-acrylic acid) membrane	Phase inversion technique in the presence of template	[100]
Polyvinylidene fluoride (PVDF) microfiltration membrane	Immersing substrate into a pre-polymerization mixture	[101]

Among them, only MIP-modified polyvinylidene fluoride microfiltration and commercial porous polypropylene membranes were used for 'fast filtration' of 10 mL of water samples at 10 mL min^{-1} (Table 1-3). In these studies, either a thin MIP layer was created on the surface of the substrate or the substrate was immersed in a pre-polymerization mixture and continued by polymerization processes. The main obstacles were clogging or reduction of the porosity of the used substrate during polymerization. In addition to that, complicated, partially expensive and time-consuming preparation methods with trained and experienced personals are needed to prepare the loaded substrate with reproducible performance. Furthermore, all these methods lack of flexibility to adjust and control the desired amount of loaded adsorbents.

Table 1-3: MIPs/membrane format used as affinity filtration.

Sample volume (mL)	Sample flow rate (ml min^{-1})	Weight of used MIP (mg)	Reference
10	10	NR	[89]
10	NR	NR	[90]
0.1	-	13	[91]
0.5	0.1	3	[92]
10	0.084	17.2	[94]
10	10	~7	[97]
10	10	40	[98]
NR	0.5	NR	[100]
10	10	~2.6	[101]

N.R.: Not Reported

In addition to the previous mentioned sample-preparation methods, miniaturized MISPE formats like MIP grafted to porous polyethylene [92], microextraction by packed sorbent [102], porous membrane protected micro-solid phase extraction [103] and molecularly imprinted monolith μ-SPE [104] were developed and optimized. The key advantage of these extraction techniques is the minimized usage of organic solvents. In some cases, on-line connection to gas chromatography and liquid chromatography is possible and allows automated operation and minimal labor effort. MIPs have been also used as selective sorbents in SPME .Various methods were developed for preparing MIP-coated SPME fibers, which were used for analysis of clenbuterol [105], triazines [106], sudan dyes [107], ascorbic acid [108], tetracyclines [109], bisphenol A (BPA) [110], chloroacetanilide herbicides [111], anabolic steroids [112] and estrogens [113]. MIP-monolithics were also synthesized and used as SPME fibers for selective determination of triazines [114], methamphetamine [115], diacetylmorphine [116], ephedrine [117] and parabens [118]. In all these applications, the limited mechanic stability of the MIP fibers may cause short life times and problems for multiple use. One approach developed to overcome this problem was in-tube SPME technique. Besides, handling an in-tube device may be easier for automated on-line methods. First report for in-tube MIP-SPME was released by Pawliszyn group [119] for selective determination of propranolol. The MIP selected particles were used to pack a polyetheretherketone (PEEK) tube. Since then, different methods like monolith in-tube MIP-SPME [120], MIP modified-polypropylene hollow fiber [121] and multiple fibers packed in-PEEK tube [122] were developed. Additionally, the advantages of MIP materials can be combined with other selective sorbents to increase their efficiency for extraction of target molecules with high enough sensitivities from aqueous samples having complicated matrices. MIP microspheres were synthesized using RAFT polymerization and then coated with an ultrathin hydrophilic shells using “grafting to” method [123]. This strategy combined the advantages of MIP and RAMs. In another example, an amino-aptamer was used as the functional monomer within the imprinted cavity (hybrid receptor) [124].

Synthesis of new imprinted polymer materials for important targets and their combination with new innovative sample-preparation strategies is an interesting research subject which is still in progress.

1.5. Ion mobility spectrometry

Since the introduction of ion mobility spectrometry (IMS) technique in 1970 as a large and expensive detector, it has been enormously developed [125]. Nowadays, IMS is available as a handheld and field-portable chemical detector in a lot of airports thought the world. It has found its way to military application for the fast and sensitive detection of chemical warfare agents and explosives. IMS was introduced primarily in a time-of-flight (TOF) configuration (Figure 1-9) which has also nowadays the most common application.

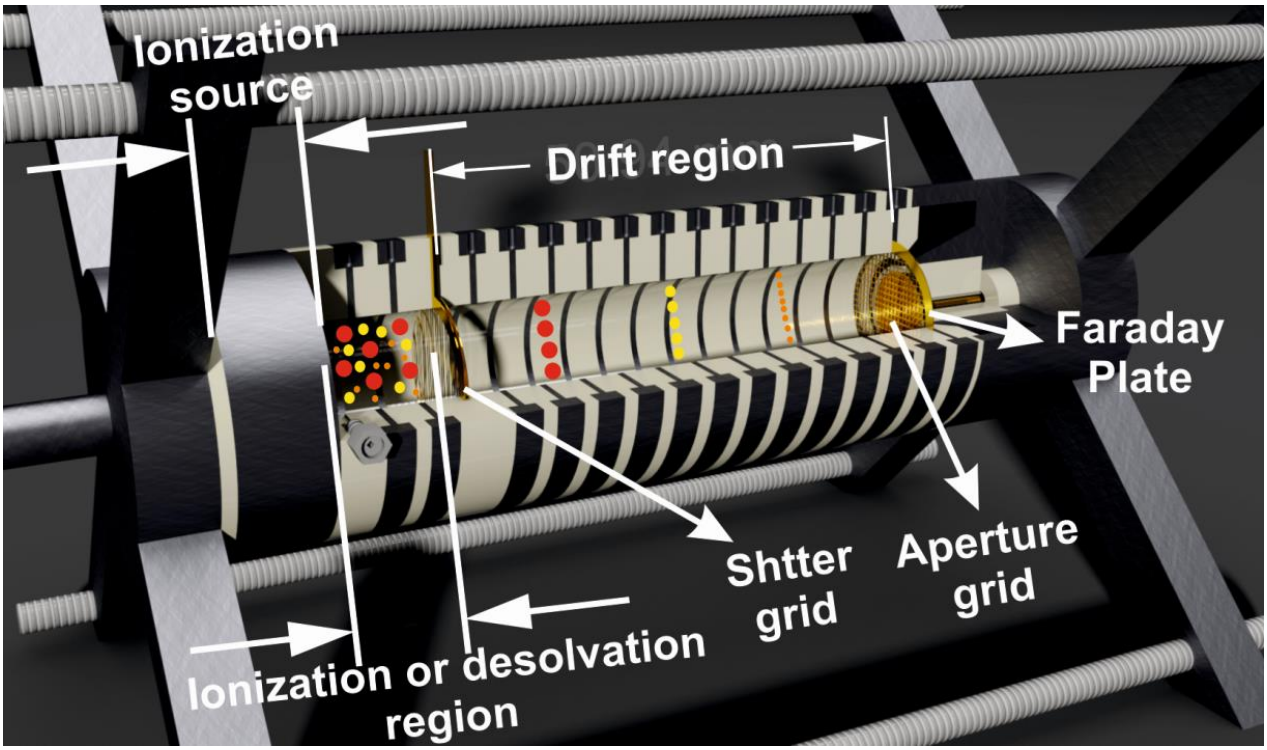


Figure 1-9: Time-of-flight ion mobility spectrometry (TOF-IMS) configuration.

Later, other miniaturized configurations like differential mobility spectrometry (DMS) or high-field asymmetric waveform ion mobility spectrometry (FAIMS) and aspirator IMS were also introduced. ^{63}Ni and ^{3}H as beta emitter have been used as the most common ion source in IMS detectors. However, these radioactive ion sources can be replaced with new developed ionization methods like photoionization (PI), corona discharge (CD) ionization, pulsed-electron ionization, ambient pressure direct current glow discharge (DCGD) ionization and electrospray ionization (ESI). After ionization of chemical molecules, ions are whether injected as an ion

swarm into separation region (drift tube) using an electrical shutter-grid in a TOF-IMS or transferred continuously via carrier gas in a DMS or an aspiration IMS. After injection of ion swarm in a TOF-IMS, ions fly towards the detector under the influence of a uniform electrical filed (200-400 V cm^{-1}). Each ion in the swarm reach a specified velocity depends on its mass, charge, and collision with drift gas molecules (drift gas is an e.g. dried pure N_2 which flows in opposite direction of flying ions to clean the drift region and help to increase ions separation based on their collision-cross-section). At the end of the drift region, ions reach a faraday detector and produce ion current which is amplified and converted to voltage using an operational-amplifier (op-amp) and finally visualized as a peak versus time (time is in the range of millisecond and starting point is the time that the ion swarm was injected into drift region). Faraday detector is simply a metal plate which is shielded by a metal mesh termed aperture grid (it is about 1 mm in front of the metal plate and an there is an electrical field of about two times more than drift-region electrical field between aperture grid and faraday detector) to remove environmental noises. In DMS and FAIMS, ions are flying continuously through conducting surfaces. Flying ions experience an asymmetric low (≈1000 V cm^{-1}) and high (10,000-30,000 V cm^{-1}) electric field. The ions with the same mobility at low and high electric fields can fly through the plat to the detector and the rest ions will be deviated from the center and neutralized In contact with conducting surfaces. A compensated voltage which is a DC voltage can be applied to the conducting surfaces to displace the flying path of ions. Using different DC voltages, all ions can pass through the conducting surfaces towards the detector successively to monitor all entering ions into the analyzer. In aspiration IMS, a carrier gas transfers the produced ions into a channel which this channel has successive perpendicular (towards the gas flow) conducting plates. These plates produce deflection filed which causes those ions with higher mobility deflect after short delay and produce a signal on the preliminary plates. The ions with lower mobility will be detected later with the subsequent plates.

1.5.1. Electrospray ionization-ion mobility spectrometry

ESI is the known ionization method for liquid samples that is commonly used in HPLC-MS. Using ESI, the flow of a liquid sample containing chemical compounds changed continuously to charged droplets. The size of these charged droplets is then progressively reduced and

transformed into charged analytes. To produce ions using ESI, the target molecules must be primarily ionized inside the liquid phase (A^+B^-). Depend to the mode of ESI (positive or negative), one ion (A^+ or B^-) is electrochemically reduced or oxidized at the metal surface of electrospray capillary. The remained counter ions which have the same charges will be sprayed within the primary aerosol droplets produced by just electrospray or by pneumatically assisted electrospray.

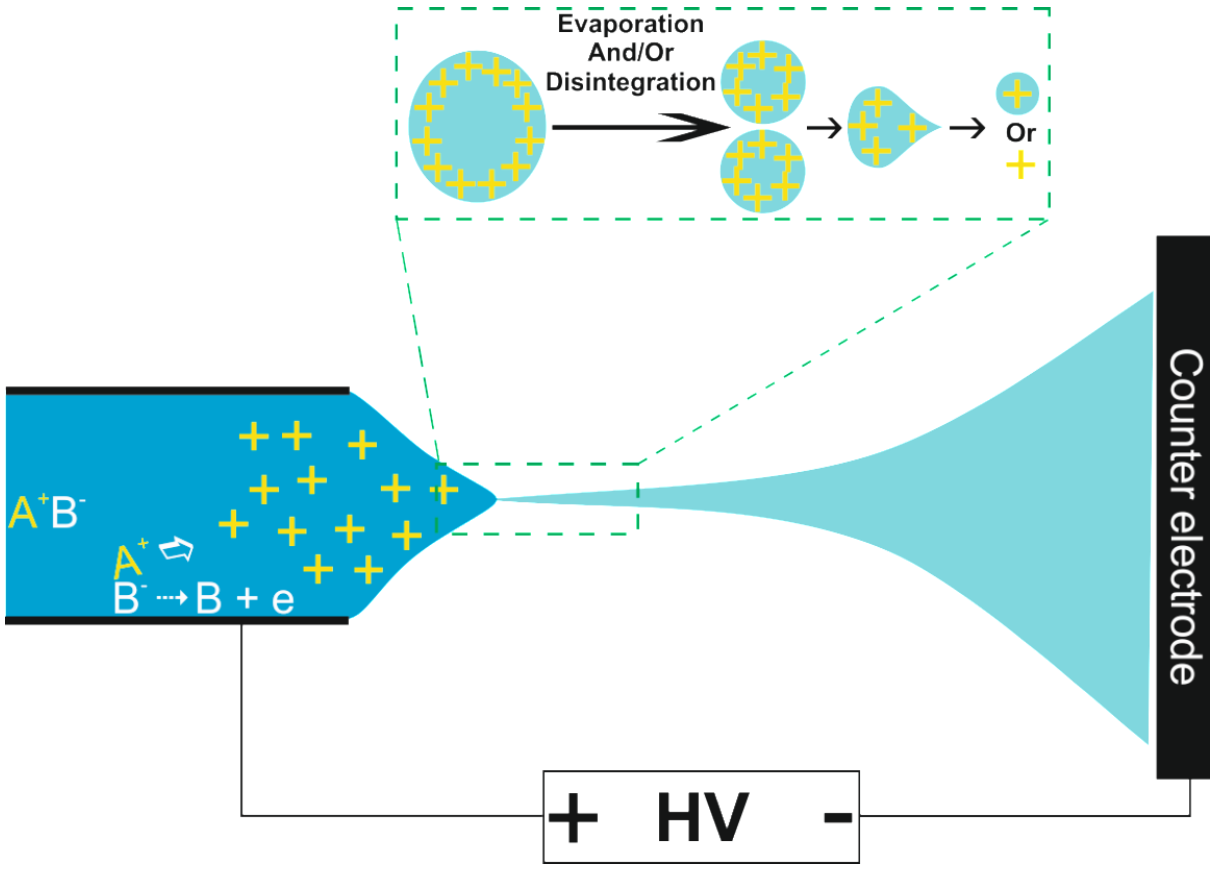


Figure 1-10: Ion production using electrospray method.

The size of these primary droplets depends on the liquid sample flow rate, solvent evaporation (which can be increased by a counter heated gas-flow) and sample composition (whether liquid matrix which controls the surface tension or electrolyte concentration). Low sample flow-rates, low surface tension and low electrolyte concentration enable the production of micrometer size droplets. It is important to mention that the increase of the size of these primary droplets affect negatively the formation of final ions which therefore reduce the ionization efficiency of the ESI method. Produced ions within the primary aerosol droplets have the same charge and repel

each other. The charge residue model (CRM) says that these charged droplets undergo contentiously a successive evaporation and disintegration process till final ion products are produced. The other ion evaporation model (IEM) says when the repulsion force within the primary aerosol droplets become stronger that the surface tension of the droplet, smaller charged droplets can leave the primary droplet. This process continue till the electric field at the surface of the droplet reach a level whereupon the charged target analytes leave the droplet into the surrounding gas [126] (Figure 1-10). Coupling the ESI ion source to IMS expanded its functionality from analyzing the gaseous samples to liquid samples. Although the first ESI-IMS couplings were introduced in the eighties, their initial use was limited due to the insufficient desolvation at atmospheric pressure and the resulting low resolution of spectrometers [127]. However, much effort has been made to increase the sensitivity and the resolution of ESI-IMS spectrometers. Different instrumental improvements such as water cooled ESI sources [128] or the incorporation of counter flow heated drift gas [129] were developed. Additionally, the design of the desolvation region in front of the drift tube was optimized, e.g. by reducing of its volume and by adjusting the position of ESI needle [130]. Another approach included the minimization of the solvent amount by attaching nano ESI to IMS [131]. However, the application of nanospray emitters goes hand in hand with limited ion transfer into the ion mobility spectrometer and therefore with reduced sensitivities [132]. Nevertheless, these technical modifications led to the availability of functional ESI-IMS couplings, which can be also configured for high-resolution measurements [133]. Additionally, advances in miniaturization of IMS hardware will further support the mobility of ESI-IMS for on-site application, for instance, for fast water monitoring.

2. Experimental section

2.1. Chemicals

The chemicals used for polymer synthesis and extraction experiments are listed in Table 2-1. Distilled water was prepared using a Milli-Q water purification system.

Table 2-1: List of chemicals used during this study.

Chemical name	Chemical formula	CAS Number	Chemical vendor
4-Nitrophenol	$C_6H_5NO_3$	100-02-7	MERCK
Acetic acid	$C_2H_4O_2$	64-19-7	MERCK
Hydrochloric acid	HCl	7647-01-0	MERCK
Methanol	CH_3OH	67-56-1	MERCK
Acetonitrile	C_2H_3N	75-05-8	MERCK
Ethylene glycol dimethacrylate	$C_{10}H_{14}O_4$	97-90-5	Sigma-Aldrich
Methacrylic acid	$C_4H_6O_2$	79-41-4	Sigma-Aldrich
2,2´-Azobisisobutyronitrile	$C_8H_{12}N_4$	78-67-1	Sigma-Aldrich
EPA 604 phenols mixture	-	-	Sigma-Aldrich
Acetone	C_3H_6O	67-64-1	MERCK
Chloroform	$CHCl_3$	67-66-3	MERCK
Dichloromethane	CH_2Cl_2	75-09-2	Sigma-Aldrich
Atrazine	$C_8H_{14}ClN_5$	1912-24-9	Sigma-Aldrich
Ammonium hydroxide solution (25%)	NH_4OH	1336-21-6	MERCK
(Vinylbenzyl)trimethylammonium chloride	$C_{12}H_{15}ClN$	26616-35-3	Sigma-Aldrich
Saccharin	$C_7H_5NO_3S$	81-07-2	Sigma-Aldrich
Bentazon	$C_{10}H_{12}N_2O_3S$	25057-89-0	Sigma-Aldrich
Ibuprofen sodium salt	$C_{13}H_{17}O_2Na$	31121-93-4	Sigma-Aldrich
Naproxen sodium	$C_{14}H_{13}O_3Na$	26159-34-2	Sigma-Aldrich
Caffeine	$C_8H_{10}N_4O_2$	58-08-2	Sigma-Aldrich
Potassium hydroxide	KOH	1310-58-3	Sigma-Aldrich
Acesulfame potassium salt	$C_4H_4KNO_4S$	55589-62-3	Fluka
Humic acid sodium salt	-	68131-04-4	Sigma-Aldrich
Silica gel S (0.063-0.1 mm)	-	112926-00-8	Riedel-de Haën

The stock standard solution of 4-Nitrophenol, Saccharin, Bentazon, Ibuprofen sodium salt, Naproxen sodium and Caffeine were prepared in water at a concentration of 500 mg L^{-1} and stored in the refrigerator. The stock standard solution of atrazine was prepared in methanol at a concentration of 2000 mg L^{-1} and stored in the refrigerator. Other standard solutions were daily

prepared via the dilution of the stock solution using pH adjusted deionized water. Whatman filter paper (185 mm ø) was obtained from Sigma-Aldrich.

2.2. Instruments

KDS100 syringe pump from KD Scientific (Holliston, USA) was used to generate defined flows through the synthesized MIP-capillaries [16] and prepared cartridges [1, 134]. A GFL water bath (Burgwedel, Germany) and VL-6LM UV-lamp (6W, 312 nm) were used for the synthesis of the imprinted polymers. 8 mL BAKERBOND SPE glass columns (inside diameter: 12 mm, length: 91 mm), 3 mL BAKERBOND SPE glass columns, polytetrafluoroethylene (PTFE) frits, speedisk cartridge (H2O-Philic DVB), SDB cartridge (Styrene-divinylbenzene; 200mg/3mL), octadecyl cartridge (C18) and 24-fold vacuum extraction box were purchased from J.T.Baker (Deventer, Holland). 10, 20 and 100 µL glass-capillary tubes were prepared from BRAND (Wertheim, Germany). 2 mL glass vials and 250 µL micro glass vials (inserts) from Sigma-Aldrich were used for collecting the samples.

2.2.1. High-performance liquid chromatography with diode-array detection

The high-performance liquid chromatography with diode-array detection (HPLC-DAD) analysis was performed using an HPLC instrument HP series 1100 of Hewlett Packard (Waldbronn, Germany) equipped with a binary pump, a membrane degasser, an autosampler and a diode-array UV detector (DAD). For separation, an Aqua C18 column (Phenomenex, Aschaffenburg, Germany) of 30 mm length and 2.00 mm I.D. (5 µm particle size) was applied. The eluent consisted of 85% water (pH 2.5, acetic acid) and 15% ACN (1% acetic acid) at 1 mL min-1. The column temperature was adjusted at 22°C. 5 µL of extracts were injected automatically. The DAD diode array detector operated at 320 nm (bandwidth: 40 nm) with a reference at 450 nm (bandwidth: 80nm). Regularly blank and 4-NP standard analyses were carried out to check carryover effects and instrument performance.

2.2.2. Gas chromatography–mass spectrometry

The gas chromatography–mass spectrometry (GC-MS) analysis was performed using an Agilent GC-7890A/MSD-5975C system coupled with an Agilent Technologies CTC Analytics Combi PAL

autosampler. For separation, a HP-1MS capillary column (30 m, 0.25 mm ID, 1 µm film) and helium gas as the carrier at a constant flow rate of 1 mL min−1 were applied. 1 µL of the samples were injected in the injection port of the GC that was maintained at a temperature of 260 °C. The oven temperature of the GC was initially held at 60 °C for 1 min, then increased to 280 °C at a rate of 15 °C min^{-1} and remained at this level for 2 min. Mass spectrometric detection was carried out using electron impact (70 eV) ionization. After recording full scan spectra, we monitored target chemicals and brombenzol-d5 (internal standard) using their typical trace ions (m/z 200 and 215 for atrazine and m/z 82, 161 and 163 for brombenzol-d5).

2.2.3. High-performance liquid chromatography-tandem mass spectrometry

High-performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) was performed using an HPLC-system "Agilent 1260" (Agilent Technologies, Waldbronn, Germany) coupled with a "QTrap 5500" triple stage quadrupole mass spectrometer (AB Sciex, Darmstadt, Germany). 5 µL of the sample extracts were injected automatically. The chromatographic separation was performed with an "Ascentis Express C18" column (10 cm x 3 mm ID and 2.7 µm particle size, Supelco, Seelze, Germany). Water with 0.1 % acetic acid (solvent A) and methanol with 0.1 % acetic acid (solvent B) were used to separate the target substances at a flow rate of 300 µL min^{-1} and a linear elution gradient started with 95 % solvent A (1 min). Within 15 min the portion of solvent A decreased to 10 % and returned to 95 % between 20 min and 25 min run time. The column oven was set at 30 °C. Electrospray ionization was operated in positive mode at 5.5 kV spray voltage and -4.5 kV in negative ionization mode. Multiple reactions monitoring mode was applied for quantification (Table 2-2).

Table 2-2: Method characteristics of the analysis of selected wastewater contaminants determined in the cross selectivity study of the new developed MIP/NIP materials.

Target substance	CAS-No.	Ion transitions (m/z) quantifier/qualifier
Acesulfam	55589-62-3 (as K- salt)	162→82* 162→78*
Bentazon	25057-89-0	239→132* 239→197*
Caffeine	58-08-2	195→138 195→110

Carbamazepine	298-46-4	237→194 237→193
Ibuprofen	15687-27-1	205→161* 205→159*
Naproxen	22204-53-1	231→185 231→115
Saccharin	81-07-2	195→138 195→110
4-Nitrophenol	100-02-7	138→108
2-Nitrophenol	88-75-5	138→108
2,4-Dinitrophenol	51-28-5	183→109
2-Methyl-4,6-dinitrophenol	497-56-3	197→180
2,4,6-Trichloro phenol	88-06-2	195→161
Pentachlorophenol	87-86-5	265→163

*Negative ESI

2.2.4. Electrospray ionization-ion mobility spectrometry

The ion mobility spectrometer used in this study was designed and constructed at the Helmholtz-Centre for Environmental Research. The spectrometer has a traditional stacked ring electrode design with stainless steel ring electrodes of 1 mm thickness and isolators of 5 mm thickness made from Macor. The inner diameter of the electrodes and the isolators within desolvation region (53 mm length) is 10 mm while the drift region has an inner diameter of 20 mm. The drift length between the inlet grid and aperture grid is 113 mm. The aperture grid is positioned a further 1 mm in front of the faraday plate. Electric fields of 400 V cm^{-1} within the desolvation region and of 410 V cm^{-1} within the drift region are created by a series of variable and constant resistive voltage dividers. The shutter grid between desolvation region and drift tube was operated with an injection time of 200 µs with a duty cycle of 24 Hz which allows high signal intensities to be measured at an acceptable resolution (sample peaks do not overlap with solvent peaks). A home-made pulse generator in combination with an Agilent 33622A waveform generator (Keysight Technologies, Santa Rosa, USA) was used to apply a pulsed voltage to the shutter grid. Unless otherwise stated, the system temperature remained constant at 55°C and nitrogen was used as the drift gas with a standard flow rate of 600 mL min^{-1}. The ion current at the end of the drift tube was registered by a Faraday plate, amplified using a STEP amplifier (STEP Sensortechnik, Pockau, Germany) and digitized by an Agilent MSO6054A oscilloscope

using an averaged signal of 16 measurements (Keysight Technologies, Santa Rosa, USA). All measurements were performed in negative mode.

For ESI, a PicoChip (New Objective, Woburn, USA) equipped a liquid-junction interface and 120 mm uncoated silica tip emitter (30 µm tip diameter and 50 µm inner diameter of tube) was located approximately 2 mm in front of the first drift ring of the desolvation region which acts as counter electrode for the emitter. This ESI voltage was optimized to 2.3 kV. A commercially available stage (PicoChip for Thermo LTQ) was modified for three-dimensional positioning of the PicoChip and its power supply.

The solvent flow is adjusted using a KDS100 syringe pump (KD Scientific, Holliston, USA) with a 100 µL 1710TLLX syringe (Hamilton, Bonaduz, Switzerland). The solvent is transported through PTFE tube with an inner diameter (ID) of 0.45 mm to a three-port valve, which is used for refilling the solvent without opening the system. Via the PTFE tube, the solvent is transported to a 6-port injection valve (A1357, Knauer, Berlin, Germany) where the sample is injected into a 50 µL sample loop. The sample solution is transferred to the PicoChip via a NanoViper tube (Thermo Fisher Scientific, Waltham, USA) with 50 µm ID and a length of 35 cm.

2.2.5. Scanning electron microscopy

The surface morphology of the polymers was analyzed using an ULTRA 55 scanning electron microscope from Carl Zeiss AG with a maximum magnification factor of up to 1,000,000. The electron energy was set to 18 keV and, to ensure high surface sensitivity, the secondary electrons were used for imaging of the sample surface. The polymer was deposited on a silicon wafer and covered with a 10 nm thick gold layer by argon magnetron sputtering to reduce charging effects.

3. Polymer synthesis and evaluation

During our study, various polymers were synthesized and imprinted for three chemical compounds with different physical-chemical properties. These chemicals are important to be monitored in environmental water samples and are of great interest for environmental institutes like UFZ. These chemicals are 4-nitrophenol (4-NP, known as a priority pollutant), atrazine (the known herbicide) and acesulfame (known as an indicator to identify the introduction of domestic wastewater into water systems). The used interaction approach between template molecules and functional monomers was non-covalent interaction due to its inherent advantages mentioned in the introduction section (1.2.2.4.).

Synthesizing imprinted polymers for highly water-soluble template molecules like acesulfame is a great challenge. For this reason, we developed a new methodology for synthesizing imprinting polymer for this target (it is worthy to mention that the proposed method can be evaluated in future for other important polar compounds like glyphosate). New synthesized polymer for acesulfame was completely evaluated to prove its selectivity. The other two template molecules (i.e. 4-nitrophenol and atrazine) have been evaluated and published in many studies. Therefore, polymers for 4-nitrophenol and atrazine were synthesized using valuable information from published manuscripts. Theses synthesized polymers were just further evaluated and optimized to accomplish our necessities for the subsequent extraction-methods optimizations.

3.1. Acesulfame imprinted polymer

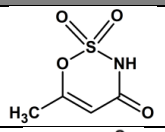
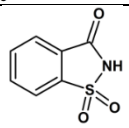
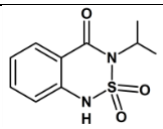
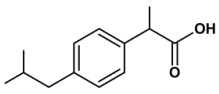
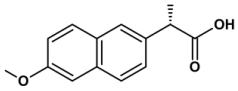
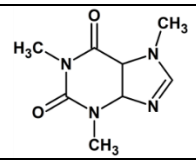
Acesulfame is a known indicator that is used to identify the introduction of domestic wastewater into water systems. It is an artificial low-calorie sweetener that is consumed in substantial quantities and can be found in various foods and beverages. After undergoing human metabolism, it passes through the system largely unaffected and as a result, is ubiquitously present in aquatic environments [135]. It may also enter soils via irrigation with wastewater-polluted surface water, fertilization with sewage sludge, or through leaky sewers [136]. Due to its large consumption levels and persistency towards molecular transformation in treatment processes, acesulfame has been accepted as an ideal indicator to identify the impact of wastewater on e.g. groundwater [137]. This is particularly the case in urban settings with

complex hydrology, where indicator substances can be used to locate pollution sources and to discover aquifer pathways. A systematic study has shown that even multi-barrier treatment systems cannot remove acesulfame completely from surface water, thus this compound was the only artificial sweetener detected in fully-processed drinking water [138]. In many studies, acesulfame has been reported as a marker which can be used to identify wastewater-related contaminations in surface water [139], sewage sludge [140], leachate [141], groundwater samples [139] and hyporheic and riparian zones [142]. Acesulfame is negatively charged and highly water-soluble at environmental pH values. The main challenge in MIP technology is to find an appropriate strategy for the synthesis of an actual valid and proper selective polymer for water-soluble compounds, such as acesulfame. In order to solve this problem, some methodologies have already been devised. To prepare 2,4,5-trichlorophenoxyacetic acid, bisphenol A, tratarzine and antibiotics/oligopeptides imprinted polymers in water and methanol–water systems, 4-vinylpyridine [143], 3-acrylamido-N,N,N-trimethylpropan-1-aminium chloride (AMTC) [144], 1-(∝-methyl acrylate)-3-methylimidazolium bromide (1-MA-3MI-Br) [68] and acryloyl-cyclodextrins [145] were used as functional monomers, respectively. An ionic template-functional monomer complex and a water-soluble crosslinking reagent were used for the synthesis of (4-tributylammoniummethyl)-benzyl tributylammonium chloride (TBTA)-imprinted polymer in water as a porogenic solvent [146]. For preparation of the pyridoxine-imprinted polymer, dodecyl sulfate ion (DS^-) was used to transfer pyridoxine ion (Py^+) from water to chloroform via ion-pair complex formation [147]. In our study, a selective imprinted polymer was synthesized for the negatively-charged acesulfame. (Vinylbenzyl)trimethylammonium chloride (VBTA) was used as a phase-transfer catalyst and a functional monomer. After the phase transfer process, methacrylic acid was used as an extra functional monomer to enhance the selectivity of the synthesized polymer. To evaluate the proposed methodology, different parameters have been assessed and optimized. After polymerization, the optimized polymers were evaluated to prove their selectivity feature.

3.1.1. Solubility evaluation of acesulfame

Acesulfame is strong polar solutes and dissolves well in strong polar solvents. It is freely soluble in water, partially soluble in methanol and insoluble in chloroform [148] (Table 3-1).

Table 3-1: Physicochemical properties of studied chemical compounds.

Compound	Log K_{ow}	pKa	Solubility in water (mg/L, at 25°C)	Structure
Acesulfame	-1.33 [149]	-0.28 [149] 5.67 [a]	5.88×10^5 [a]	
Saccharin	0.910 [149]	1.60 [149]	3.45×10^3 [a]	
Bentazon	2.8 [a]	3.3 [a]	500 [a]	
Ibuprofen	3.97 [a]	4.91 [a]	21 [a]	
Naproxen	3.18 [a]	4.15 [a]	15.9 [a]	
Caffeine	-0.07 [a]	14.0 [a]	21.6×10^3 [a]	

[a] Values are obtained from: http://toxnet.nlm.nih.gov/

Due to the serious drawbacks of polar solvents, non-polar solvents such as chloroform are commonly used for MIP synthesis [147]. For the synthesis of MIP materials using a non-covalent interaction strategy, the porogenic solvents should have relatively low polarity, to reduce any interference during the complex formation processes that take place between the template and functional monomers [150]. In order to increase acesulfame solubility in chloroform, two procedures were tested and evaluated. Both methods are based on ion-pair formation between acesulfame and VBTA. VBTA is a derivatized styrene compound with a benzylic trimethylammonium group. In order to improve the solubility of acesulfame in chloroform, VBTA was examined as an ion pairing reagent: (I) to improve the solubility of acesulfame directly in chloroform or (II) alternatively, via extraction from water into chloroform (whereas VBTA supported the phase transfer).

In the first series of experiment (I), 201 mg (1 mmoL) of acesulfame-K and 211 mg (1 mmoL) of VBTA were weighted together in a glass vial and 10 mL of chloroform was added. The mixture was sonicated for 15 min and subsequently shaken at 300 min^{-1} for 1 h. In comparison, the same procedures were applied to dissolve 201 mg acesulfame-K in 10 mL chloroform.

In the second series of experiments (II), 201 mg of acesulfame-K and 211 mg of VBTA were weighted together and dissolved in 3 mL water. 10 mL chloroform was added to the prepared mixture and stirred vigorously for 1 h. The two phases were separated and the concentration of acesulfame in chloroform was analyzed. As a comparison, 201 mg of acesulfame-K was dissolved in 3 mL water. Then 10 mL of chloroform was added to the mixture and was stirred vigorously for 1 h. The two phases were separated in the same way and the concentration of acesulfame in chloroform was analyzed.

Results from the first series of experiments (I) showed that acesulfame solubility increased from 0.01 % to 41 % using VBTA-addition into chloroform. It was also observed that adding VBTA to chloroform containing acesulfame resulted in a cloudy suspension.

Using the second series of experiments (II), in which ion-pair complex was formed between acesulfame and VBTA in the water phase and then transferred into chloroform phase, the extractability of polar and ionic acesulfame from water into chloroform was enhanced from 0 % (just acesulfame in water phase) to 53 % (both acesulfame and VBTA in water phase). Using this second strategy, both acesulfame and VBTA can be easily dissolved in water, where the ion pairing process can occur quantitatively. Thus, from a practical point of view, extraction of the acesulfame-VBTA ion pair complex from water into chloroform was more efficient and simpler to realize. Therefore, the ion-pair transfer from water into chloroform was selected for the subsequent future evaluations.

In order to evaluate the transfer efficiency of acesulfame into the chloroform phase, the ratio between acesulfame and VBTA was changed in a series of experiments. The amount of acesulfame was kept constant at 1 mmoL and the amount of VBTA was increased from 0.5 to 1 and 1.5 mmoL. For each Acesulfame/VBTA ratio, the extraction process was repeated 3 times (E1, E2 and E3) successively. The concentration of acesulfame in chloroform for each of the extracted fractions was analyzed.

Figure 3-1 shows the results obtained for our efficiency evaluation of the acesulfame/VBTA ratio in acesulfame transfer from water into chloroform during three successive extraction steps.

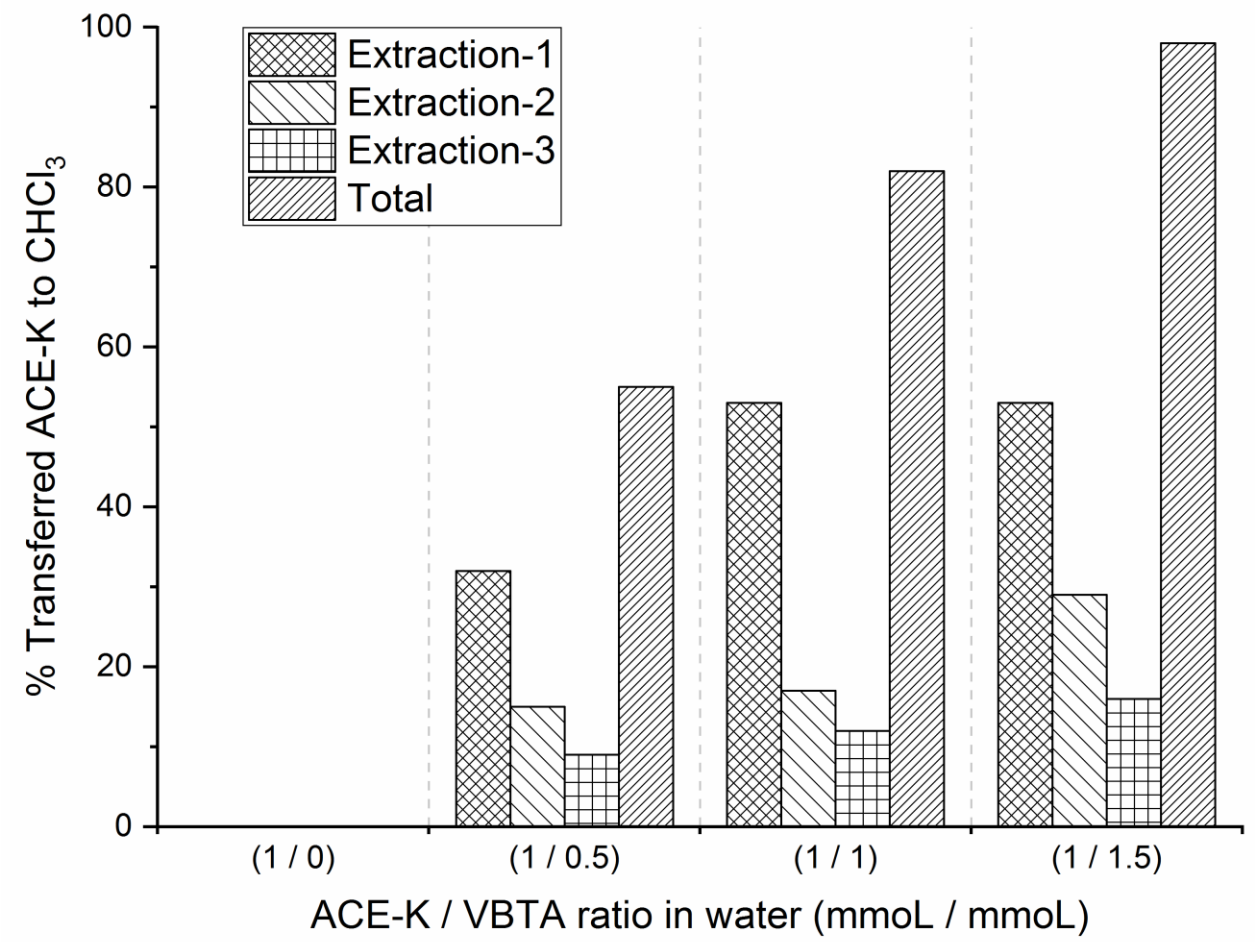


Figure 3-1: Solubility evaluation of acesulfame in chloroform using different Acesulfame/VBTA ratios in water during three successive extraction procedures.

Increasing the acesulfame/VBTA ratio resulted in an increase in the amount of totally transferred acesulfame. Both amounts 1 and 1.5 mmoL VBTA extracted the same amount of acesulfame (53%) during the first extraction step, while when using 1.5 mmoL VBTA, nearly all (98%) 1 mmoL acesulfame was extracted after all 3 extraction steps.

3.1.2. Synthesis of acesulfame imprinted polymers and equilibrium rebinding experiments

In order to evaluate the efficiency of VBTA and MAA as functional monomers, 4 different polymers were prepared. Table 3-2 outlines the composition of reagents for MIP synthesis using the precipitation method.

Table 3-2: Different polymerization mixtures used for preparation of MIPs for acesulfame.

	Acesulfame-K in water (mg)	VBTA in water (mg)	Extracted acesulfame to Chloroform (mg)	MAA (mg)	EGDMA (mL)	AIBN (mg)	Porogen type	Porogen volume (mL)
MIP1	201	211	106.5	0	2	10	Chloroform	10
MIP2	201	318	106.5	0	2	10	Chloroform	10
MIP3	201	211	106.5	182	2	10	Chloroform	10
MIP4	201	318	106.5	182	2	10	Chloroform	10

All polymer mixtures were deaerated with helium for 15 min and photochemically polymerized at 15℃ for 3 h using a UV-lamp at 312 nm. For all of the MIPs mentioned above, the corresponding non-imprinted polymers (NIPs) were prepared in the same way without the target molecule. Following polymerization, the materials were washed with methanol/NH_4OH 25% (80:20, v/v) and the supernatant was separated by centrifugation. Washing processes were checked and verified using a high-performance liquid chromatography-tandem mass spectrometry (HPLC-MS-MS) instrument and were continued until target molecules were no longer detectable. Afterwards, polymer particles were activated during 24 h washing process using Soxhlet extraction. The upper edge of an extraction thimble was cut so that it was lower than the siphon-top of the extractor. Methanol/acetic acid (99:1, v/v) mixture was used as the extraction solvent. Finally, the polymer particles were dried under vacuum conditions and stored in a desiccator at room temperature for further evaluation.

The synthesized MIPs and NIPs were evaluated using static adsorption experiments in water to investigate their rebinding capacity towards acesulfame. For this purpose, 10 mg of synthesized polymer was weighed and 50 µL of methanol was added to condition the weighed polymer. The conditioned polymer was equilibrated with 5 mL of water containing acesulfame-K at concentrations ranging between 5 to 200 mg L^{-1}. The resulting mixture was shaken for 12 h at 25 °C. Finally, the water phase was separated and used for further analysis by HPLC/MS-MS. The adsorption capacity of polymer materials, A ($mg_{Acesulfame}\ g_{Polymer}^{-1}$) was calculated using the following equation:

$$A = \frac{C_\circ - C_e}{m} \times V \qquad (3-1)$$

where $C_\circ$ and C_e represent the initial and the equilibrium concentration (µg mL^{-1}) of acesulfame-K in a water solution, respectively. V is the volume (mL) of water sample and m is the amount (mg) of polymer. Figure 3-2 represents the binding isotherms of acesulfame at concentrations ranging between 5 to 200 mg L^{-1} for various synthesized polymers.

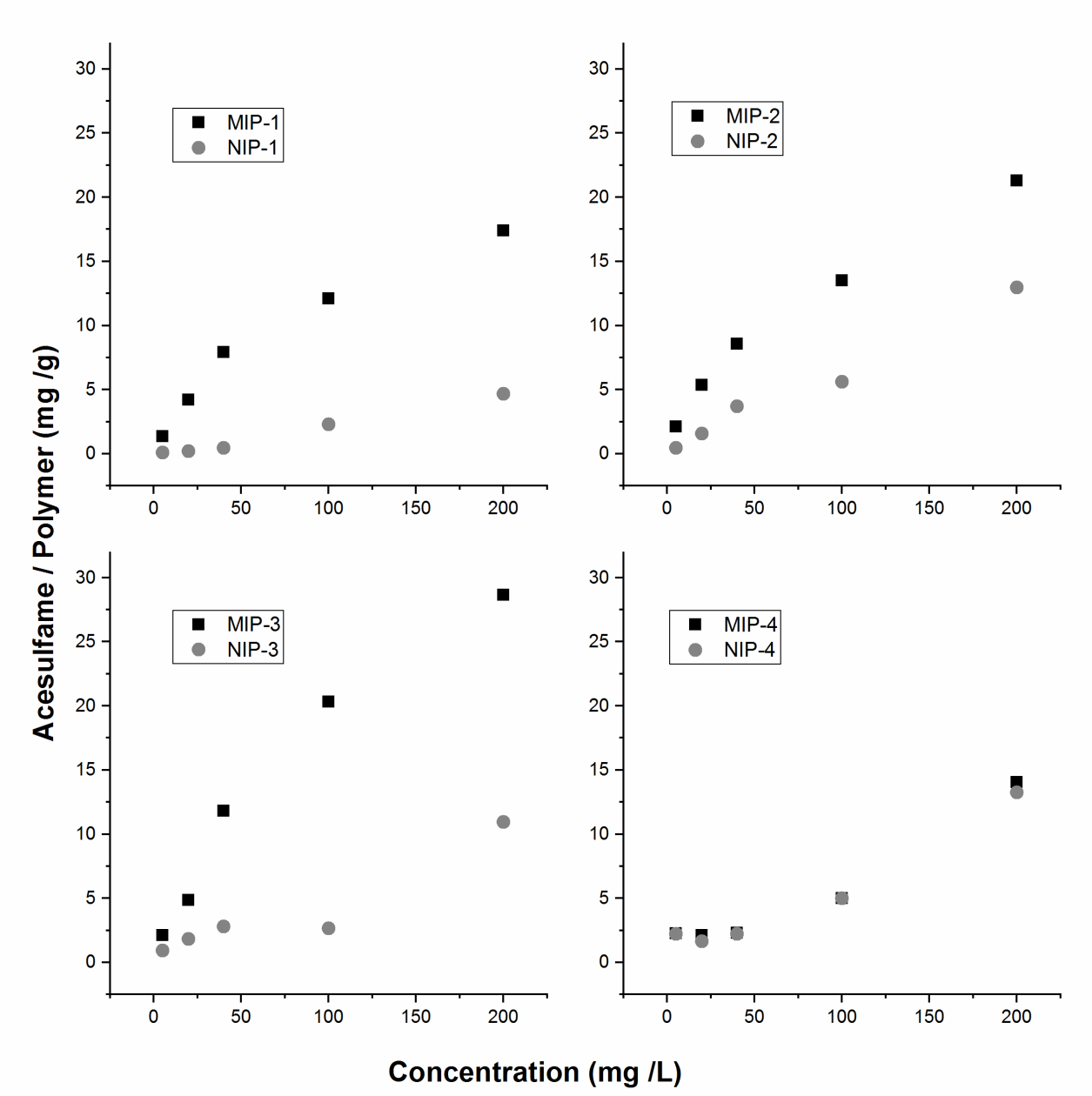


Figure 3-2: Binding isotherms of synthesized polymers. MIP1, MIP2, MIP3 and MIP4 compositions are shown in Table 3-3.

Binding isotherms for MIP1 and MIP2 indicated a reduction in selectivity for MIP2 that could be related to the surplus amount of VBTA transferred during the phase transfer process. This excess amount of VBTA in chloroform for both MIP and NIP would produce more non-specific sites. While MIP4 exhibits the worst selectivity, the binding isotherm for MIP3 actually achieves a capacity and selectivity enhancement for the synthesized polymer by adding MAA, which operates as an additional functional monomer in MIP3. Due to the high maximum adsorption capacity (A_{max}) and the selectivity of MIP3, this polymer was selected for further selectivity experiments.

3.1.3. Selectivity experiments

Concerning selectivity assessment of the synthesized MIP3, two series of experiments were performed. In the first series of selectivity experiment (I), equilibrium rebinding experiments at concentration of 200 mg L^{-1} (A_{max}) were investigated for acesulfame structural-analogues as followes: bentazon, saccharin, ibuprofen sodium salt, naproxen sodium salt and caffeine. The selectivity coefficients were calculated using the following equations:

$$D = \frac{A}{C_e} \qquad (3-2)$$

$$\alpha_{Acesulfame/X} = \frac{D_{Acesulfame}}{D_X} \qquad (3-3)$$

where D, A and C_e represent the distribution factor, polymer adsorption capacity and equilibrium concentration in the liquid. $\alpha_{Acesulfame/X}$, $D_{Acesulfame}$ and D_X are the selectivity factor in batch mode, the distribution factor of acesulfame and lastly the distribution factor of other studied chemical analogues, respectively.

Acesulfame, bentazon and saccharin have a sulfonyl group connected to an amine group that can be easily deprotonated at environmental pH values (Table 3-1). Ibuprofen and naproxen have a carboxyl group that can be also deprotonated at environmental pH values (Table 3-1). Unlike the aforementioned chemical compounds, caffeine cannot be deprotonated in water but has been used as an anthropogenic marker [151].

Calculated selectivity factors for acesulfame in comparison to bentazon, saccharin, ibuprofen, naproxen and caffeine at Amax concentration were as follows:

Table 3-3: Obtained selectivity factors for MIP3 polymer towards acesulfame in comparison to its structural analog

$\alpha_{\frac{Acesulfame}{Bentazon}}$	$\alpha_{\frac{Acesulfame}{Saccharin}}$	$\alpha_{\frac{Acesulfame}{Ibuprofen}}$	$\alpha_{\frac{Acesulfame}{Naproxen}}$	$\alpha_{\frac{Acesulfame}{Caffeine}}$
1.96	2.05	3.69	4.04	6.69

Obtained results from the first series of selectivity experiments, shows clearly the selectivity feature of synthesized polymer towards acesulfame target molecules.

In the second series of selectivity experiments (II), the column method was evaluated. An 8 mL SPE cartridge packed with 200 mg of MIP3 and used for selectivity evaluation in column mode. A 50 mL water sample containing acesulfame-K, bentazon, saccharin, ibuprofen sodium salt, naproxen sodium salt and caffeine (where the concentration of each solute was 0.05 mg L^{-1}) was passed through the conditioned MISPE cartridge.

After sample loading, 2 successive washing steps and immediately 2 successive elution steps were used as followes: 5 mL methanol (as the first washing step, Washing-1), 5 mL methanol (as the second washing step, Washing-2), 5 mL methanol/NH_4OH 25% (99.5:0.5, v/v) (as the first elution step, Elution-1) and 5 mL methanol/NH_4OH 25% (99.5:0.5, v/v) (as the second elution step, Elution-2) were used to remove the adsorbed compounds completely from the sorbents. The results (Figure 3-3) showed that caffeine was hardly retained at all by the MIP sorbent. The adsorbed caffeine was washed out completely during the first washing step.

It appears that caffeine was adsorbed via non-specific adsorption on the non-imprinted sites in the polymer matrix. In comparison to caffeine molecules, MISPE cartridge showed higher adsorption affinity towards ibuprofen and naproxen. This can be explained by the negatively charged carboxyl groups available in the structure of ibuprofen and naproxen molecules. Nevertheless, much of both pharmaceutical compounds were washed out during the washing steps (W1 and W2) and the remained targets were eluted during the first elution step (Elution-1). Despite having the highest adsorption affinity for acesulfame, the MISPE cartridge showed

noticeable adsorption efficiencies towards the pesticide bentazon and the sweetener saccharin. This is probably related to their common negatively-charged sulfonamide functional group.

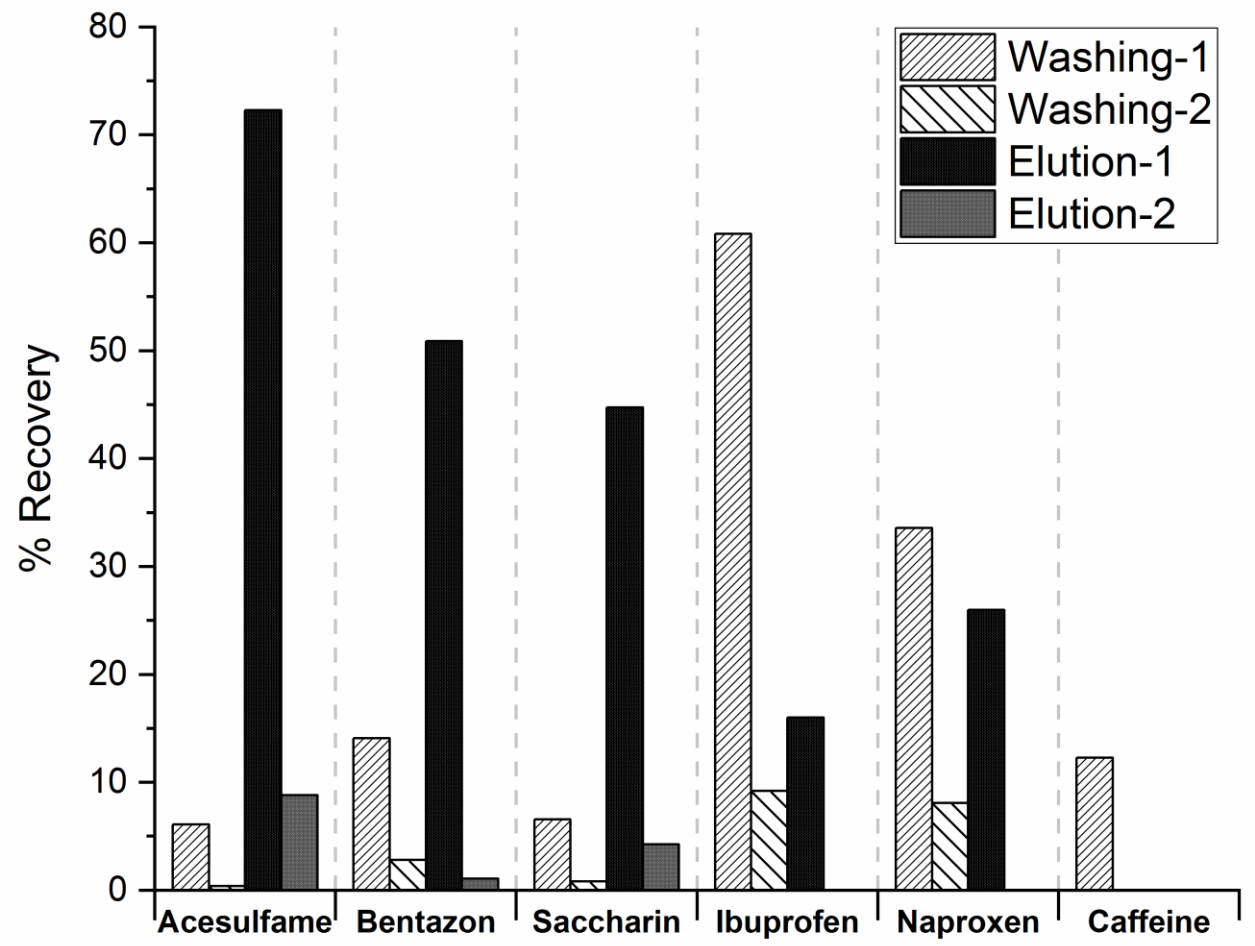


Figure 3-3: Selectivity evaluation using column method. Spiked water sample with six different and similar chemical compounds was used to load the MISPE cartridge. Two washing steps (Washing-1 and Washing-2) and two elution steps (Elution-1 and Elution-2) were used successively to remove the adsorbed targets.

3.1.4. Scanning electron microscopy images

Figure 3-4 shows the SEM micrographs of MIP3 and NIP3 materials prepared with VBTA and MAA at different magnification levels. The images highlight extraordinary differences in the morphology of the imprinted material MIP3 and its non-imprinted counterpart NIP3. While the non-imprinted polymers had a completely inhomogeneous rigid structure, the imprinted polymers are homogeneous in size and had porous structure.

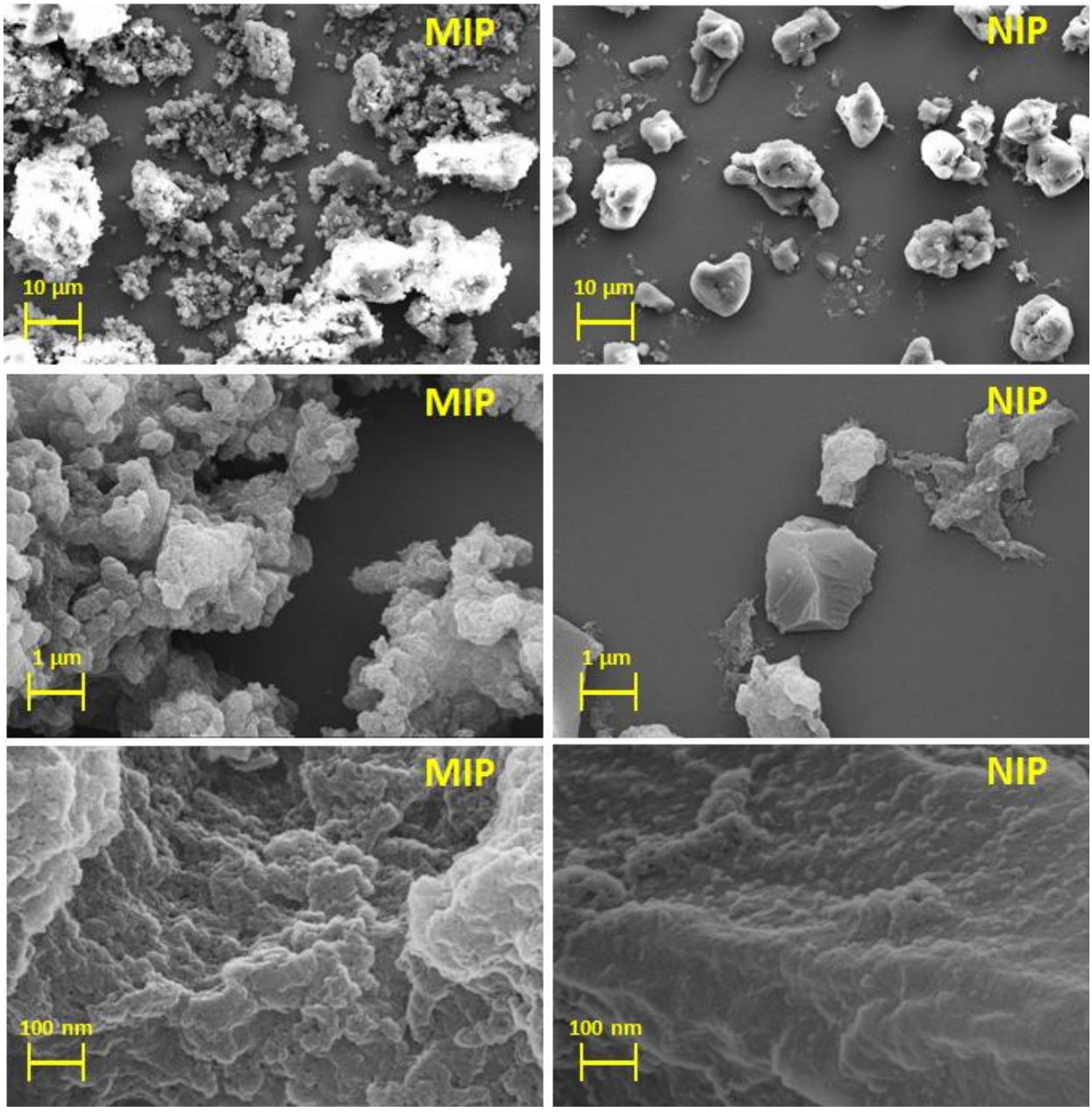


Figure 3-4: SEM images of synthesized MIP3 and NIP3 for acesulfame.

This could indicate the presence of recognition sites in MIPs which can be ascribed to the removal of template molecules. Due to solvent evaporation during SEM analysis, the MIP particles look agglomerated.

Figure 3-5 shows a schematic representation of MIP3 synthesis which was selected for MISPE investigation.

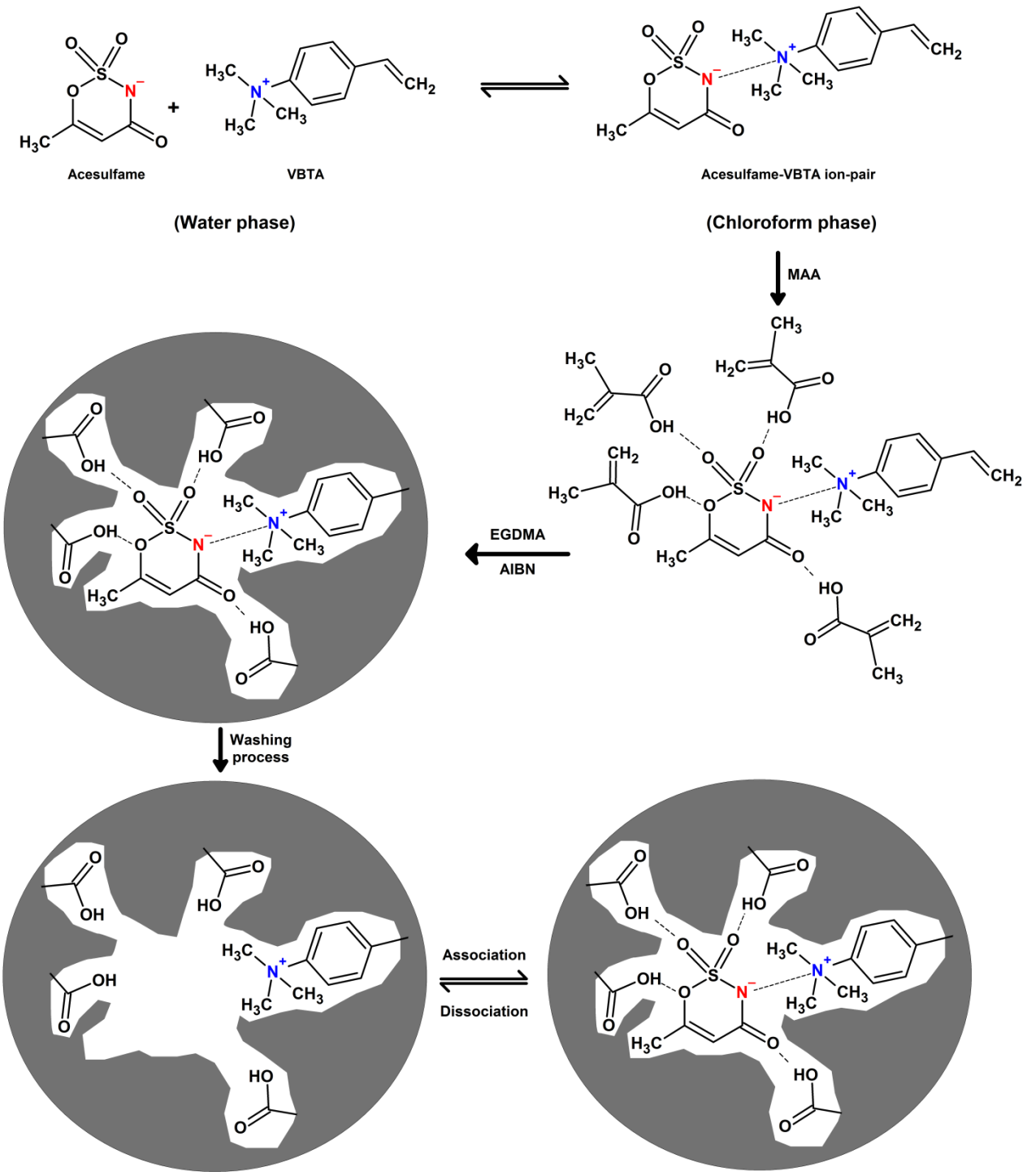


Figure 3-5: Schematic representation of imprinted polymer synthesis for acesulfame. VBTA, MAA, EGDMA and AIBN represent (vinylbenzyl)trimethylammonium chloride, methacrylic acid, ethylene glycol dimethacrylate (EGDMA) and 2,2´-azobisisobutyronitrile, respectively.

3.2. 4-nitrophenol imprinted polymer

Due to the high toxicity impact of 4-nitrophenol (4-NP) on environment and human health, it is regulated as one of the priority pollutants by the US Environmental Protection Agency (EPA) [16].

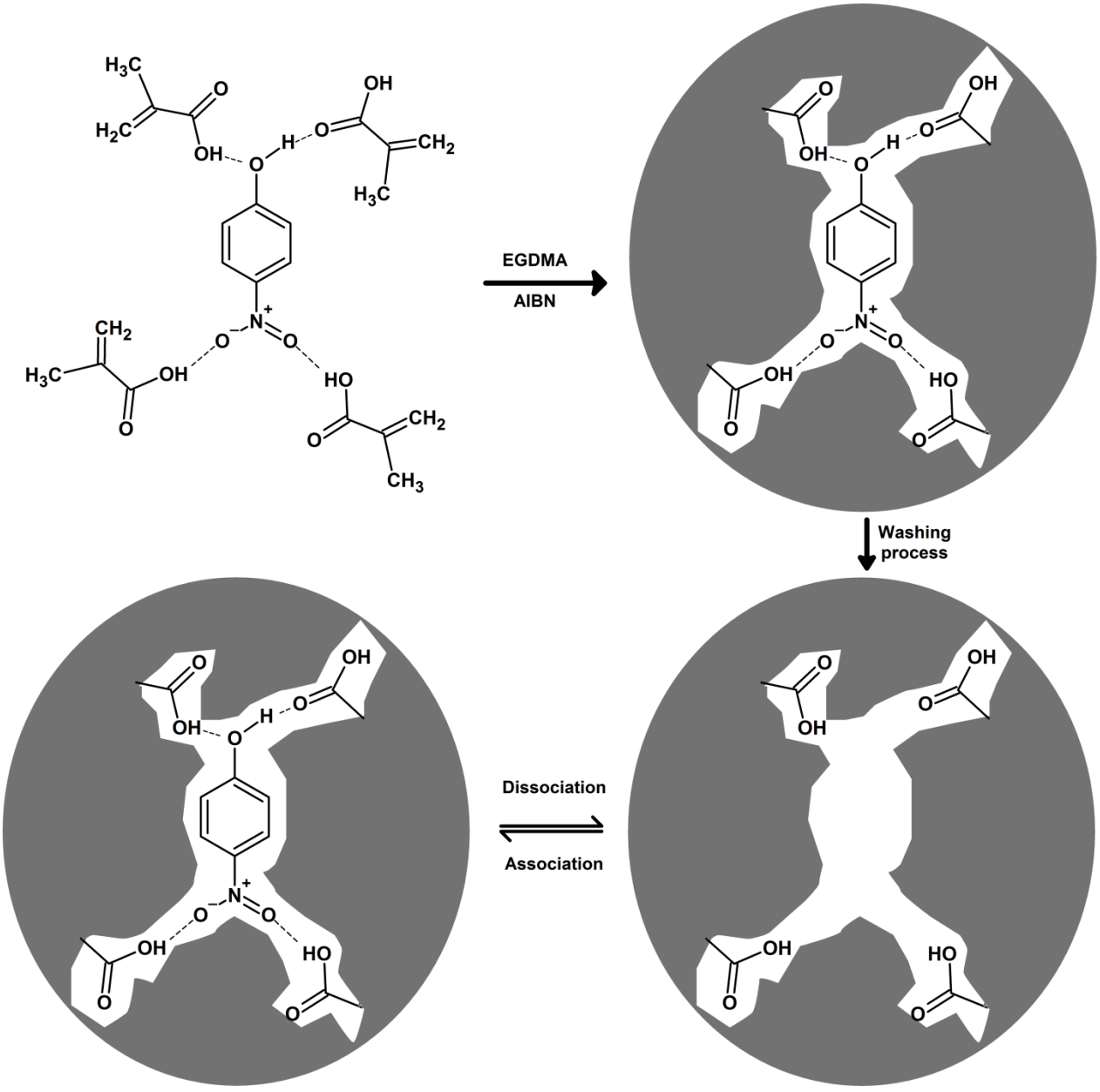


Figure 3-6: Schematic representation of imprinted polymer synthesis for 4-nitrophenol. MAA, EGDMA and AIBN represent methacrylic acid, ethylene glycol dimethacrylate (EGDMA) and 2,2´-azobisisobutyronitrile, respectively.

4-Nitrophenol has been used widely as an intermediate or precursor for the production of insecticides, pharmaceuticals and dyes. Therefore, determination of 4-nitrophenol in complex samples like environmental waters has attracted a lot of attention from environmental researchers. Commercially available SPME fibers [152, 153] and new generated SPME fibers [154] were used for its determination in water samples. Molecularly imprinted polymers were synthesized for 4-nitrophenol and evaluated for its determination in honey [155] and water [156, 157] samples.

The aim of our study was to use 4-nitrophenol imprinted polymer in a new sample preparation format to extract selectively and efficiently the target compound from environmental water samples. Due to valuable information available in published manuscript, the polymer mixture precursors and their ratios were adopted from [156] and [157]. In these studies, MIP polymers were synthesized and used to pack analytical columns which were later coupled on-line to an HPLC instrument. The authors proved the selectivity of synthesized polymers when they were washed and eluted with suitable solvents. In summary for MIP preparation, 0.041 g of 4-NP, 0.01 g of AIBN, 1.17 g of EGDMA and 100 µL of MAA acid were dissolved in ACN as porogen (Figure 3-6). Non-imprinted polymer (NIP) mixture was prepared in the same way without the target molecule. In both [156] and [157] studies, the synthesized hard monolith polymer was crushed, ground and wet-sieved to separate polymer particles with desired sizes to for further experiments. In our study, the polymer particles were synthesized in an in-tube SPME format using a simple and effective strategy developed by us. It includes polymerization within a glass capillary while a metal rod was inserted in its middle. The method which is used for the preparation of MIP tubes is schematically shown in figure 3-7. In summary, both tips of a 10 or 20 µL glass-capillary were coned with flame to the diameter sizes of 100 and 480 µm and were checked carefully with hand lens (Figure 3-7A). Then, a one-side closed 100 µL capillary was filled with the precursor polymer mixture and deaerated with helium for 10 min. The coned glass-capillary was placed inside the deaerated polymer mixture and the related metal rod was inserted in the middle of it (Figure 3-7B). This technique allows a bubble-less load of the glass-capillary with the polymer mixture. The open side of 100 µL capillary was closed immediately and placed in a water bath which was adjusted at 60°C. After polymerization, the outer glass

capillary was broken carefully; the excess polymer was removed from outside the coned glass-capillary which used later as open tubular MIP/NIP device. The metal rod defining the internal diameter of the MIP tube was removed, whereupon a uniform polymer tube was remained inside the coned glass capillary (Figure 3-7C). 50 mL of methanol was passed through the tube with a flow of 5 mL h^{-1} to remove the template molecules and other unreacted precursors.

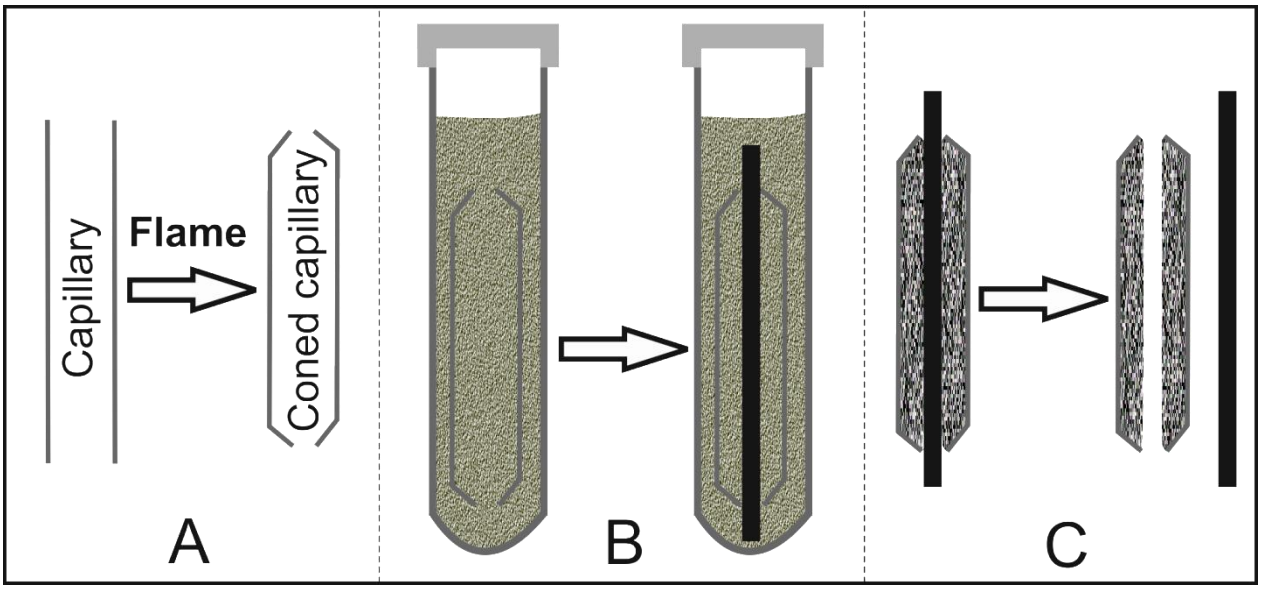


Figure 3-7: Open-tubular MIP-capillary preparation. (A) The both tips of a glass-capillary were coned with flame to the diameter size of a desired metal rod. (B) The coned capillary was placed in a bigger one-sided closed capillary which contained polymer mixture and then the related metal rod was inserted into coned capillary. (C) After the polymerization, the metal rod was removed from the polymer matrix synthesized within coned capillary.

Using above mentioned strategy, synthesized polymer materials can be produced in an in-tube SPME format. Various polymer materials in different conditions were synthesized to obtain the most efficient SPME device. In order to have the highest extraction efficiency for synthesized MIP particles in the new in-tube SPME format, different parameters were evaluated and optimized as follows: I) the volume and inner surface area of in-tube SPME, II) porogen volume used for synthesizing the polymer, III) swelling and shrinking of polymer and IV) the polymerization time. During optimization of MIP polymer particles, an extraction setup (shown in figure 4-1 and explained in detail in section 4.1.) was implemented. In summary, MIP capillary was dried completely at the first step using pure nitrogen which was purged through the tube with a flow of 300 mL min^{-1}. Then, 1 mL of ACN was passed through during 5 min. 2 mL of

freshly prepared standard sample with a concentration of 0.05 mg L^{-1} was passed through conditioned tubes with a flow of 2 mL h^{-1}. The pH of the solution was adjusted at 3, thus 4-nitrophenol is neutralized (pKa: 7.15) allowing efficient extraction. 0.5 mL of water with pH 3 with a flow of 2 mL h^{-1} was used for washing the tube. The extracted 4-NP was eluted with 50 µL ACN containing 0.1 % (v/v) of acetic acid. To collect the elution solution, a 250 µL micro glass insert was placed in a 2 mL glass vial which could be sealed with a silicone-rubber septum cap for storing the eluted fraction. The collected samples were stored in a refrigerator till further analysis. The MIP tubes were washed with ACN until no template was detected by HPLC-DAD. Both MIP and NIP materials were evaluated with the same procedures and blank analyses were performed irregularly between samples. All the experiments were done in triplicate otherwise it is mentioned.

3.2.1. Amount of active sites and porogen volume

For the extraction, the type and the amount of the adsorbent are among the most important parameters which must be considered carefully to get a high recovery and a complete and fast desorption [158]. Coating-Water distribution constants (Kcw) of polydimethylsiloxane (PDMS) and polyacrylate (PA) for phenol compounds are listed in Table 3-4, where nitrophenol compounds are among the chemicals that need polar coatings for efficient extraction.

Table 3-4: Coating-Water distribution constants (K_{cw}) [152]

Chemical compound	Polydimethylsiloxane -Water	Polyacrylate -Water
Pentachlorophenol	370	170
2,4,6-Trichlorophenol	15	60
2,4-Dichlorophenol	4.6	47
4-Chloro-3-methylphenol	2.4	16
2-Chlorophenol	0.2	9.3
2,4-Dimethylphenol	1.3	9.1
2-Methyl-4,6-dinitrophenol	6	7.3
2-Nitrophenol	4.8	3.7
4-Nitrophenol	0.1	2.4
2,4-Dinitrophenol	0.1	1.7
Phenol	1.3	1.3

Furthermore, the volume and the surface area of the sorbent influence the extraction efficiency as proved by Turiel et al. [114] for the determination of propazine by MIP-monolith SPME fiber. In order to evaluate the volume and the surface effectiveness of the polymer for adsorbing 4-NP, different MIP-capillaries were synthesized. Glass capillaries of 10 and 20 µL volume (same length and different capillary inner diameters) were used as holder and shape defining form for the MIP-capillaries. The internal diameter of 100 µm was defined by the related metal rod inserted during polymerization. The volume of synthesized polymers was calculated 9.749 and 19.749 mm3 for 10 and 20 µL glass capillaries, respectively. Both tubes had the same inside surface area of 10.1 mm^2. The recovery for both synthesized MIP-capillaries was 23.5 ± 2.1%. Apparently, at chosen extraction conditions and similar inside surface area of 10.1 mm^2, the volume of 9.749 mm^3 was high enough for extraction of 4-NP. For evaluating the effect of polymer surface on the recovery of 4-NP, MIP-capillary with wider inner diameter of 480 µm was prepared. For this purpose, the 100 µm metal rod was replaced by a 480 µm one, positioned before polymerization in the middle of the 20 µL glass capillary. The volume and the surface for this new synthesized MIP-capillary were 14.481 mm^3 and 48 mm^2, respectively. The percent recovery increased from 23.5 ± 2.5% to 32 ± 3.1% as the inner surface increased from 10.1 mm^2 to 48 mm^2. Turiel et al. [114] also showed a linear increased recovery for polymer volume enhancement from 0.079 to 2.21 mm^3. In their study for area evaluation, the recovery was increased from 0.4 to 3.6% for 3.14 to 10.1 mm^2 surface enhancement, while, it was increased from 3.6 to 12.2% for 10.1 to 16.7 mm^2, respectively. In our study, additional to the benefits of larger polymer volume and surface, the handling of bigger capillaries is easier. Furthermore, a bigger inner diameter would allow the use of salt in the extraction solution without the risk of clogging. Glass capillary with 20 µL volume and a metal rod of 480 µm diameter were therefore found to be most efficient for 4-NP extraction and were used for further optimization.

Porogen volume along with nominal cross-linker ratio are the two most important experimental parameters governing the physical nature of the final synthesized polymer [159]. When the porogen volume is increased beyond that normally used in polymerization, the primary polymer particles form gel-type polymers and precipitate when they reach the threshold size. Due to this

fact that precursor ratios have been optimized previously [156, 157], we investigated the porogen volume enhancement from 1.5 to 6 mL and its effect on the extraction yield of 4-NP. During polymerization, the polymer particles precipitate in the free space between the glass capillary and the metal rod like 'bricks in a wall' to produce a MIP-capillary. The percent recovery increased from 32 ± 3.1% to 39.3 ± 3.5% with increasing volume which can be attributed to the high porosity and surface area.

3.2.2. Swelling, shrinking effects and polymerization time

According to the accepted theory of MIP, cavities are imprinted in the polymer matrix which shows affinity toward the template molecule. The monomers are fixed inside these three-dimensional cavities with proper cross-linker in the positions which were predestinated with template-monomer complex. After template removal, these cavities can be affected by milieu variation. Swelling and shrinking effects can transform the shape of the imprinted cavity and the spatial positioning of the monomers, which might influence the affinity of the imprinted cavities [160]. In order to examine these effects, nitrogen was passed through the tubes with a flow of 300 mL min^{-1} for 15 min and caused the tubes to shrink noticeably. The time which is needed for shrinking, differs for tubes synthesized in 1.5 mL and 6 mL porogen volume from 5 min to 5 seconds, respectively. Figure 3-8 shows the magnified cross section of the polymer capillary tube before and after the drying step. The shrinking effect becomes visible by the free capillary glass wall in Figure 3-8a. Basically, MIP shows best affinity to the target molecule when the extraction is carried out with the same solvent as used for polymer synthesis. This process guarantees that the imprinted shape of cavities and the spatial positioning of monomers within the cavities are remained complementary of template molecule during extraction. So after the drying step, 1 mL of ACN (porogen) was passed through the tubes for 5 min continuously. Due to solvent absorption, the polymer material swelled nearly immediately to the size where the cavities and the monomers have the right shape and orientation to interact with the template molecule. After this conditioning step, 2 mL water solution containing 4-NP was loaded over 60 min. During the extraction, 4-NP and water molecules diffuse into the cavities and replace the ACN molecules which were loaded during conditioning. Due to this ACN replacement, the polymer inclusive the cavities start to shrink gradually. As a result of such gradual shrinking, the

cavities act like a specific trap for the 4-NP. By adding drying step, the recoveries for the tubes which were synthesized in 1.5 and 6 mL porogen were increased from 32 ± 3.1% and 39 ± 3.5% to 38 ± 3.2% and 58 ± 3.4%, respectively.

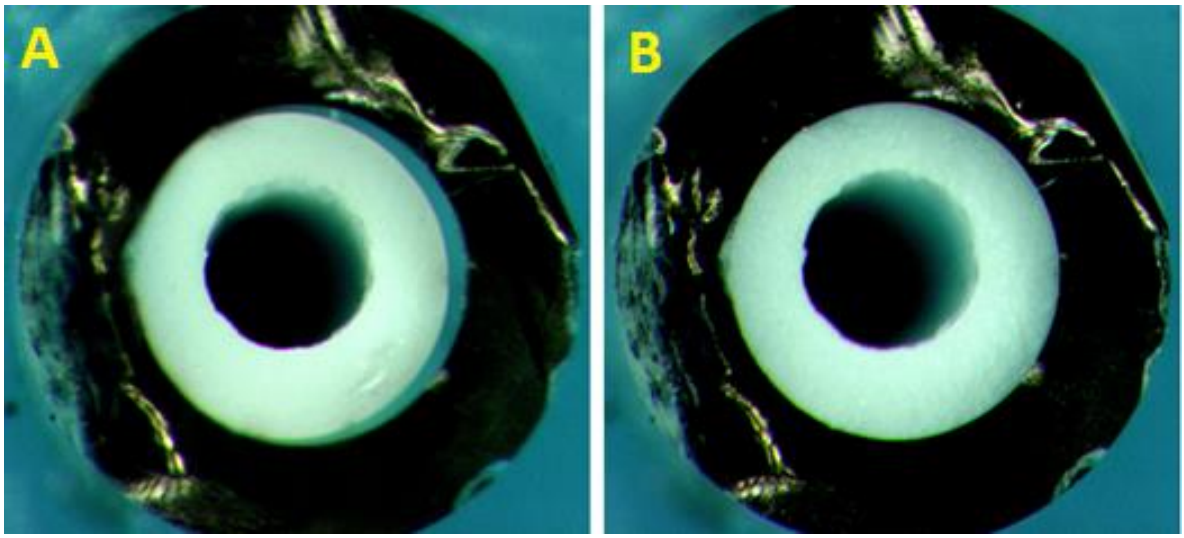


Figure 3-8: Magnified cross section of the polymer tube inside the 20 µL capillary glass. (A) Shrinking effect. (B) Swelling effect after adding ACN.

Finally, the polymer capillary tube synthesized with 6mL porogen was selected for further analysis owing to the enhanced recovery of 4-NP and the fast shrinking effect of the polymer.

Polymerization time is an important factor which can modify the porosity of the synthesized polymer [114]. Polymerization inside the glass capillary was performed during 2, 4 and 21 hours. The obtained results showed that the recovery of 4-NP was increased from 2 to 4 hours of polymerization, but decreased when it lasted for 21 hours. Besides, the polymer which was synthesized for 21 hours indicated bleeding of 4-NP which required extra solvent for cleaning. Recovery enhancement from 2 to 4 hours can be attributed to increased number of binding sites, while, reduced recovery from 4 to 21 hours probably attributed to the excessive binding beads, leading to a less porous structure and preferably non-specific interactions with 4-NP. Therefore, 4 hours was preferred as an optimized polymerization time.

In summary, optimized values for the construction of 4-NP imprinted polymer particles in an in-tube SPME format using our strategy were as follows: I) glass capillary with 20 µL volume and a metal rod of 480 µm diameter II) 6mL porogen volume and III) 4 hours was selected for polymerization time.

3.3. Atrazine imprinted polymer

Atrazine, as one of the most widely used pesticide in the world, belongs to the herbicide group of triazine class. It is assumed that atrazine could increase the corn production yield of up to 6%. Some other research shows that atrazine is a persistent contamination in groundwater and has carcinogenic effect. However, the safety issue for atrazine is an ongoing arguing debate when it is consumed in accordance with regulations [161]. Due to its importance, various synthesis methods have been evaluated using atrazine as the target of interest. Synthesized polymers were used in different extraction formats like membrane protected micro-solid-phase extraction (μ-SPE) [162], MISPE [163], SPME [164] for determination of atrazine in different matrix samples like beef liver [165], soil [166], tea [162], rice [164] and environmental water [163] samples. Besides traditional extraction formats, various innovative new extraction methodologies were developed using the same synthesized polymers. For example, a novel online extraction methodology [167] was developed to determine a group of target analytes including atrazine in river water samples without matrix interference. In this method, a SPE column was packed with RAMs sorbent materials and used as a preliminary step to remove the matrix chemicals with molecular weights of more than 15000 from water samples. Then, the adsorbed targets to the RAMs column were eluted with a suitable organic solvent and transferred directly to a MISPE column for further selective extraction of target analytes. In our study, the ratio for atrazine/monomer/crosslinker was adopted from Matsui et al. [168]. In summary, MIP was prepared using 0.032 g of atrazine, 0.011 g of AIBN, 0.827 g of EGDMA and 50 μL of MAA dissolved in chloroform as porogen. Polymer mixture was deaerated with helium for 15 min and polymerized photochemically (at 25℃ for 4 h using the UV-lamp at 312 nm) or thermally at 60 °C. The non-imprinted polymer (NIP) mixture was prepared in the same way without the target molecule. Figure 3-9 shows schematically the used preparation procedure for atrazine imprinted polymer via the non-covalent approach. Matsui et al. [168] proved that their suggested polymerization precursors and their related ratios provide a selective polymer towards atrazine. However, besides the polymerization precursors and their ratios which control the final selectivity of synthesized polymer, there are two other important factors which can affect the selectivity and capacity of the synthesized molecularly imprinted polymers.

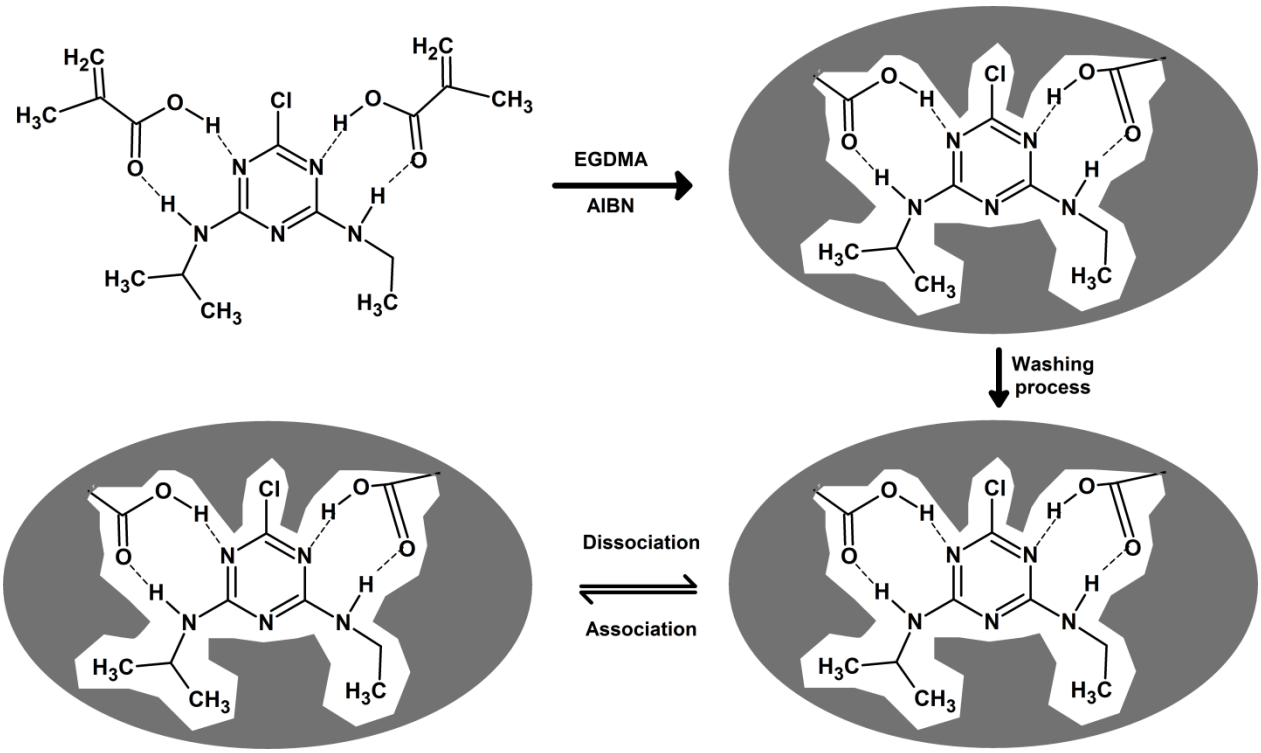


Figure 3-9: Schematic representation of imprinted polymer synthesis for atrazine. MAA, EGDMA and AIBN represent methacrylic acid, ethylene glycol dimethacrylate (EGDMA) and 2,2´-azobisisobutyronitrile, respectively.

These two factors are the temperature in which the polymerization is carried out and the volume of porogen [169]. Therefore, using adopted polymerization precursors and their ratios, we evaluated the polymerization temperature and porogen volume.

Polymerization was carried out using thermally polymerization at 60°C (MT) and photochemically polymerization at 25°C (MU1) for 4 h. In order to evaluate the effect of the porogen volume on the selectivity and efficiency of extraction, it was increased from 2.2 mL (MU1) to 4.4 mL (MU2). Following polymerization, the material was ground and sieved through a 40-µm sieve, followed by soxhlet extraction with methanol and acetic acid (99:1). Finally, the polymer particles were dried under vacuum conditions and used for further assessment using traditional SPE cartridge. In order to prepare a conventional 3 mL MISPE, the cartridge is generally packed with about 50 mg of sorbent. 3 mL cartridge that was packed with 50 mg of synthesized polymers resulted in very high back pressure. Therefore, 10 mg MIP and NIP materials were used to pack 3 mL cartridges. A 10 mL water sample with 0.4 mg L^{-1} atrazine was passed through the conditioned sorbents with a flow rate of 2 mL min^{-1}.

Figure 3-10 shows atrazine recovery of polymers synthesized in different conditions. The results (Figure 3-10) indicated that synthesis at 25 °C (MU1) provided a MIP material with the best and most reproducible recovery and selectivity for atrazine. As previous studies discovered [169], a higher temperature has a negative impact on the complex stability during the imprinting process and the polymerization reaction is hard to control resulting in low reproducibility of MIPs [150]. Thus, a polymerization temperature of 25 °C under ultraviolet irradiation was preferred.

Furthermore, the experiments indicated a lower recovery and selectivity when a higher porogen volume (4.4 mL chloroform) was used for MIP synthesis. It is known that the nature and the amount of porogenic solvent determine the strength of the non-covalent interactions between the target substance and the monomers. Additionally, the porogen influences the polymer morphology that affects the performance of MIP [150]. Under our synthesis conditions, a higher porogen volume caused more non-specific sites whereby the MIP materials reflected lower selectivity compared to relative NIP materials. Finally, the porogen volume of 2.2 mL and the polymerization temperature of 25 °C under ultraviolet irradiation were selected for the synthesis due to the enhanced recovery and selectivity of the synthesized polymers. This polymer will be implemented to develop a new sampling format termed mixed-bed SPE (4.3.)

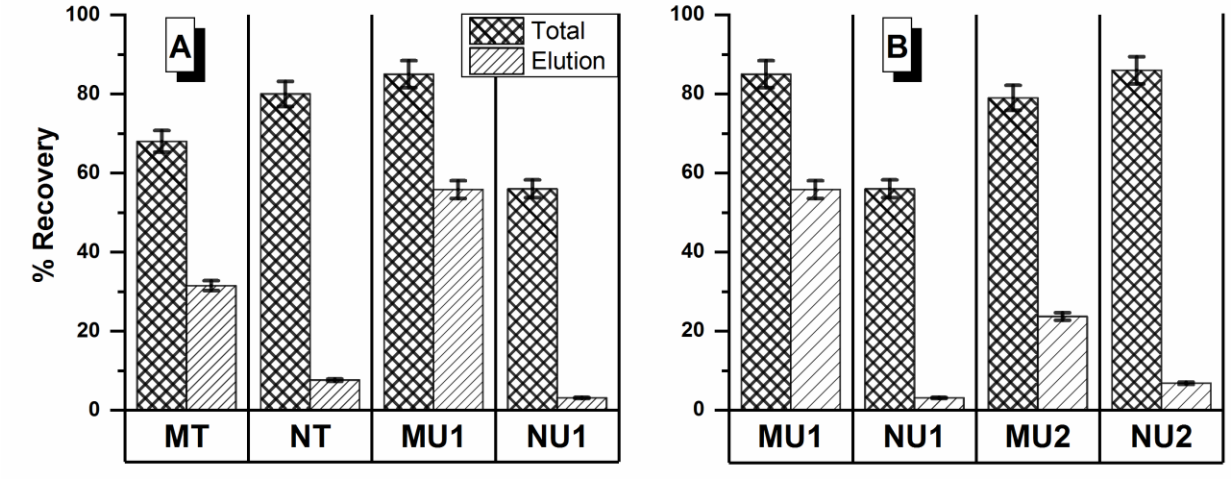


Figure 3-10: Influence of the polymerization conditions of imprinted polymers for the recovery of atrazine. (A) Temperature effect. (B) Porogen volume effect.

4. Sample-preparation methods

Using synthesized polymers whether as particles or particles which were molded in a capillary format, different sample-preparation techniques were developed. These sample-preparation techniques were evaluated and optimized to find, step by step, a method which can be used for the field-application purposes. An ideal method should support satisfactorily sample-preparation of relatively large volumes of water samples and at relatively high flow-rates.

To this aim, the selective imprinted polymer materials were used as sorbent materials in combination with the known extraction methods such as SPME and SPE. In the first step, SPME was selected due to its known potential for field-application. 4-Nitrophenol imprinted polymer materials which were molded in the in-tube SPME format were used to optimize an extraction procedure for the template molecules from environmental water samples. However, the obtained results showed that SPME format could not satisfactorily support our aim.

Therefore and in the next steps, SPME was replaced with SPE method which in known as exhaustive extraction method and applicable for the larger sample volumes. New synthesized acesulfame imprinted polymer materials were used in SPE format to extract template molecules from real water samples. The sample volume was increased due to higher amount of active sites. However, the packed SPE cartridge faced the back-pressure which limited the flow-rates. To increase the micro channels within the packed SPE bed and therefore flow-rates enhancement, two new SPE format was developed as follows: mixed-bed SPE and selective filter-paper disk.

After all optimizations, the obtained results for the selective filter-paper disk method showed that this new simple, cost-effective and efficient methodology can be used for the preparation of selective filed-applicable sample-preparation method.

4.1. In-tube solid-phase microextraction

As mentioned above, in the first attempt 4-nitrophenol-imprinted polymer materials which were molded in an in-tube solid phase microextraction (SPME) device was used to optimize a selective extraction method for 4-nitrophenol as a priority pollutant from environmental water samples. As shown in figure 4-1, a hypodermic needle was simply connected to the MIP-capillary tube via a Teflon sheath. This assembly can be easily connected to different needle sizes of syringes. A syringe pump was used for liquid delivery along the tubes. For the target molecules like 4-nitrophenol which can dissociate into the ionic from at higher pH, optimization of acidity or basicity of sample is important. On the other hand, in a SPME method 'salting out' effect can be used to enhance the extraction efficiency of polar target compounds from water samples. Extraction time, as a general parameter in extraction methods, and serial connection of two developed in-tube device were also evaluated and optimized.

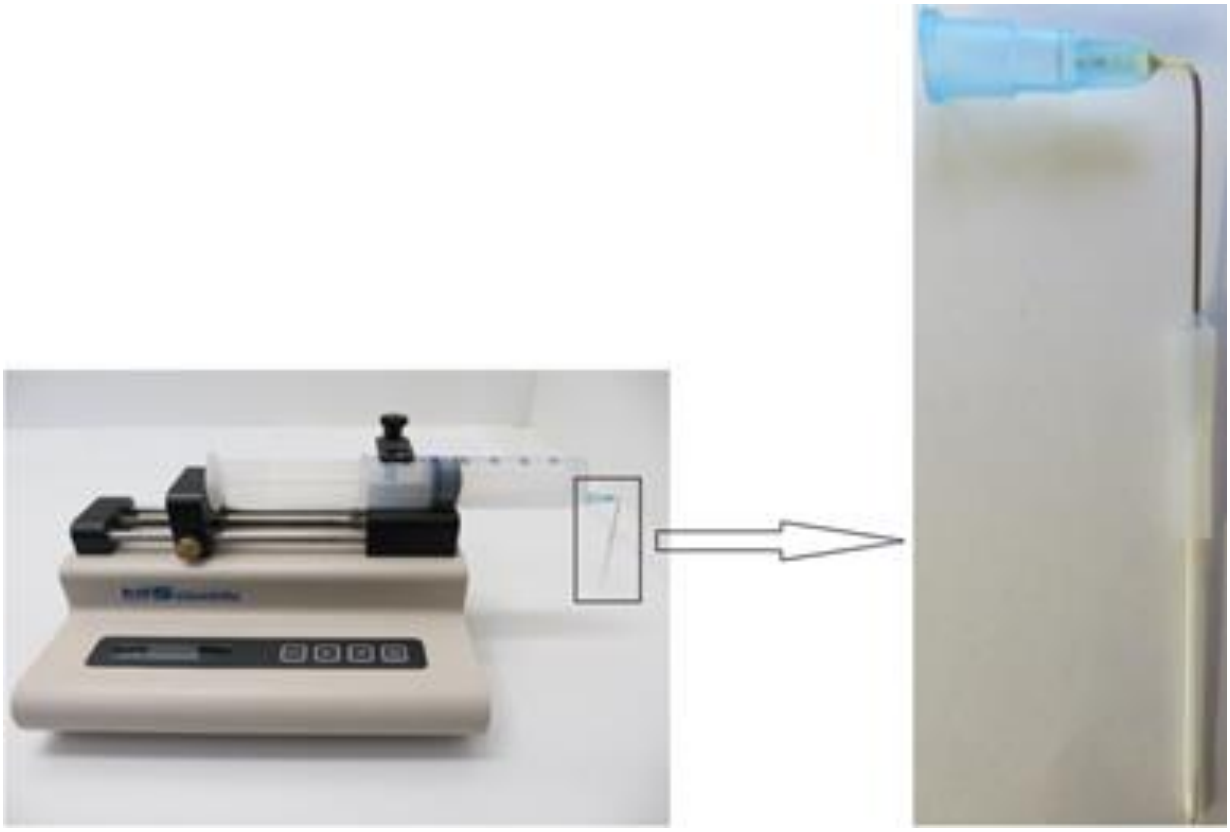


Figure 4-1: Extraction device used during the in-tube SPME procedure.

4.1.1. pH of the sample solution

Due to the dissociation constant of 4-nitrophenol (pKa: 7.15), the pH of its solution is an important parameter which must be optimized. For efficient extraction of 4-nitrophenol (Table 4-1), water samples must be acidified and salt saturated at the same time. Pawliszyn et al. [152] reported a decrease of extracted amount by a factor of 0.6 for separate acidification and 0.1 for separate salt saturation while an increase factor of 3 was observed for addition of both at the same time.

Table 4-1: SPME extraction of phenol compounds from water samples

Method	Adsorbent	Extraction condition	Reference
SPME-GC-MS	PA, 85-µm	Salt saturated, pH 3.0, 40 min	[158]
SPME-GC	PA, 85-µm	Salt saturated, pH 4.8, 40 min	[170]
SPME-GC-MS	PA, 85-µm	Salt saturated, pH 2.0, 45 min	[171]
SPME-HPLC	PA, 85-µm	Salt saturated, pH 2.5, 30 min	[172]
SPME-GC	PA, 85-µm	Salt saturated, pH 2.0, 40 min	[152]
SPME-GC-MS	PA, 85-µm	Salt saturated, pH 2.0, 40 min	[153]
SPME-GC	SU-8 2100, 18-µm	Salt saturated, pH 1.0, 60 min	[154]
SPME-GC-MS	β-Cyclodextrin	Salt saturated, pH 1.5, 40 min	[173]
SPME-GC	MWCNTs/Nafionwas	pH 5-9, 15 min (not equilibrium)	[174]

* MWCNTs: Multiwalled carbon nanotubes

In our investigation, the pH of the standard solution of 4-nitrophenol was adjusted to values ranged from pH 2 to pH 8. The percent recovery decreased at higher pH and highly acidified solution. The cavities of the polymer were imprinted for the neutral 4-nitrophenol molecule, while at higher pH, 4-nitrophenol dissociates into its anionic form and does not fit completely to the cavities. Furthermore, the higher water solubility of the anionic 4-nitrophenol reduces its affinity for adsorption. On the other hand, at strong acidic conditions, the carboxyl groups as active sites in the cavities might be protonated and are lost for effective interactions with 4-nitrophenol. According to these results, pH of 3 was chosen as an optimum for the 4-nitrophenol extraction by the MIP tubes.

4.1.2. Extraction time

Extraction time is an important parameter in SPME that influences the extraction yield of an analyte (Table 4-1). In our investigations, 2 mL of the standard 4-nitrophenol solutions were passed through the tube for allocated times which correspond to different flow rates of the sample solution. The percent recoveries were 20 ± 2.5%, 58 ± 3.4% and 64.3 ± 3.6% for 30, 60 and 90 min load time, respectively. For standard SPME of phenols, Pawliszyn et al. [152] showed that alkylated and chlorinated phenols and also 2-nitrophenol required 15 min to equilibrate with a PDMS-coated fiber but for the efficient extraction of 4-nitrophenol, 2,4-dinitrophenol and 2-chlorophenol, the PDMS coating was proved to be not suitable. The more polar PA-coated fiber provided higher partition coefficient also for 4-NP but the equilibrium time was prolonged to 40 min. The authors explained this longer equilibrium time according to the solid phase nature of the PA-coated fiber whereas PDMS-coated fiber is a polymeric liquid which the analytes can easily diffuse into and through. According to our results and in applied conditions, increasing the extraction time resulted in the enhancement of percent recovery and an approximate plateau is reached for extractions longer than 60 min. Recovery enhancement can be explained by improved mass transfer of analyte to the adsorption sites of MIP at slower extraction rate [119]. However, the extraction time can be reduced via serial connection of MIP-capillaries (section 4.1.4.), whereupon shorter total analysis time would be achievable.

4.1.3. 'Salting out' effect and washing step

'Salting out' is an effect that increases the ionic strength of a solution when salt is added to the matrix solution and, consequently, reduces the solubility of the polar compounds in water. Due to this reduction of the solubility of polar compounds, their extraction efficiency can be enhanced (Table 4-1).

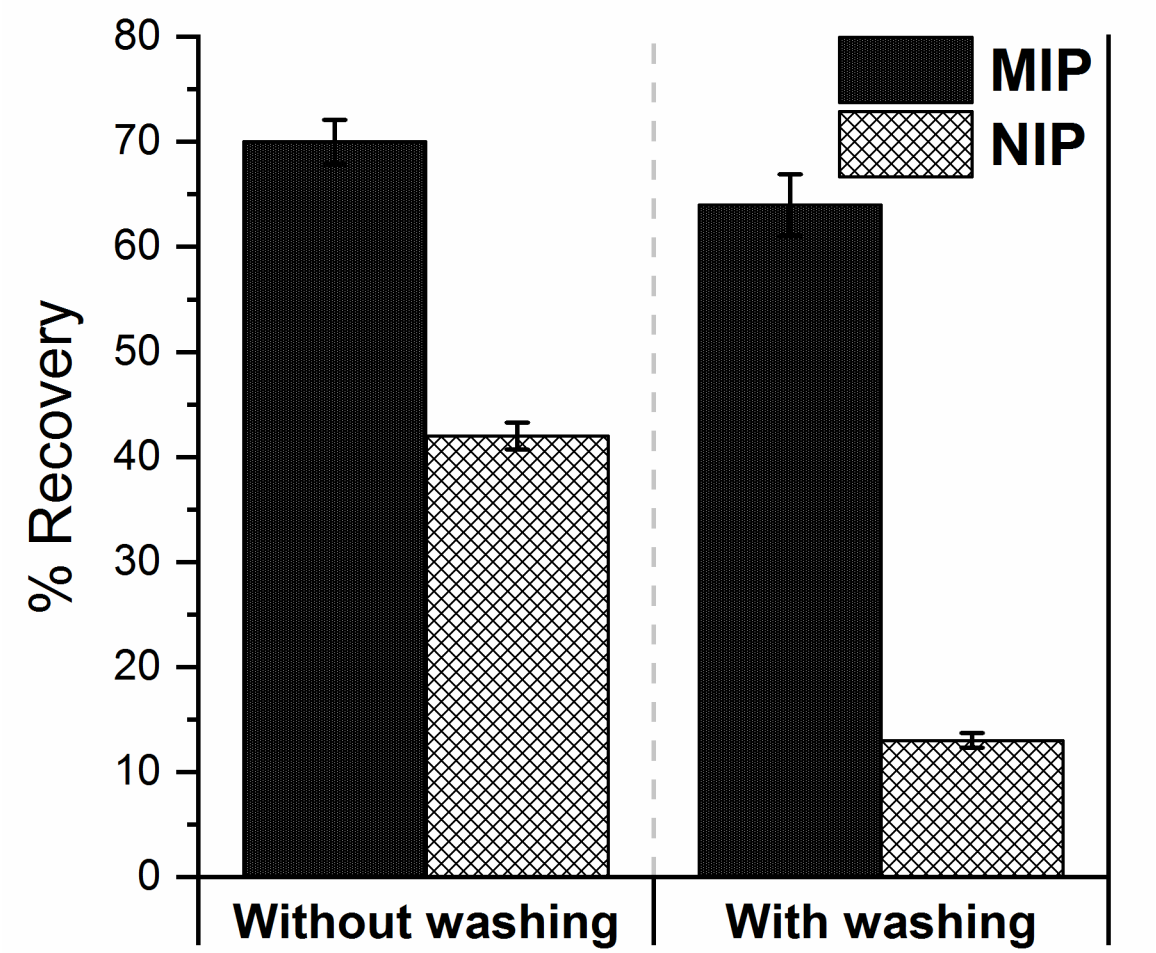


Figure 4-2: 'Salting out' effect and washing process evaluation for MIP and NIP

Depending on the type of the ad/absorbent and the properties of the target substance, the 'salting out' effect can support a more efficient extraction but has to be optimized carefully [159]. As previously demonstrated [152], salt addition to the sample and acidic condition can intensively improve the extraction of phenols. However, salt can be co-extracted unfavorably resulting in inadequate conditions like clogging of the extractor tubes during elution. In order to examine the influence of salt addition, 2, 3.5 and 5 g of NaCl was dissolved in 25 mL of the standard sample solutions and 2 mL of each solution was passed through the MIP-capillary tube with a flow 2 mL h^{-1}. During the washing step, 0.5 mL of water (pH 3) passed through the tube for 15 min. In this way, not only the remained salt was removed, but also target molecules and contaminations less tightly adsorbed due to irregular cavities and non-specific interaction. Obtained results showed that adding the salt could enhance the extraction efficiency of 4-

nitrophenol, from water sample. On the other hand, high amount of salt could close the capillary. In order to avoid the negative effect of tube clogging by saturated salt solution (360 g L^{-1}), 3.5 g NaCl in 25 mL sample (140 g L^{-1}) was selected for the 4-nitrophenol extraction. As shown in Figure 4-2, adding a washing step after sampling process could also highlight the difference between MIP and NIP.

4.1.4. Serial connection of MIP-capillaries

In order to increase the recovery, two polymer capillary tubes were connected easily with a Teflon sheath and used for extraction of 4-nitrophenol solutions at range of 0.001-10 mg L^{-1}. After the sample loading and washing steps, the tubes were disconnected and analyzed separately. Obtained results showed that the first tube could extract about 0.07 µg of the total 4-nitrophenol available in 2 mL sample at 0.05 mgL^{-1}. On the other hand, when the concentration was increased to 10 mg L^{-1}, 9.49 µg of 4-nitrophenol was extracted by the same tube. Here an interesting question can arise: why is the first tube not able to extract the total 0.1 µg of 4-nitrophenol at 0.05 mg L^{-1} while 9.49 µg of the analytes were extracted at 10 mg L^{-1}? It is known that when the liquid moves through the tube, a thin boundary layer is created from the steady molecules at the polymer surface to the free stream away from the surface which controls the mass transfer of 4-nitrophenol from free stream to the surface. Depending on the dimensions of our tube and the flow rate of 2 ml min^{-1}, a Reynolds number of 1.47 was calculated which clearly represents a laminar flow in the tube. In order to reach the surface, target molecules must diffuse through this boundary layer. So, the amount of extracted analyte might be proportional to the concentration gradient in the boundary layer. Considering the concentration gradient effect on the amount of extracted analytes, the question can be answered. Due to the higher concentration gradient for 10 mg L^{-1}, target analytes diffuse faster than those in a 0.05 mg L^{-1} solution.

The other question can come to mind when we draw the extracted analyte versus 4-nitrophenol concentrations in water samples. The regression equation for the first tube was y=0.9475x+0.0037 and for the second tube was y=0.5537x+0.0109 which indicates a less steep curve and a bigger constant for the second one. If both tubes are the same, why does the regression equation of tubes differ? The differences in the curve are simply explainable due to

the lower concentration, entering the second tube which needs a correction by the adsorbed analytes in tube 1. That means, the concentration which enters the second tube is lower than that we considered primarily to draw and conclude the y=0.5537x+0.0109 for the second tube.

4.1.5. Analytical evaluation and real samples

To validate the proposed method, standard 4-nitrophenol solutions at range of 0.001-10 mg L^{-1} were prepared and analyzed at optimized conditions with the developed in-tube MIP-SPME-HPLC/DAD method. Linear regression analyses were performed using the peak area obtained by in-tube MIP-SPME against the corresponding concentrations. As shown in figure 4-3, the linear range of the method was 0.001-10 µg mL^{-1} with a regression equation y=591.9x+9.837 and correlation coefficient r=0.9999. The RSDs were 1.5-6.1% in this linear range.

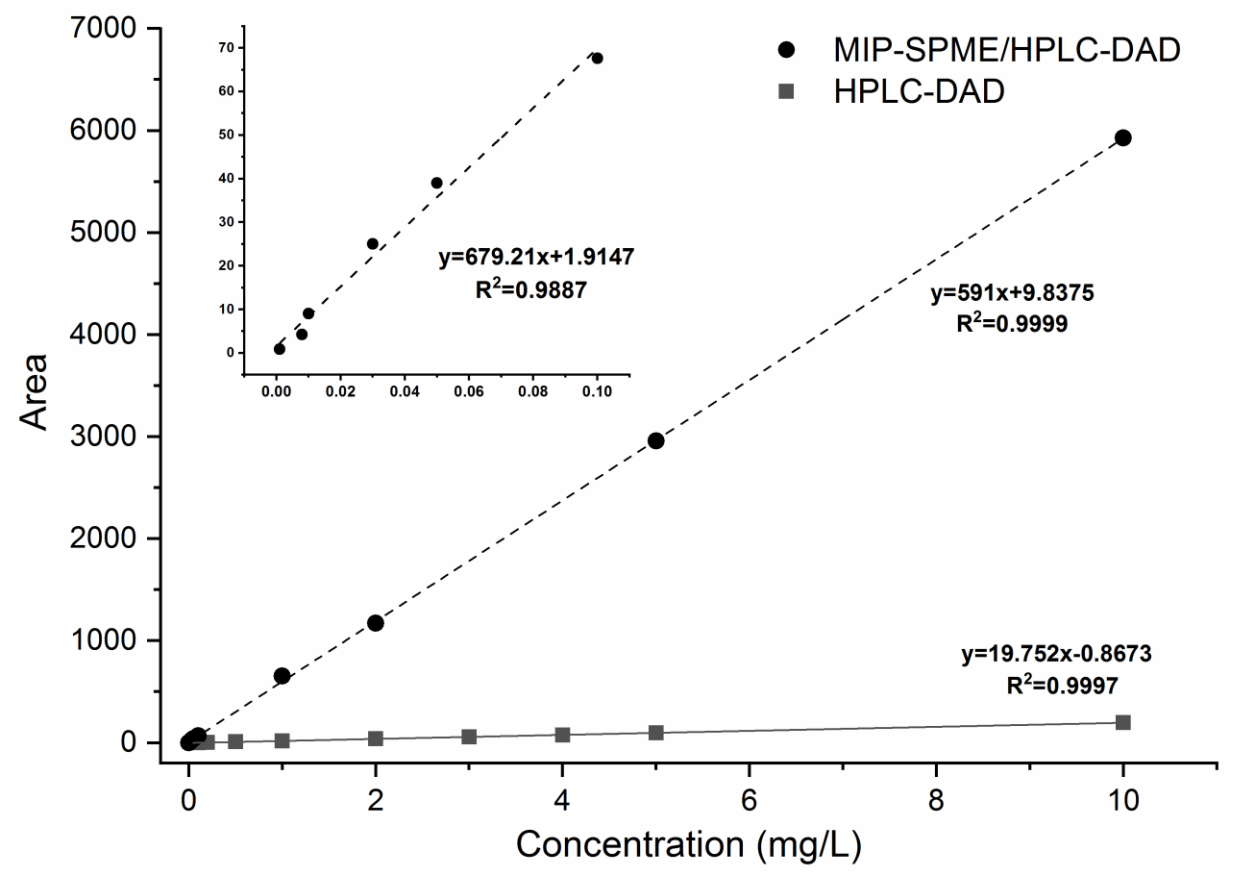


Figure 4-3: Calibration curve for 4-nitrophenol obtained using HPLC-DAD with and without developed sample-preparation method

The limit of detection (LOD) and limit of quantification (LOQ) were calculated at signal-to-noise ratios of 3 and 10, respectively. The LOD and LOQ for this linear range were 0.33 and 1.1 µg L^{-1}, respectively. Table 4-2 summarizes the LODs and linear ranges of some other reported methods for 4-nitrophenol determination in different samples by various procedures. Although our introduced method is very simple, lower detection limit with appropriate linear range were established.

Table 4-2: Comparison of the obtained detection limit of presented work with different other reported works for 4-nitrophenol determination

Method	Linear range (µg L^{-1})	LOD (µg L^{-1})	Reference
SPME–HPLC–UV	10 - 30000	3.6	[172]
SPME-GC-FID	80 - 8000	7.8	[152]
SPME-GC-MS	7 - 700	0.75	[152]
SPME-GC-MS	50 - 1000	10	[173]
Our study	1 - 10000	0.33	[16]

In order to evaluate the cross selectivity of the prepared polymer, EPA 604 phenols mixture was analyzed with the optimized method. This mixture contains alkylated, chlorinated and nitrophenols including the 4-nitrophenol (4-NP) which is listed as priority pollutant by US EPA. Obtained results (Table 4-3) shows that just 2-nitrophenol (2-NP) and 2-methyl-4,6-dinitrophenol (2M-4,6DNP) can be detected at higher concentration and the other phenols could not be detected above their LODs.

Table 4-3: Selectivity evaluation of prepared tube with diluted EPA phenols mixture

(µg L^{-1})	4-NP (Area)	2-NP (Area)	2M-4,6DNP (Area)
10	9	-	-
30	25	2.3	6
50	40	8	14.5

The analytical utility of the method was evaluated for the determination of 4-nitrophenol in different water samples. Tap water and waste water from the effluent of the municipal plant of Leipzig (Germany) was used for preparing the 4-nitrophenol solutions applied in the experiments to show the influence of matrix components on the 4-nitrophenol extraction. Both water samples were tested before addition of 4-nitrophenol with the optimized method and it

was found that all of them were 4-nitrophenol free. Tap water without any other treatment was used for preparing the 4-nitrophenol solutions applied in the experiments. Prior spiking, the waste water was filtered with a 45 µm glass fiber filter and was stored in refrigerator till experiments. In preliminary analysis it was proved that 4-nitrophenol was not retained by the filter material. Then, they were spiked with the EPA 604 phenols mixture to adjust concentration at 0.05 mg L^{-1}. Each spiked sample was prepared freshly before the extraction experiments. The final extracts were analyzed with HPLC-DAD detector. The results of the analyses summarized in table 4-4 indicate a low matrix influence on the extraction process. Even the complex composition of municipal water did not reduce the recovery of 4-NP (98 % ± 4.3%).

Table 4-4: Determination of 4-nitrophenol spiked into samples with different matrices

Sample	Spiked (µg L^{-1})	Found (µg L^{-1})	Recovery (%)	RSD[a] (%)
Tap water	50	51.5	103	2.5
Waste water	50	49	98	4.3

[a] Number of sample = 5

Furthermore, water was sampled from a well within a contaminated military site in Germany which is monitored regularly for residual explosives and their degradation products. This water was used for a method comparison applying standard SPE and the new MIP tubes for 4-nitrophenol extraction in combination with liquid chromatography and mass spectrometry. Water samples from this well which is located in an old military site are regularly monitored for residual amounts of TNT- and RDX-related pollutants. This sample was used to compare the performance of the new MIP procedure with that of a standard protocol involving SPE on Oasis HLB. In summary for the SPE experiments with Oasis HLB, 500 ml of the filtered water (45 µm glass fiber filter) were acidified to pH 2.5 with formic acid and extracted by solid phase extraction (SPE) using Oasis HLB in accordance to OASIS Applications Notebook. In comparison to the SPE, MIP extraction was performed with 2 mL of the real water sample using optimized procedure. The optimized MIP protocol was applied and all extracts were analyzed with HPLC-MS-MS with negative electrospray ionization.

The total ion chromatograms in figure 4-4 indicate a very complex mixture of ionizable substances at negative electrospray conditions. However, the corresponding full scan analysis of

the MIP extract indicated less signals (Figure 4-4A) in the total ion chromatogram in comparison to the SPE extract (Figure 4-4B).

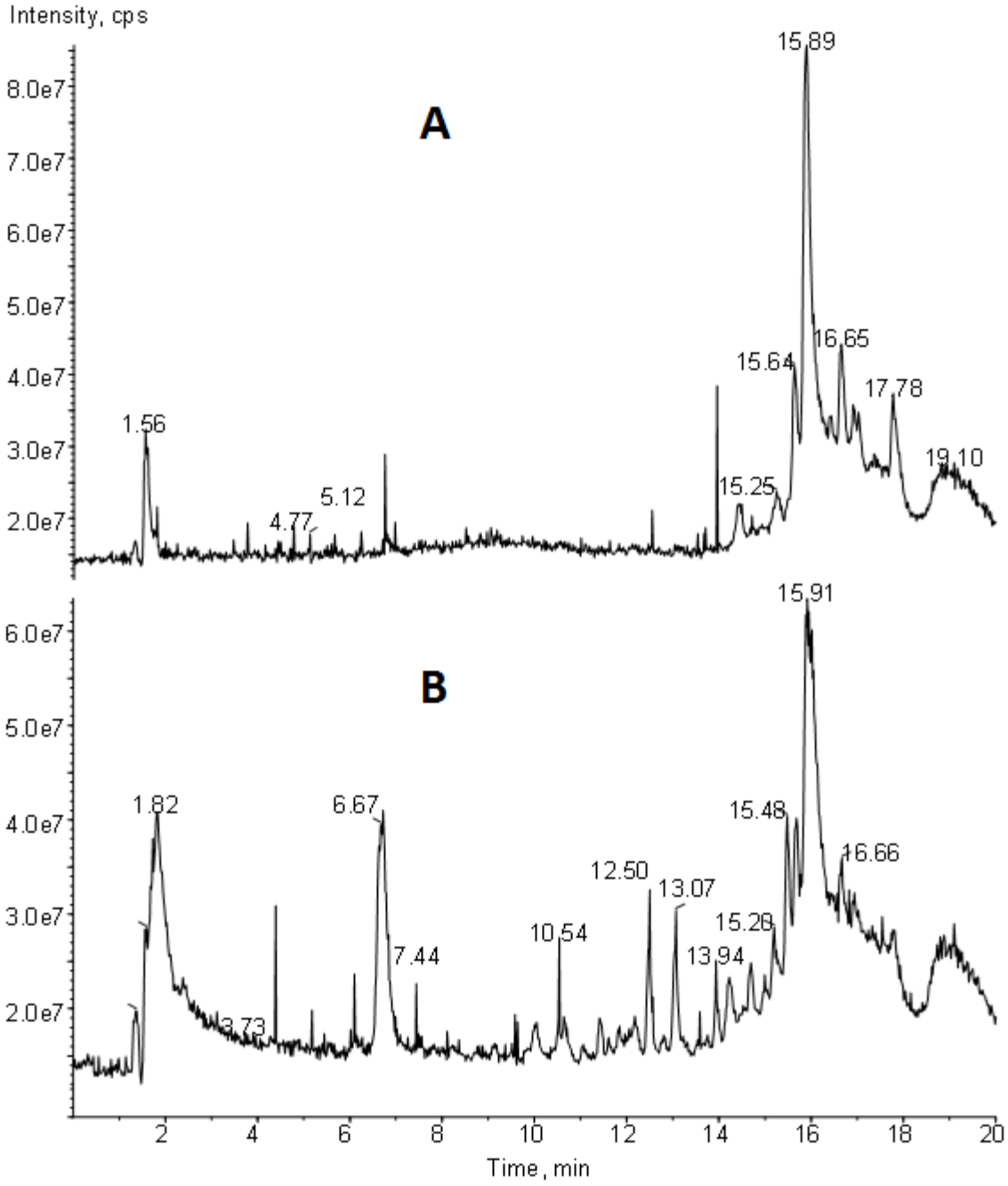


Figure 4-4: Total ion chromatograms of the full scan analysis of the MIP extract (A) and the SPE extract (B) of the real sample taken from a military site

The cleaner chromatogram for MIP extract is caused I) by the smaller sample volume used for the MIP extraction (2 mL) compared to 500 mL for SPE and II) by the specific molecular interactions enabled by the MIP polymer which reduces the extraction of matrix molecules.

Figure 4-5 shows the target analysis of selected nitro- and chlorinated phenols in the SPE extract and the MIP extract of the real sample.

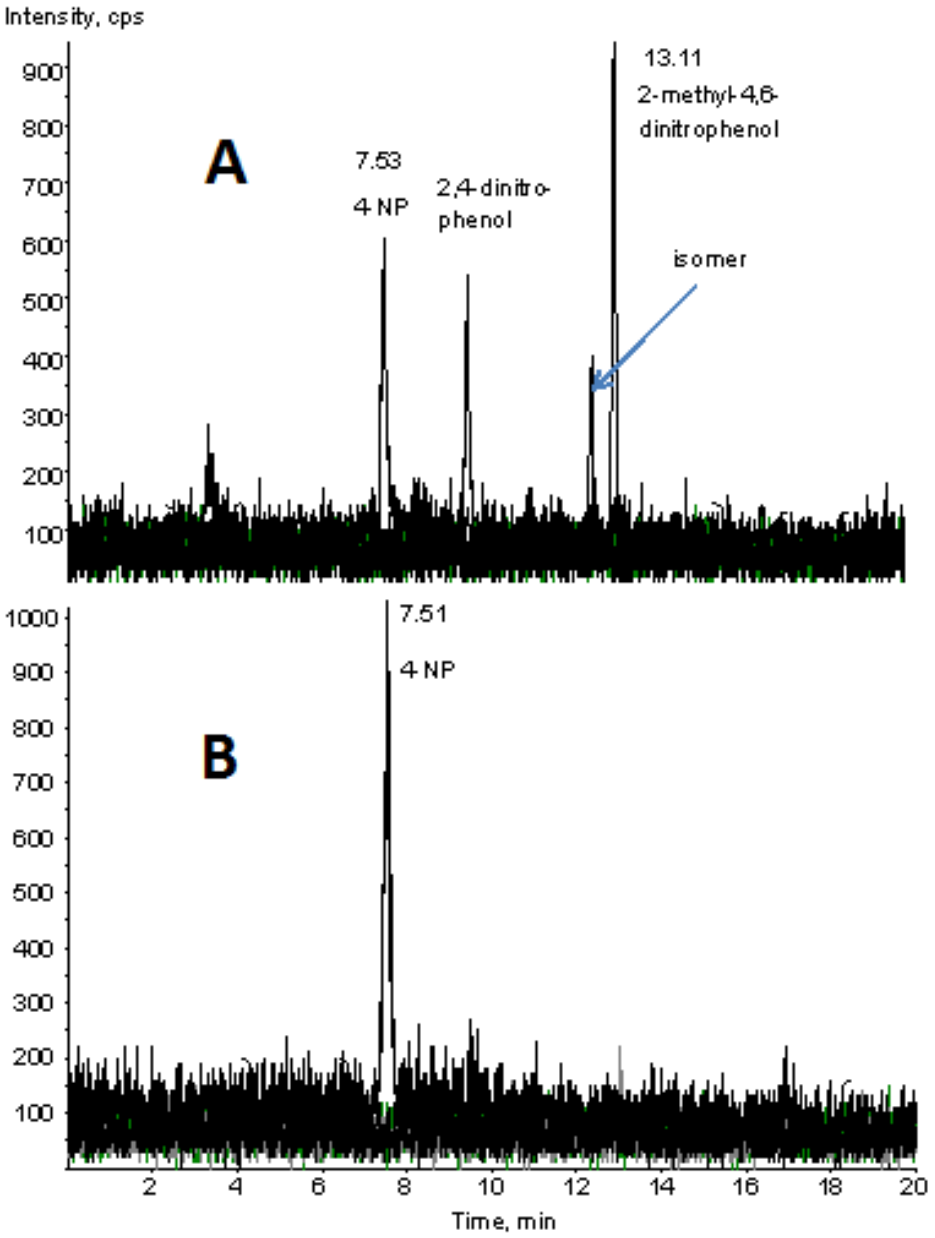


Figure 4-5: Target analysis of selected nitro- and chlorinated phenols in the SPE extract (A) and the MIP extract (B) of the real sample

While by LC-MS-MS (ESI-) in the MIP extract only one signal was recorded corresponded to 4-nitrophrnol (Figure 4-5B), the target analysis of the SPE extract determined 4-nitrophenol, 2,4-dinitrophenol and two isomeric methyl-dinitrophenols. The clear chromatogram of the MIP extract analysis emphasizes the high selectivity of the new MIP tube. Even the interference by 2-NP can be excluded due to MIP extraction experiments applying both 4-NP and 2-NP individually where 2-NP was not recovered above the LOD.

The water from the former military site pose a challenging sample with complex matrix including a variety of organic substances with different properties as well as compounds with nitro aromatic moieties provoking the selectivity of the MIP tube extraction. Due to the complex matrix of the sample, for 4-nitrophrnol quantification a standard addition method was preferred adding 4-nitrophenol at amounts of 5, 25 and 50 µg to 50 mL sample. From the SPE extraction a concentration of 20 µg L^{-1} ±4% 4-nitrophenol was calculated and using the MIP procedure, a concentration of 22 µg L^{-1} ±1% was obtained. Thus, the comparison with a standard protocol indicated good agreement of quantification results for 4-nitrophenol.

4.1.6. Conclusion

A novel preparation protocol was developed for the construction of MIP-capillaries and evaluated via in-tube MIP-SPME extraction of polar compound from water. Besides its simple preparation and because of the glass cover, this new polymer tube has high robustness and mechanical stability. Depending to the experimental conditions, an array of the tubes can be connected to increase the extraction recovery and number of concerned targets. Despite the interesting results obtained during this study including easy maintenance, reduced matrix influence, high selectivity, good precision and low LOD compared to LOD of standard HPLC-DAD without enrichment and also the inherently potential of the tubes for automation, optimized sample volumes were up to 2 mL and sample flow was 0.033 mL min^{-1}. These values cannot satisfactory support in-field sampling and sample preparation of target of interests. Therefore we had to change SPME strategy to a faster extraction method. In comparison to SPME method, SPE is known as an exhaustive extraction method which can be used for larger sample volumes and at higher sample flow-rates.

4.2. Solid-phase extraction

In the second step, synthesized MIP materials for acesulfame were used to fill a traditional solid phase extraction (SPE) cartridge. An 8 mL SPE glass column was cut from 91 to 45 mm and filled with 200 mg of the acesulfame imprinted polymer. The open part of the prepared cartridge was closed with a Teflon nozzle that can be easily connected to the different syringe needle sizes. A syringe pump was used for liquid delivery from the lure tip of the prepared molecularly imprinted-solid phase extraction (MISPE) cartridges at specified flow rates. In a MISPE procedure, the optimization of conditioning, loading, washing and elution steps pre-determine final extraction performance. As mentioned in synthesis section (3.1.) The imprinted polymers are expected to contain ammonium groups (VBTA involved in the imprinting process as an acesulfame-VBTA ion pair) that can operate as an anion-exchanger. As reported for ion chromatographic analysis of sweeteners from foods and beverages [175], alkaline conditions are needed for complete elution of acesulfame. In our first experiments, methanol containing 15 mM KOH was used as an elution solvent but the extraction recovery of acesulfame dropped rapidly over a set of repeated extractions. It was assumed that the high concentration of hydroxide used during the elution step blocks and disrupts the ammonium and carboxyl functionalities of the MIP sorbent. Therefore, optimization of the elution step was evaluated along with an activation step which performed after elution. For this purpose, 5 mL of methanol and a 10 mL mixture of methanol/acetic acid (10%) were passed through the used cartridge, followed by drying of the MIP sorbent using a gentle nitrogen stream. For the conditioning step, 5 mL methanol and 5 mL distilled water were passed through the activated cartridge, respectively. The conditioned MIP cartridge was loaded with 5 mL of a water sample containing $acesulfame^-$ at a concentration level of 0.5 µg mL^{-1} and subsequently dried with a nitrogen stream. Two successive fractions of 5 mL methanol/KOH (15mM) were used as an elution step. Interestingly, the idea of adding an activation step solved the low recovery problem and all of the analyte was recovered in the first fraction of the elution step. It appears that the carboxyl groups of the polymer matrix could be protonated and the retained hydroxide anions from the elution step were replaced by acetate, using high concentrations of acetic acid. An acetate anion, with its delocalized negative charge, could be more easily replaced by molecules with

anion functionalities. However, a major disadvantage of elution with methanol/KOH was a drastic decrease in the analytical response of acesulfame in methanol/KOH (15mM). Signal response was reduced to about 5%, compared to that of distilled water/methanol (90/10, v/v). Furthermore, the LC-MS instrument and the operational lifetime of MISPE cartridges may suffer as a result of these harsh alkaline conditions. Thus, alternatively, 5 mL of methanol/NH4OH 25% (99.5:0.5, v/v) was applied in two successive fractions to elute acesulfame from the MIP. The activation step was also changed to 5 mL methanol/acetic acid (5%). Results showed that 69% of the total loaded analyte was recovered during the first fraction of the elution step and the remaining 31% was eluted during the second fraction. Therefore, 10 mL of methanol/NH4OH 25% (99.5:0.5, v/v) was used for complete elution of the retained analyte molecules. In the next optimization step, the influence of the sample volume on the extraction yield of acesulfame was examined. The sample volume was increased from 5 mL (0.5 µg mL^{-1}) to 50 mL (0.05 µg mL^{-1}) and contained the same total amount of acesulfame. Additionally, after sample loading, 10 mL of methanol was used for washing.

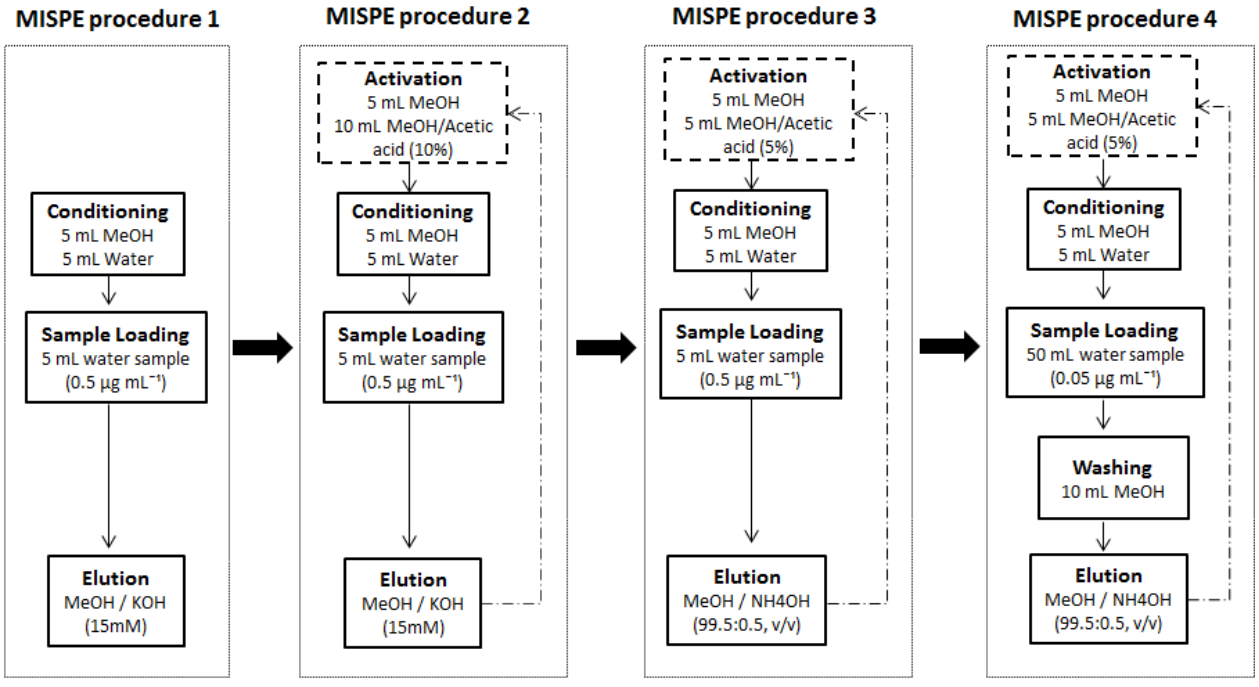


Figure 4-6: Molecularly imprinted solid phase extraction procedures

This was to remove any remaining sample parts and to elute polar non-bonded substances. This protocol ensured acesulfame recovery of up to 94±2.6% in a 50 mL of sample. Figure 4-6 illustrates the different MISPE procedures used during SPE optimization.

As we have seen already in the previously developed selective extraction method [16], imprinted cavities in the polymer matrix can be affected by milieu variations. Swelling and shrinking effects could result from changes in the pH of the solvents used or may be caused by drying of the polymer. These effects can transform the morphology of the imprinted polymer (Figure 3-4 and Figure 4-7) and/or the shape of the imprinted cavities [16, 160]. The results obtained showed that, when using an acesulfame imprinted polymer in the MISPE process, drying of the polymer in the activation step has a big effect on the repeatability of recovery in further experiments. It seems that the cartridge is reseted by its complete drying. On the other hand, and probably due to polymer morphology changes resulting from drying using gentle nitrogen stream (Figure 4-7) and/or harsh pH conditions used during the SPE process, the MISPE cartridge was affected by the back pressure problem after some usage. This was intensified for those samples with a more complex matrix.

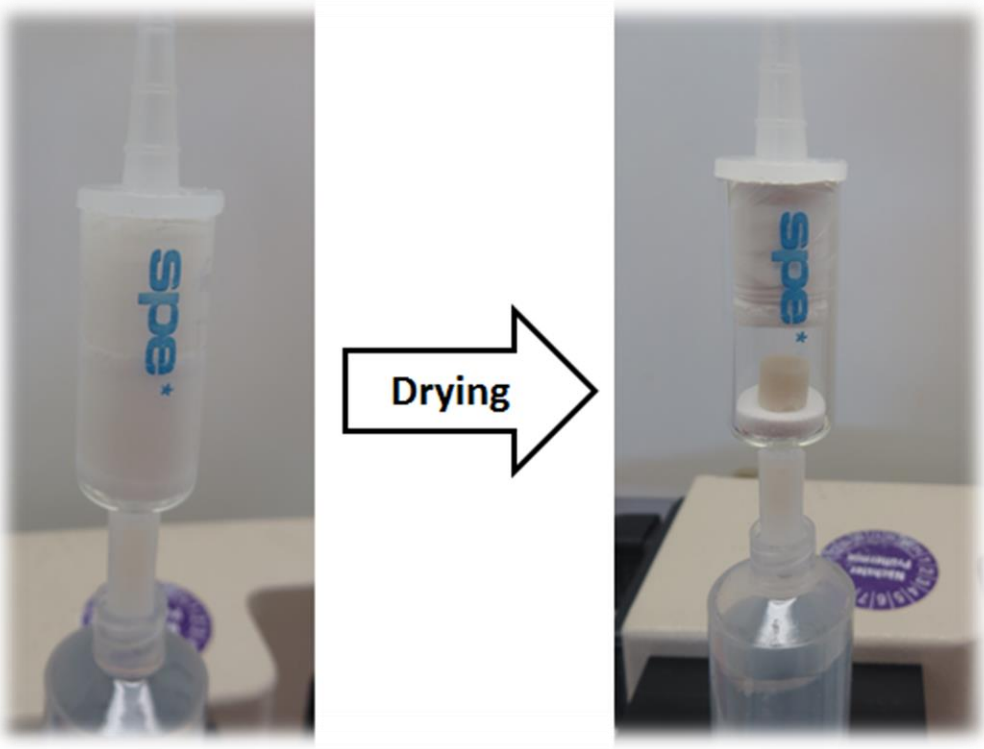


Figure 4-7: Drying of the imprinted polymer using gentle nitrogen stream.

After optimization of extraction parameters, standard acesulfame-K solutions in distilled water at a concentration range of 1 to 100 µg L^{-1} were prepared and analyzed at the optimized conditions using the developed MISPE-LC/MS-MS protocol. The experiments were carried out in triplicate using three SPE cartridges filled with the MIP material synthesized in different batches. The calibration curve for the aforementioned concentration range was linear, with the coefficient of determination of R^2 = 0.9976. The relative standard deviations (RSDs) and the recoveries for the above-mentioned concentrations, ranged between 3.8-8.7% and 81-95%, respectively. LOD and LOQ for the MISPE were calculated 0.12 µg L^{-1} and 0.35 µg L^{-1}, respectively. Using developed method, standard concentration at calculated LOQ was determined 0.358±8.4% which was within the precision and the accuracy of the calibration curve. The results showed that the prepared MISPE cartridges were reusable. Together with subsequent experiments or a new prepared cartridge, standard acesulfame-K solution in distilled water at 50 µg L^{-1} was analyzed that remained in parameters rage of the calibration curve. Table 4-5 represents the LODs and LOQs for acesulfame determination in other reported literatures. Despite the high-enough sensitivity of our method in comparison to the other reported methods; we actually intended to develop a selective pre-concentration method which is strongly needed in the case of acesulfame analysis in highly contaminated wastewater samples.

Table 4-5: Limit of detection (LOD) and limit of quantification (LOQ) for acesulfame determination in other reported literatures

Determination	LOD (µg L^{-1})	LOQ (µg L^{-1})	Reference
HPLC-MS/MS	10	30	[176]
SPE-LC-MS/MS	-	1 and 0.2	[149]
SPE-HPLC-MS/MS	0.3	1	[177]
HPLC–CAD–UV/DAD	200	610	[178]
MISPE-HPLC-MS/MS	0.12	0.35	[134]

When analyzing real water samples like wastewaters with HPLC-MS/MS, matrix effects are believed to be a problem that greatly affects the accuracy of the method due to signal suppression or enhancement during the ionization process. Furthermore, matrix compounds can affect SPE pre-concentration performance [149, 179, 180]. Two common methods including

direct injection of an aqueous sample or treating sample by various SPE techniques are used to transfer targets into HPLC-MS/MS instrument. Without using a suitable pretreatment to minimize interferences of sample matrix constituents, the use of internal standards is mandatory for correct quantification [181]. Despite the fact that the labeled-standards usage for the methods involving SPE are recommended, it is not mandatory [181]. Scheurer et al. [179] showed that except for sucralose, all other evaluated sweeteners including acesulfame could be quantified by external standard calibration in a diluted wastewater samples using a SDB cartridge. They reported [179] that the dilution of wastewater samples could eventuate a matrix, which approximately matches the external standard calibration. So, influent and effluent samples were diluted with distilled water by a factor of 10 and 5, respectively. In order to assess method recovery reduction and ion suppression in the ESI process resulting from matrix effects, samples were spiked both before and after MISPE, respectively [149, 179, 180]. It has already been reported [149, 180] that the two previously mentioned effects caused by matrix compounds can be calculated using the following equations:

$$\%ME = \frac{(R_A)_{spiked} - R_{non-spiked}}{(R_{STD})_{pure}} \times 100 \qquad (4-1)$$

where $\%ME$ represents the matrix effect percentage. $(R_A)_{spiked}$, $R_{non-spiked}$ and $(R_{STD})_{pure}$ are obtained response factors from samples spiked over the extract, a non-spiked sample and a pure standard.

$$\%R_{SPE} = \frac{(R_B)_{spiked} - R_{non-spiked}}{(R_A)_{spiked} - R_{non-spiked}} \times 100 \qquad (4-2)$$

where $\%R_{SPE}$ represents "the recovery accounting exclusively from the sample preparation [149, 180]". $(R_B)_{spiked}$, $(R_A)_{spiked}$ and $R_{non-spiked}$ are obtained response factors from sample spiked before the extraction process, sample spiked over the extract and non-spiked sample.
Considering equation 4-1 and due to the fact that added targets in the extract were not subject to any variation due to the SPE procedure, using the response factor of the pure standard seems to be completely logical.

However, we believe that using equation 4-2 is the best option; where two completely different parameters, namely SPE procedure and the matrix effect on SPE procedure, are considered together. In order to specify $\%R_{SPE}$ for the matrix effect on the SPE procedure, we modified equation 4-2 using a normalization of response factors as follows:

$$\%R_{SPE} = \frac{\frac{(R_B)_{spiked} - R_{non-spiked}}{(R_{STD})_{column}}}{\frac{(R_A)_{spiked} - R_{non-spiked}}{(R_{STD})_{pure}}} \times 100 \qquad (4-3)$$

and with a simple rearrangement of the equation:

$$\%R_{SPE} = \frac{(R_B)_{spiked} - R_{non-spiked}}{(R_A)_{spiked} - R_{non-spiked}} \times \beta \times 100 \qquad (4-4)$$

$$\beta = \frac{(R_{STD})_{pure}}{(R_{STD})_{column}} \qquad (4-5)$$

where $(R_{STD})_{column}$ is the response factor of standard after the SPE procedure and β is the inverse function of SPE performance under standard conditions.

Wastewater samples were preparaed from a local municipal wastewater treatment plant (WWTP) at Langenreichenbach (Germany, with equivalent usage by a population of 80,000 people). Common water quality parameters of the samples analyzed are listed in table 4-6. The influent wastewater sample was filtered with a 45 µm glass fiber filter and was stored in the refrigerator until the experiments were carried out. The effluent wastewater sample was applied in the experiments without any other form of pre-treatment. The pH value of all samples was in the range of 6 to 7.

Table 4-6: Water quality parameters of the wastewater from the pilot constructed wetland plant in Langenreichenbach used for MIP testing

	pH	Dissolved oxygen (mg/L)	COD[a] (mg/L)	BOD[b] (mg/L)	TOC (mg/L)	Ammonia (mg/L)	NO_3^- (mg/L)	NO_2^- (mg/L)
Influent	7.5	0.2	358	227	147	45	0.4	0.01
Effluent*	7.2	0.2	133	84	32	59	0.2	0.02

[a] COD: Chemical oxygen demand, [b]BOD: Biochemical oxygen demand

*effluent of non-planted horizontal flow subsurface wetland

Using developed equations, matrix effects on ionization process in LC/MS-MS (% ME) and on SPE recovery (% R_{SPE}) were evaluated using influent water sample. To evaluate the % ME, the obtained extract from MISPE cartridge spiked with acesulfame-K, bentazon, saccharin, ibuprofen sodium salt, naproxen sodium salt and caffeine each at 2.5 µg mL^{-1}. The results (Figure 4-8A, % ME) show that the extracted matrix, using developed MISPE cartridge, causes signal enhancement for caffeine and signal suppression for the rest of spiked targets. Nevertheless, it is obvious that the matrix effect for acesulfame has been minimized by employing a selective extraction method.

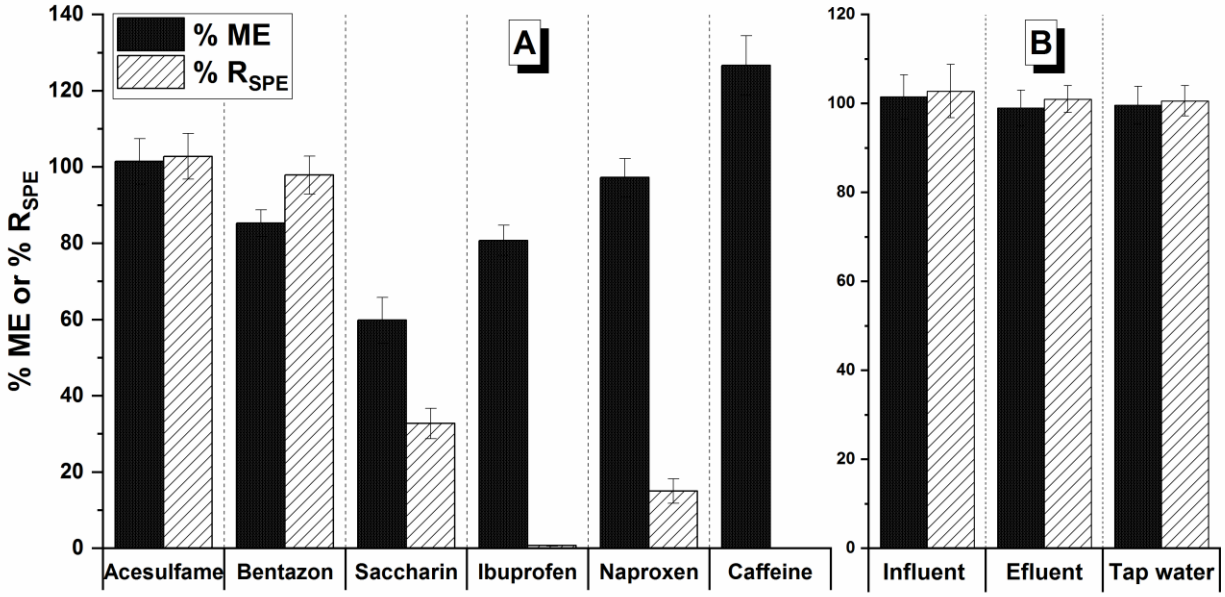


Figure 4-8: Evaluation of matrix effects on ionization process in LC-MS/MS (% ME) and on SPE recovery (% R_{SPE}) in A) influent using 6 target and in B) influent, effluent and tap waters using acesulfame

In the next step, the influence of matrix compounds on the method recoveries were determined by spiking (where the concentration of each solute was 0.05 µg mL^{-1}) the influent water sample before the extraction. Using MISPE cartridge (Figure 4-8A, % R_{SPE}), nearly no effect from matrix compounds on the acesulfame and bentazon recoveries was observed. Besides the highly contaminated influent sample, effluent water sample the tap water sample were spiked with asceulfame-K before and after extraction using MISPE cartridge. The results (Figure 4-8B, % ME and % R_{SPE}) shows that the MISPE cartridge could extract the target compound with nearly no effect from matrix compounds. Influent and effluent water samples were analyzed using developed MISPE method and a SDB-SPE extraction procedure to highlight the selectivity of our

developed method. The extraction procedure using the SDB-SPE cartridge was adapted from Scheurer et al. [179]. In summary, the pH of samples was adjusted to 3. The SDB cartridge was conditioned with 9 mL of MeOH and 9 mL of deionized water set to pH 3. A 50 mL water sample was passed through the conditioned cartridge and then dried using a gentle nitrogen stream. The analytes were eluted with 9 mL of methanol. The resulting eluate was evaporated under a gentle stream of nitrogen at 25 °C and then reconstituted with 1 mL of distilled water/methanol (30:70, v/v). Obtained chromatograms for the target substances are shown in figure 4-9.

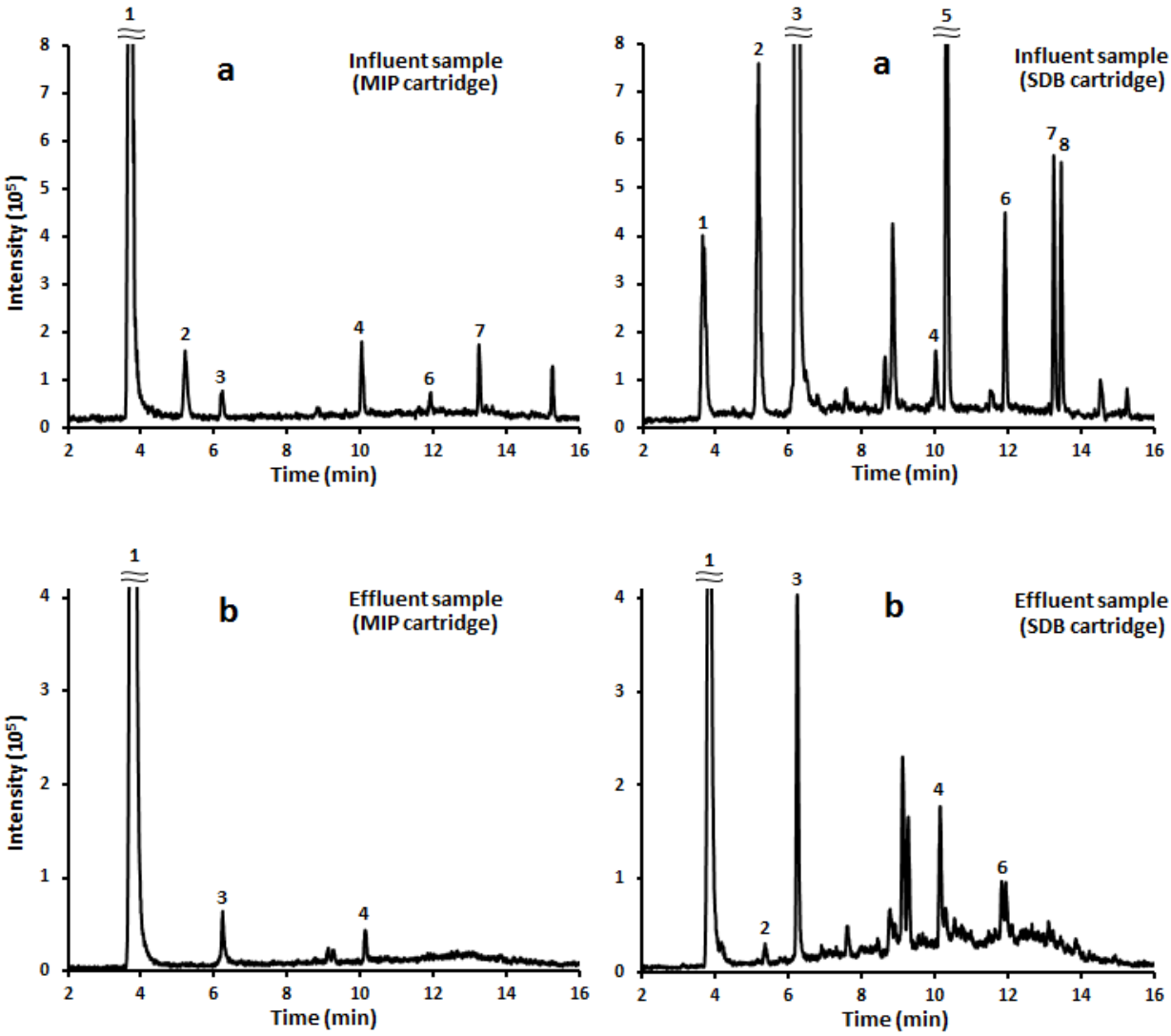


Figure 4-9: Wastewater results using MIP and SDB cartridges. a) influent and b) effluent samples. Numbers represent special targets. 1: Acesulfame, 2: Saccharin, 3: Caffeine, 4: Bentazon, 5: Carbamazepine, 6: Naproxen, 7: Ibuprofen, 8: Diclofenac.

Comparing the obtained results (Figure 4-9) for both MISPE and SDB-SPE procedures, the high selectivity of MISPE is reflected by a clearer chromatogram, indicating the effective exclusion of matrix compounds using MISPE. Figure 4-10 compares the obtained areas for detected targets using MISPE, NISPE and SDB-SPE sample-preparation procedures. The results highlighted the selectivity of MISPE and indicated that the respective non-imprinted polymer detected only traces of the target substances demonstrating that retainment without imprinting was not efficient.

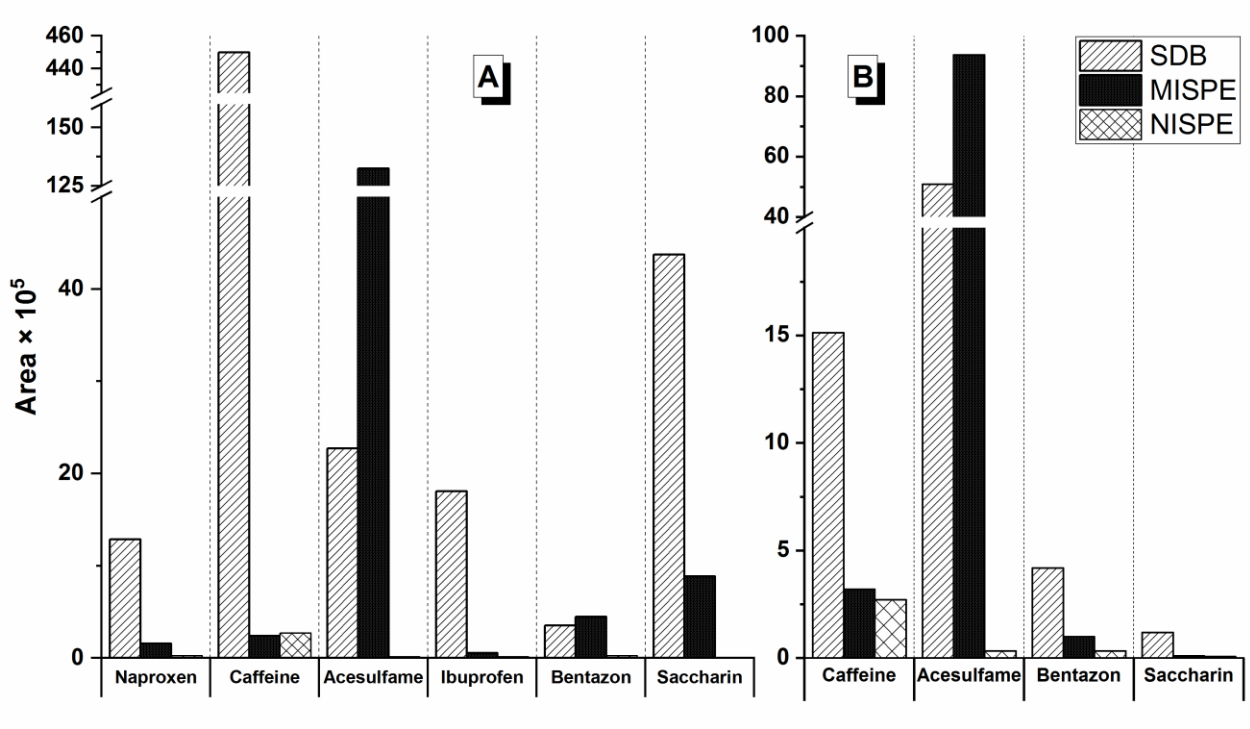


Figure 4-10: Obtained area with HPLC/MS-MS instrument using different sample-preparation for A) influent and B) effluent water samples

It has been shown [179] that the dilution of wastewater samples could eventuate a matrix, which approximately matches the external standard calibration for acesulfame measurement using SDB cartridge. Due to the usage of a selective MISPE for diluted influent and effluent samples, external standard calibration of entire sample-preparation procedure was used to calculate the acesulfame concentrations in influent and effluent water samples. Influent water sample contained 304 ±11.9 µg L^{-1} and effluent had 99 ±4.5 µg L^{-1}. The results are in agreement with previously reported studies which showed WWTP cannot eliminate acesulfame completely

from influent water samples and therefore acesulfame can be used as a suitable anthropogenic marker.

In conclusion, the novel MIP sorbent material was integrated successfully in an optimized MISPE protocol which proved to be highly selective for the extraction of acesulfame from water samples such as influent and effluent wastewater of WWTPs with highly complex matrix. However, the prepared traditional MISPE cartridge faced back-pressure problem which limited the sample flow-rats up to maximum 0.5 mL min^{-1}. Therefore, sample-preparation of 50 mL water sample took more than 100 min which is not suitable for field-applicable and high throughput sample-preparation method.

Till this stage, we have optimized two known analytical sample-preparation formats using our synthesized imprinted polymers as selective sorbents, SPME and MISPE as selective SPE format. However, developed methods could not support fast sampling of large sample volumes neither SPME method nor MISPE method.

During the evaluation and optimization procedures, a solution arose to mitigate or perhaps eliminate the back-pressure problem in MISPE. The solution was based on a simple question: is it possible to produce micro-channels within the packed MIP sorbents in a SPE whereupon liquid samples including water sample and washing and elution solvents can pass through at relatively higher flow-rates. This question guided us to develop a new SPE format termed mixed-bed SPE.

4.3. Mixed-bed solid phase extraction

The idea of mixed-bed solid phase extraction (SPE) is blending the synthesized irregular atrazine-imprinted polymer particles (synthesis protocol in section 3.3.) among with more regular and rigid commercially available ingredients. The MIP materials are irregular and could strongly swell when they are in contact with liquid especially organic solvents. This can cause elevated high back-pressure during SPE procedure. The question was: could extra rigid and regular ingredients provide micro-channels through which liquid solvents can pass at relatively higher flow-rates? To answer the question, various parameters were evaluated to find the nature and the ratio of mixing elements for mixed-bed. Then, extraction conditions were optimized. Finally, the extraction efficiency of optimized mixed-bed (contained 10 mg polymer) was compared with its relative MISPE which contained 10 mg polymer, MISPE which contained 200 mg polymer and a SPE cartridge packed with commercial SDB sorbents.

4.3.1. Selection of matrix ingredient to be mixed with the MIP material

The type and amount of matrix ingredients are factors that could directly affect the final performance of the packed column. The matrix ingredients should preferably be inert as much as possible towards the target molecule. Therefore, the affinity of PTFE and silica gel particles for extracting atrazine was evaluated. PTFE (< 100 µm) particles were prepared by grinding and sieving the commercial PTFE frits. Silica gel S (S) (63-100 µm) was obtained from Riedel-de Haën (Seelze, Germany). SPE glass columns were packed with 10 mg of the aforementioned ingredients and used for the extraction of atrazine from standard water samples. A 10 mL water sample with 0.1 mg L^{-1} atrazine was passed through the previously-conditioned cartridges at a flow rate of 2 mL min^{-1}. Both silica gel and PTFE particles showed very low attraction (recovery < 2%) for the extraction of atrazine (which was washed completely during washing step). In comparison to PTFE, the silica gel consists of more rigid and uniform particles, therefore allowing higher flow rates at lower back pressure when packed in a cartridge. Therefore, silica gel was selected for our further investigations.

4.3.2. Optimization of the MIP-silica gel composition

In SPE, the mass and type of sorbent predetermine the performance of SPE, its capacity, extraction time and the type and volume of required organic solvents. As mentioned before MIP materials swell when they come into contact with solvents while silica gel particles remained unchanged. This elevated packing effect can cause a reduction in the number of available selective sites for interaction with the target analytes. That can also cause to increase the channeling and voiding effects, to elevate the back pressure and run times. Reducing the amount of MIP material as SPE sorbent can reduce back pressure and the required volume of organic solvents for conditioning, washing and elution steps. In this case, the time required for extraction decreases. However, the cartridge's capacity may also decrease as well. For this purpose, different ratios of MIP/silica gel material mixtures were evaluated. The best ratio guarantees that the MIP particles are totally homogenized and dispersed in the silica gel matrix, making it possible to operate with a higher capacity per milligram of sorbent.

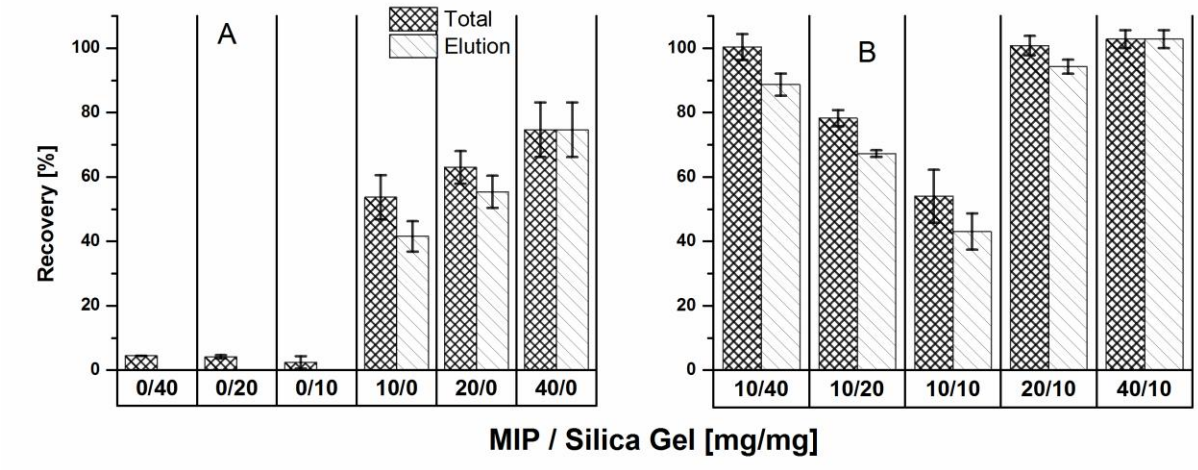


Figure 4-11: Percentage recovery evaluation of different imprinted polymer to silica gel ratios. (A) Pure silica gel and imprinted polymer. (B) Mixture of silica gel and imprinted polymer.

A 10 mL water sample with 0.1 mg L^{-1} atrazine was passed through the conditioned sorbents at a flow rate of 2 mL min^{-1}. As shown in figure 4-11A, pure silica gel up to 40 mg could extract less than 5% of total atrazine molecules. On the other hand, increasing polymer materials from 10 mg to 20 and then 40 mg increased the extraction recovery from about 54 to 63 and 75%, respectively. However, the obtained results in figure 4-11B shows that adding 10 mg of silica gel

to 20 mg and 40 mg of imprinted polymers could increase the recovery of atrazine up to about 100 %. As shown in figure 4-11A, 10 mg of pure silica gel could extract just about 2% of atrazine. Therefore, recovery enhancement by adding 10 mg silica gel into 20 and 40 mg MIP are attributed to the porosity enhancement of SPE bed and accordingly increased selective sites accessibility. As shown, adding 40 mg of silica gel could enhance the total extraction efficiency of 10 mg MIP polymer from about 54% (10 mg pure MIP) into about 100% (10 mg polymer+40 mg silica gel). Again, adding 40 mg silica gel could increase enormously the accessibility of imprinted sites within the SPE bed.

Despite the high enough extraction efficiency obtained for 10 mg MIP when the MIP particles have been homogenously dispersed in 40 mg silica gel, reduced amount of MIP sorbent can help to reduce required organic solvents for washing and elution steps. For further method developments, a ratio of 10:40 was applied allowing higher flow rates, less back pressure and the possibility of percolating the real samples directly without any other pre-filtration.

4.3.3. Extraction efficiency performance of mixed-bed MISPE in comparison with commercial sorbents

In order to validate the extraction efficiency of the new prepared mixed-bed MISPE, different commercial sorbents commonly used for atrazine were compared. For this purpose, 10 mg of the following commercial sorbents: styrene-divinylbenzene copolymere (SDB) (43-123 µm), octadecylsilane bonded to silica gel (ODS) (47-60 µm) and H2O-Philic divinylbenzene (DVB) (15 µm) were packed into SPE cartridges and utilized for the extraction of 1 µg atrazine from 10 mL water sample. As depicted in figure 4-12, from all evaluated sorbents, mixed-bed MISPE shows the best extraction efficiency for atrazine.

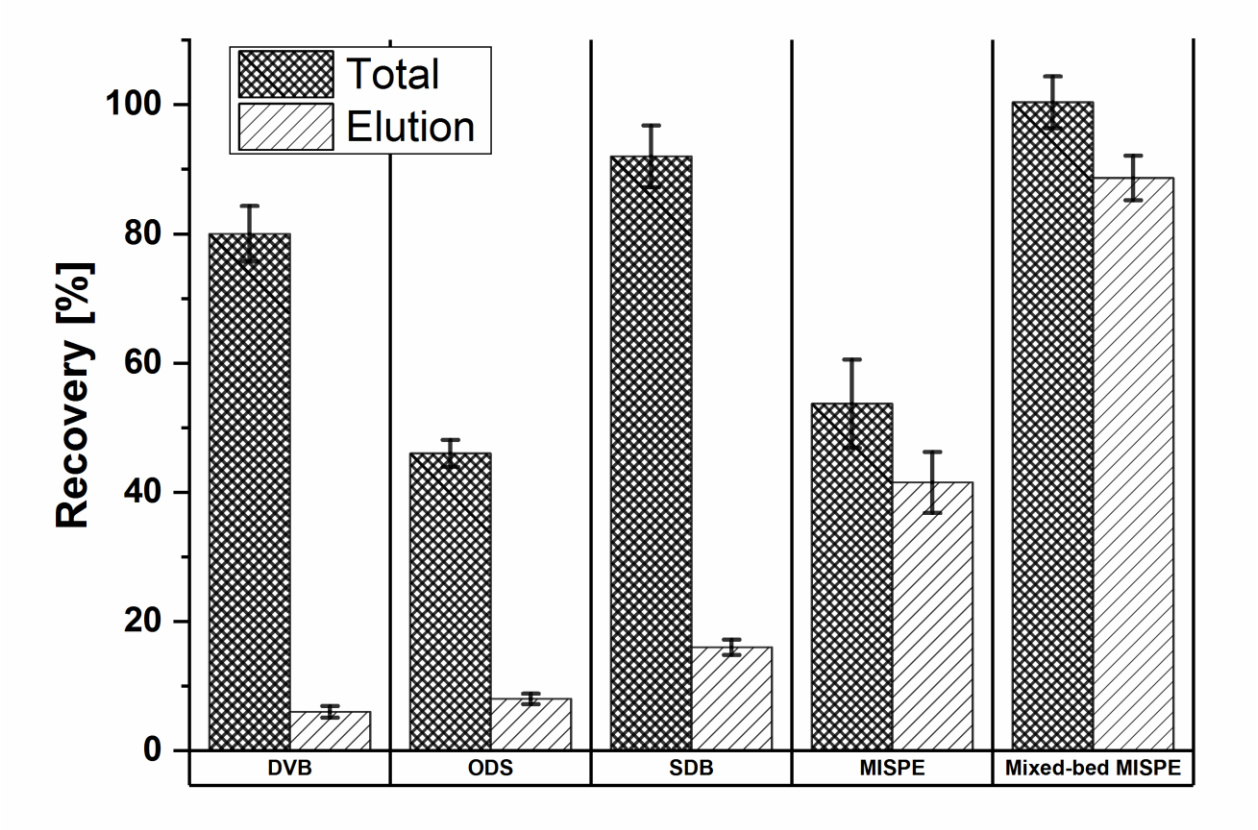


Figure 4-12: Percentage recovery evaluation of different sorbents. (SDB: styrene-divinylbenzene, DVB: divinylbenzene, ODS: octadecyl group bonded type silica gel, PTFE: polytetrafluoroethylene).

4.3.4. Optimization Extraction conditions for mixed-bed MISPE

4.3.4.1. pH of the sample solution

The rebinding of atrazine molecules to slectiv sites of MIP particles is mainly based on hydrogen bonds that are expected to be formed between atrazine and MAA. In order to strengthen these interactions, the pH condition of the sample has to be properly adjusted. Therefore, pH value of the sample solution is one of the most important parameters for the selective extraction of atrazine. In our investigation, the pH of the standard solution was adjusted to values ranging from pH 2 to pH 9. The recovery of atrazine decreased at higher pH and in highly acidified solutions. These conditions vary strongly from those applied for the imprinting process carried out under neutral conditions. Thus, the cavities of the polymer are shaped for the neutral atrazine molecule. At basic or acidic conditions, the ionic form of atrazine and the functional groups of the MIP cause that target molecules fit less well in these polymer selective sites. For instance, under strong acidic conditions, the carboxyl groups as active sites in the cavities are in

their protonated form as atrazine itself. On the other hand, basic conditions can deprotonate the carboxyl groups inside the selective site of polymer matrix and atrazine molecules. The recovery of atrazine with NIP particles was very low for all pH conditions, which shows non-specific adsorption of analyte. According to these results, a pH range of 6 to 7 was selected as the optimum for further experiments.

4.3.4.2. Sample flow-rate and breakthrough volume

Sample flow rate is an important parameter that influences the extraction yield of an analyte and the extraction time. A 10 mL water sample with 0.1 mg L^{-1} atrazine was passed through the conditioned mixed-bed MISPE cartridges at flow rates of 1, 2, 3 and 5 mL min^{-1}. Lower flow-rate means more time for mass transfer, which was reflected in an increased recovery of atrazine. An approximate plateau at 89 % recovery is reached for flows less than 3 mL min^{-1}. With the intension of reducing the extraction time, the flow rate was set at 3 mL min^{-1} for subsequent developments.

In order to determine the optimal volume for sample loading; 10, 20, 30 and 40 mL water samples, each containing 1 μg of atrazine, were loaded onto the mixed-bed sorbent at 3 mL min^{-1}. The recovery of atrazine decreased with increasing volume to more than 10 mL, which can be attributed to the promoted diffusion of the analyte in small sample volumes [92]. A loading volume of 10 mL was therefore found to be most efficient for atrazine extraction and was used for further optimizations.

4.3.4.3. Optimization of washing and elution steps

Although MIPs offer the highest selectivity when samples are administrated in the solvent used for the polymerization [182], in our experiments water was used as the loading solvent to avoid any other pre-concentration step. Due to the retention of the analytes via non-specific hydrophobic interactions during percolation of water samples, the washing step plays an important role in demonstrating the selectivity of the synthesized polymer [183]. Non-selective sites and those cavities with incomplete or irregular shape are able to adsorb molecules less tightly than the specific imprinted areas of the polymer. So, optimization of the washing step is essential because it simultaneously allows the interactions of target molecules with the specific

cavities and removal of the co-extracted non-target substances [183]. Besides the washing step, the drying step after sampling could also affect the selectivity of MIPs. Pap et al. [182] showed that small amounts of water that remain after sample application due to incomplete drying can change the binding affinity of the MIP material to atrazine. The authors reported that at least 30 min were needed to remove the adsorbed water from 50 mg of MIP. Furthermore, the application of the organic solvent before complete drying caused high variance in their experiments [182]. In our experiments and after sampling, within 20 seconds, 10 mL distilled water was passed through the atrazine-loaded mixed-bed sorbent via a SPE vacuum manifold and was further dried until no water loss was measured (by weighting). 5 min was required to completely dry the mixed-bed. To optimize the washing step, different solvents were examined and dichloromethane, as a weakly polar and aprotic solvent, was found the best choice to highlight the selectivity feature of used MISPE for water samples [182, 183]. The dried cartridge was then connected from its lure tip to a syringe containing dichloromethane. For washing, 1 mL dichloromethane was passed through the cartridge at a flow rate of 2 mL min^{-1} and easily collected from the reservoir part of mixed-bed MISPE cartridges for further analysis. A vacuum was then applied for 30 sec to remove any remaining dichloromethane via the SPE vacuum manifold. In order to elute the mixed-bed, methanol-acetic acid (99:1) was used as eluent solvent. Successive portions of 0.2 mL eluted solvent were collected from the lure tip of the cartridge and used for further analysis. The results showed that 89 % of atrazine was contained within the first elution fraction. In order to be sure about complete elution of target analyte, 0.4 mL of methanol-acetic acid (99:1) was finally selected as the eluent volume.

4.3.5. Analytical evaluation and real samples

To validate the proposed method, standard atrazine solutions in water (pH 6) at a range of 5 to 200 µg L^{-1} were prepared and analyzed at optimized conditions with the developed mixed-bed MISPE-GC/MS. In comparison, atrazine was extracted by pure MIP sorbent in SPE mode (MISPE). Standard atrazine solutions in chloroform at concentrations ranged 0.52 to 2.06 mg L^{-1} were also prepared and analyzed directly with GC-MS. Linear regression analyses were performed using the peak area obtained by mixed-bed MISPE, MISPE and directly-injected samples against the corresponding concentrations. The limit of detection (LOD) and the limit of quantification

(LOQ) were calculated at signal-to-noise ratios of 3 and 10, respectively. The calibration curve obtained for the concentration ranges mentioned above was linear with correlation coefficients of R^2 = 0.9988 for directly injected sample, R^2 = 0.9651 for MISPE and R^2 = 0.9925 for mixed-bed MISPE. LOD and LOQ for the mixed-bed MISPE were 1.34 µg L^{-1} and 4.5 µg L^{-1}, respectively. The relative standard deviations (RSDs) for the concentration range of 5 to 200 µg L^{-1} were 3.5-12.1% for MISPE and 1.8-6.3% for mixed-bed MISPE. Besides sensitivity enhancement and the improvement of the RSD with the developed method, column-to-column reproducibility was also evaluated. 10 different cartridges were prepared for each of the MISPE and the mixed-bed techniques and assessed via loading the water sample containing atrazine at a concentration of 0.1 mg L^{-1}. The results obtained showed that the percentage recovery levels (±RSD %) were 48 (±53 %) and 82 (±16.1 %) for MISPE and mixed-bed MISPE techniques, respectively.

The operational parameters and analytical characterization of mixed-bed MISPE, corresponding MISPE, conventional MISPE and the SPE procedure with SDB sorbent are summarized in table 4-7. The data emphasized that with the new mixed-bed approach, the LODs could be improved while the amount of organic solvent and the extraction time can be significantly reduced.

Table 4-7: Operational parameters and analytical performance of extraction techniques for atrazine in water samples

	SDB-SPE	MISPE	Reduced mass-bed MISPE	Mixed-bed MISPE
Adsorbent (mg)	200	200	10	10 (MIP); 40 (Silica gel)
Conditioning Solvent (mL)	2	25	0.5	0.5
Drying time (min)	n.a.	> 20	5	5
Elution solvent (mL)	2	3	0.4	0.4
RSD (%)	12	n.a.	3.5-12.1	1.8-6.3
Column-to-column RSD (%)	n.a.	n.a.	53	16.1
LOD (µg L^{-1})	10	n.a.	2.25	1.34
Reference	[48]	[183]	[1]	[1]

n.a. data not available from literature

The applicability of the method was evaluated for the determination of atrazine in real water samples with different complex matrix backgrounds. Laboratory tap water, river water and influent from the municipal wastewater treatment plant in Leipzig (500,000 PE) were used for

preparing the atrazine solutions which were applied in the experiments to show the influence of matrix components on atrazine extraction. Tap water without any other treatment was used for preparing atrazine solutions applied in the experiments. River water and wastewater were filtered with a 45 µm glass fiber filter and were stored in refrigerator for experiments. The pH of all samples was checked to be in the range of 6 to 7. Both wastewater and the river water proved to be challenging examples for samples with complex matrices provoking the selectivity and efficiency of the developed mixed-bed MISPE extraction. Prior to spiking, all samples were analyzed for atrazine using styrene-divinylbenzene column described in [184] with slight modification. In summary, the SDB cartridge containing 200 mg sorbent was conditioned by the passage of 3 mL of acetone and 5 mL of deionized water. A vacuum was applied to draw the acetone and water through the cartridge. A 10 mL sample was passed through the previously-conditioned cartridges at a flow rate of about 3 mL min^{-1}. The loaded cartridge was washed with 10 mL of deionized water and dried using the SPE vacuum manifold for 20 min. The dried cartridge was eluted with 5 mL acetone. The resulting eluate was evaporated under a gentle stream of inert gas at 25 °C and then reconstituted with 0.1 mL of chloroform. All real samples were found to be atrazine-free.

Tap water, river water and an influent obtained from the central municipal wastewater treatment plant in Leipzig were spiked with atrazine to adjust a concentration of 5 µg L^{-1}. The results of the analyses summarized in table 4-8 indicate a low matrix influence on the extraction process. Even the complex composition of wastewater did not significantly reduce atrazine recovery.

Table 4-8: Determination of atrazine spiked into samples with different matrices

Sample	Spiked (µg L^{-1})	Found[a] (µg L^{-1})	Recovery[a] (%)	RSD[a] (%)
Tap water	5	5.15	103	0.353
River water	5	4.9	98	1.131
Wastewater	5	4.4	88	1.562

[a] Number of sample = 3

The wastewater sample was used as an example to compare the selectivity and “cleanup” performance of the new MIP procedure with that of a commercial SPE protocol involving SDB

sorbent. The extracts of both methods were analyzed by GC-MS in full scan mode to obtain an overview of the extracted components. The total ion current chromatogram of the extract obtained by the standard SPE procedure using a commercial SDB cartridge (Figure 4-13) indicates a very complex mixture of substances, while the corresponding chromatogram of the mixed-bed MISPE extract contained much less signals, therefore emphasizing the higher selectivity of the extraction process. The specific molecular interaction enabled by the MIP polymer reduces the number of co-extracted matrix molecules significantly.

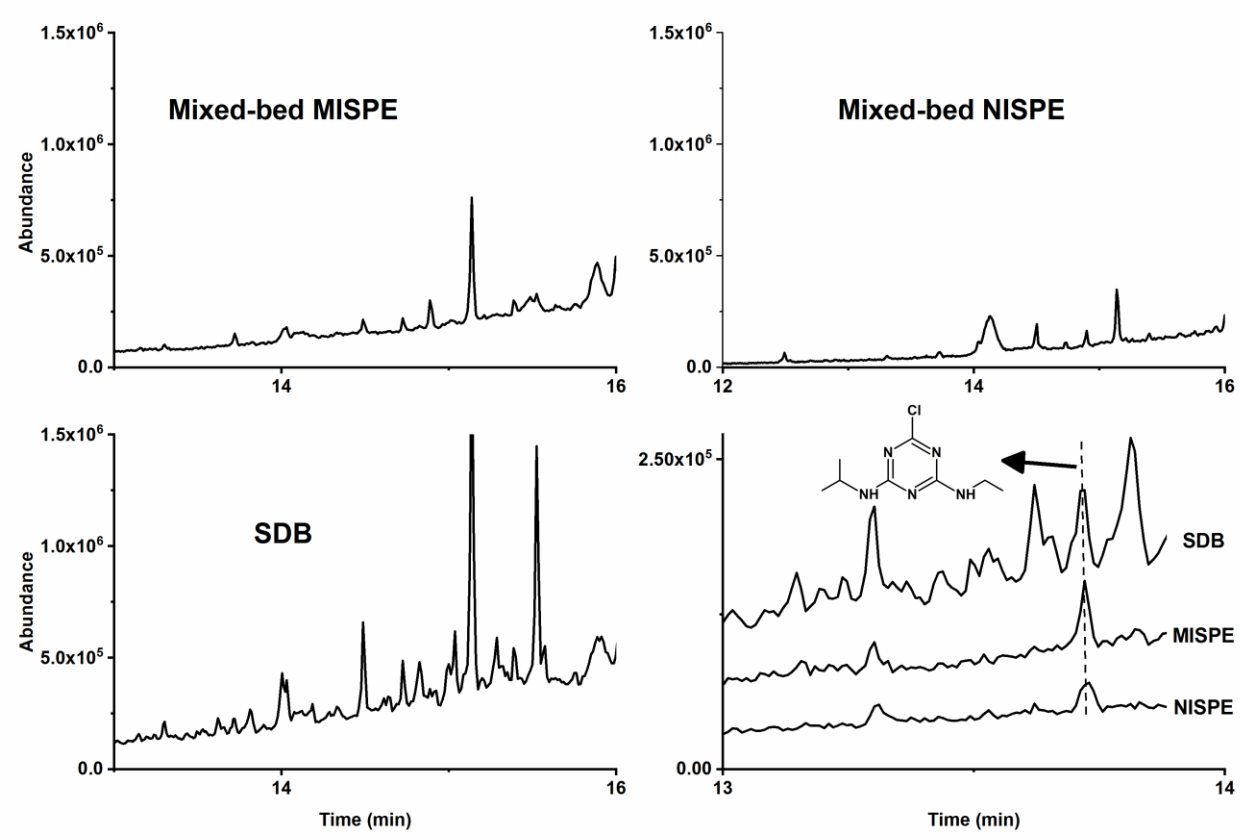


Figure 4-13: GC-MS chromatograms of spiked wastewater (5 µg L^{-1}) obtained by mixed bed MISPE and NISPE and styrene-divinylbenzene (SDB) columns.

4.3.6. Conclusion

Obtained results proved that the idea of dispersing MIP materials among a relatively inert and stable commercially sorbets can help to increase the flow-rate up to 3 mL min^{-1}. It is also enhanced substantially the accessibility of imprinted sites which synthesized within the polymer matrix. In comparison to its relative MISPE method, the new strategy improved the LODs, LODs, RSDs and column-to-column reproducibility. Due to the reduced bed-mass sorbent and at optimized conditions, the total amount of organic solvents required for conditioning, washing and elution steps reduced from more than 25 mL for conventional MISPE to less than 2 mL for mixed-bed MISPE. Besides reduced organic solvent consumption, total sample preparation time of the mixed-bed MISPE method relative to the conventional MISPE was reduced from more than 20 min to less than 10 min. However, 10 mL water sample was the obtained optimized sample volume for 10 mg of polymer particles which disperses among 40 mg silica gel. In order to determine trace concentration of target molecules in groundwater and surface waters at low part-per-billion range, the larger sample volumes are preferred. It could be concluded that for larger sample volumes, the ratio of MIP/silica gel can be simply multiplied by a constant factor. However, increasing the amount of bed materials (MIP as adsorbent and silica gel as disperser) in column format in a SPE cartridge can simply produce a physical barrier which could reduce the flow-rates. In order to solve this problem, inspired from solid phase extraction disk method, column format was changed to disk format and cellulose fibers were used as disperser materials (Figure 4-14). The new methodology is termed selective filter-paper disk.

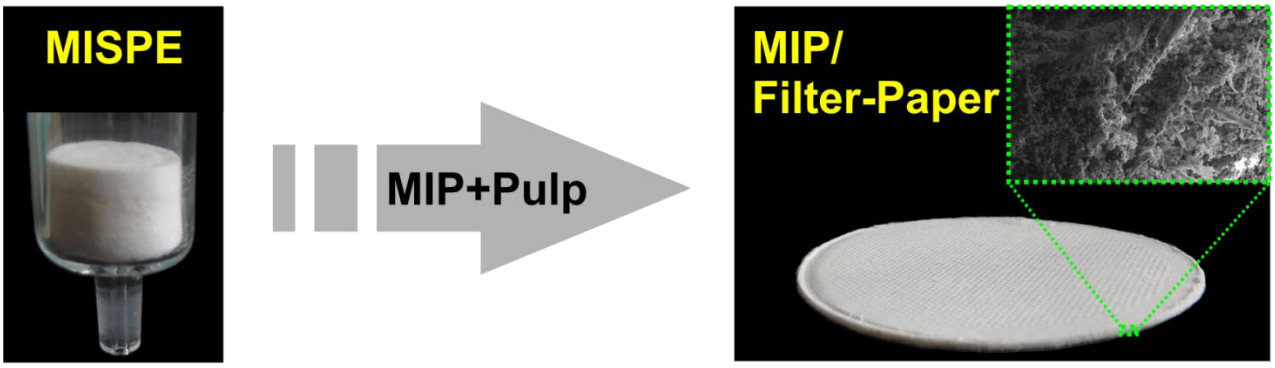


Figure 4-14: Inspired from solid phase extraction disk, polymer materials; used to pack a SPE cartridge, were disperses among cellulose fibers

4.4. Selective filter-paper disk

As mentioned in the introduction section, solid phase extraction disk (SPED) devices were introduced [21, 22] to extract chemicals from large sample volumes at higher flow rates with significant reduction of channeling and voiding effects and highly efficient mass transfer and reduced risk of clogging. Nevertheless, the uses of SPDE devices are not as widespread as SPE cartridges. SPED devices are only commercially available for a limited range of sorbent types [185] whereas none of them are selective to facilitate the investigation of complex samples and simplify the associated data processing. Furthermore, SPED devices are significantly more expensive than SPE cartridges [185]. Therefore, we tried to develop a new simple and cost-effective concept in which the selectivity feature of MIP sorbents can be combined with the advantages of SPED devices. Paper which is an inexpensive stable natural polymer was used as the substrate, disperser and protector to fabricate low-cost selective extraction disks, which are robust, reproducible and easy to handle also under field conditions. Acesulfame imprinted polymer, which was synthesized using our new strategy, was used as the selective sorbent. This polymer was evaluated already in tradition SPE format (4.2.). The obtained results showed that the SPE cartridge packed with 200 mg polymer particles could selectively extract the target molecules from 50 mL of water samples. However, the sample flow-rate was restricted to 0.5 mL min^{-1}.

In the new study, we tried to disperse 200 mg of acesulfame imprinted polymer among cellulose fibers in disk format using very simple but effective strategy. Again, extraction parameters for the new disk format were evaluated and the final optimized values were compared with those obtained with the traditional SPE cartridge. Alongside all these optimizations, laboratory-based HPLC-MS/MS detector was used for determination of targets in final eluates.

4.4.1. Preparation of selective paper-based solid-phase disk device

The used procedure for the preparation of the filter paper is schematically shown in figure 4-15.

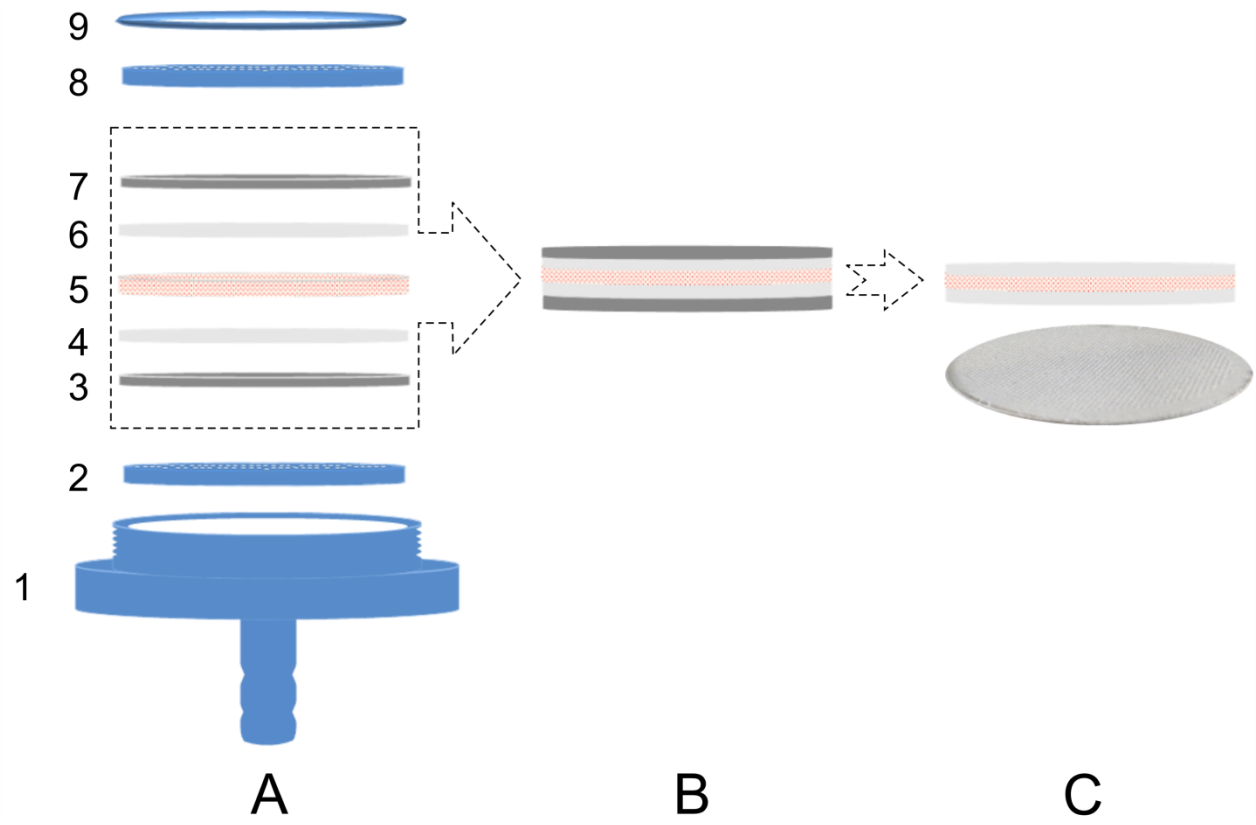


Figure 4-15: Schematic representation of polymer-loaded filter-paper preparation. 1: PTFE funnel, 2: PTFE filter funnel plate, 3: Commercial filter paper, 4: Paper pulp, 5: The mixture of paper pulp and MIP polymer particles, 6: Paper pulp, 7: Commercial filter paper, 8: PTFE filter funnel plate, 9: PTFE O-Ring. A) Selective filter paper (4+5+6) is pressed between commercial filter papers (3 and 7). B) After complete drying, (3+4+5+6+7) is taken out of PTFE assemblies. C) After removing the commercial filter papers (3 and 7), prepared selective filter paper (4+5+6) is ready to be used.

To prepare the paper pulp mixture which contains cellulose fibers, we used a very simple procedure. In summary, 19.5 g of commercial whatman filter paper (185 mm ø) was torn up to get small pieces and put with 900 mL distilled water in a blender to produce cellulose pulp. An "ULTRA-TURRAX T-25" digital homogenizer was used at a speed of 9500 min^{-1} to homogenize the prepared pulp (Pulp, Stock A). 120 mL of this pulp was diluted by 780 mL distilled water to reduce the pulp density of the suspension. This new less-dense mixture (Pulp, Stock B) was homogenized again by the ULTRA-TURRAX at a speed of 9500 min^{-1} for 1 h. The prepared pulp

mixture in stock B was used for further experiments to prepare layers 4, 5 and 6 shown in figure 4-15.

For the preparation of the middle composite layer (shown as number 5 in figure 4-15), 200 or 400 mg of polymer particles were weighed and mixed with 20 mL methanol. After complete swelling of the polymer and the thorough penetration of the organic solvent among and inside the polymer particles, the mixture was sonicated gently to fragment aggregated polymer particles. Afterwards, the pure pulp from stock B was added and stirred vigorously to mix them completely. This composite mixture will be used to construct the middle composite layer numbered 5 in figure 4-15.

A PTFE Büchner funnel (1+2 in figure 4-15) was connected to a SPE vacuum chamber and leveled using a spirit level instrument. A circular filter-paper with a 47 mm diameter (3 in figure 4-15) was cut from the commercially filter paper (Whatman, 185 mm ø) and put on the PTFE Büchner funnel plate to serve as a holder for building up the multilayer extraction disk. In order to prepare a very uniform and homogenously dispersed filter-paper, the first protecting layer (4 in figure 4-15) was prepared using 30 mL of pure cellulose fiber from the stock B which was coated layer by layer (two parts and each 15 mL) on the surface of the Whatman filter paper (47 mm ø). Among each segment, the previously coated layer was pressed carefully to stabilize it as much as possible.

Then, the prepared composite mixture (described above) was coated on the top of the first protecting layer (4 in figure 4-15) using a layer-by-layer method. The prepared new layer contains MIP particles as active selective sorbents and cellulose fibers as disperser (5 in figure 4-15). It is worthy to emphasize that the prepared composite solution must be coated in the layer-by-layer procedure. In the sample preparation process, liquid samples prefer to pass through the parts of the filter which have the least resistance towards them, especially at higher flow-rates. This effect has already been known as ‘channeling’ in conventional SPE cartridges. These shortcuts can negatively affect the performance of the prepared filter paper by low recoveries and reduced repeatability. Therefore, in order to overcome this negative effect, (I) layers in selective filter paper must be constructed layer-by-layer and also (II) during the preparation of

composite mixture, MIP materials must be swelled and disperssed in an organic solvent like methanol before adding the pure pulp solution.

Finally, another 30 mL of pure cellulose fiber from the stock B was coated (shown as 6 in figure 4-15) on the top of the previously coated composite layer as described for the layer numbered 4 in figure 4-15).

Finally, a commercial filter paper (Whatman, 47 mm ø), a PTFE filter funnel plate and a PTFE O-Ring (shown as 7, 8 and 9 in figure 4-15, respectively) were put on the top of the system and pressed by a fixing PTFE-tube. 10 mL of distilled water and 10 mL of methanol were passed through the assembled system consecutively and then it was dried by a gentle stream of nitrogen. Figure 4-16 shows the top-view of the assembled system after complete drying.

Figure 4-16: Top-view of the assembled 1 to 9 shown in figure 4-15.

After complete drying, the two Whatman filter papers (47 mm ø) (3 and 7 in in figure 4-15) were removed from both sides and the prepared selective filter-paper disk was weighed and stored in a laboratory desiccator.

Figure 4-17 shows the cross-sectional SEM image of a MIP-loaded paper-filter.

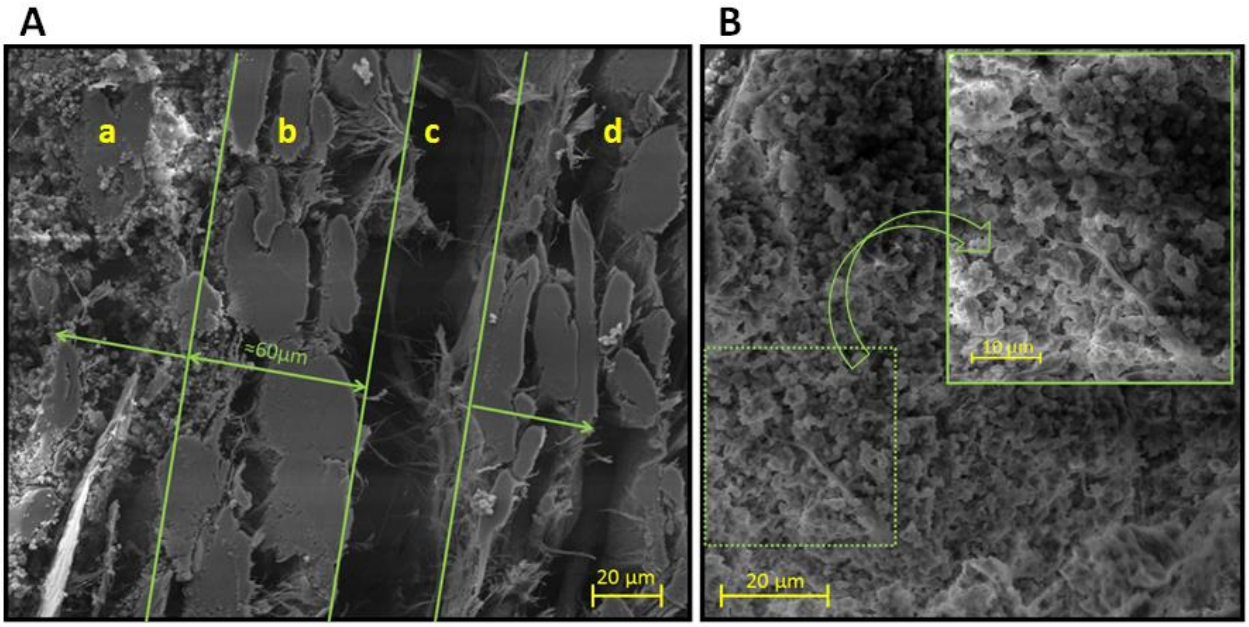


Figure 4-17: Prepared selective filter-paper at the top surface of a laboratory filter-paper was cut from its cross section A) Cross-sectional SEM image of MIP-loaded paper-filter (layer structure) a) Cellulose fibers and MIP particles (small white particles) b) first protecting layer c) Gap between prepared filter and commercial paper filter d) commercial filter paper B) Cross-sectional SEM image of Cellulose fibers and MIP particles (middle active layer of the extraction disk 'a) in A)')

Microscopic images of the final extraction disk confirm the homogenous distribution of the MIP particles in the composite layer. Figure 4-17A exhibits the layered structure of the filter disk which is still mounted on the commercial filter paper (here are already slightly separated from the new disk 'gap in sector c'). This gap is probably produced by cross cutting the filter for microscopic recording. That shows that the commercial filter paper which served as a holder for building up the disk layers that can be easily removed from the new MIP paper disk. In figure 4-17B, the focused-view on the composite layer containing cellulose fibers and MIP particles, represents the high porosity and surface area available for extraction process.

In order to produce an integrated filter-paper which can tolerate and support high enough flow-rates, the amount of cellulose fibers in the protecting layers and also cellulose fibers/MIP particles ratio in the middle layer were optimized. For 200 to 400 mg of MIP particles, about 73

mg cellulose fibers for each two protecting layers (located at both outer surfaces of the final product and are shown as 4 and 6 in figure 4-15) and about 147 mg cellulose fibers for the extracting composite layer (located in the middle of the final product and is shown as 5 in figure 4-15) were sufficient to attain a stable uniform selective extraction disk.

The density of the produced paper-filters containing 200 mg and 400 mg of polymer particles were 397 and 402 gr m^{-3}, respectively. Developed filter-papers were less dense than the commercial laboratory filter-paper used to produce the paper pulp which was about 484 gr m^{-3}. The thickness of the commercial filter-paper was 0.37± 0.03, of filter-paper without polymer particles: 0.30±0.04, of filter-paper with 200 mg non-imprinted polymer (NIP) particles: 0.54±0.02, of filter-paper with 200 mg MIP particles: 0.61±0.03 and filter-paper with 400 mg MIP particles was 0.89±0.06 mm. Our developed filter-papers were highly stable towards the acidic (pH 2) and alkaline conditions (pH 9) which were used during elution and activation steps.

The loss of polymer particles and/or cellulose fibers from the filter-paper after a series of extractions was checked by weighting. The filter paper lost less than 2% of its total weight after 15 times extraction/reactivation cycles.

It has been reported [87] that the percent swelling degree (%Sd) of the developed filter-papers can be calculated by measuring the percent weight-changes of the filter-papers immersed in a desired solvent. Filter-paper containing 200 mg MIP particles had Sd_{Water}: 229±4.3, $Sd_{Methanol}$: 213±5.8 and the one containing 200 mg NIP particles had $\%Sd_{Water}$: 250±7.4, $\%Sd_{Methanol}$: 271±5.3. The percent weight-changes for the commercial whatman filter-paper were Sd_{Water}: 190±4.7, $Sd_{Methanol}$: 125±6.8. The results indicated the prepared filter papers possess a higher hydrophilicity and/or porosity than the commercial Whatman filter paper.

4.4.2. Selective paper-based solid-phase disk extraction

Using the preparation procedure proposed above, selective filter-papers can be easily prepared in different geometries and sizes. We adjusted our circular-disk diameter to 47 mm in order to use it with a manual extraction device which is suitable for field application (Figure 4-18).

Figure 4-18: Filed-applicable devices which can be used in combination with developed selective filter paper disk

The selective disk was assembled in the desired disk-holder and conditioned with 10 mL of methanol and 10 mL of distilled water to wet the polymer completely. Afterwards, 100 mL of distilled water samples containing 25, 50, 100, 200, 1000 and 5000 ng of acesulfame potassium were passed through at 10 mL min^{-1}. The loaded disks were washed with methanol and then eluted with methanol/NH4OH 25% (80:20, v/v). In order to clean and reactivate the used selective disks for further analysis [134], 10 mL of methanol and 10 mL methanol/acetic acid (80:20, v/v) were passed through the filter. The reactivated filter-paper was dried using the inert gas stream and stored in a desiccator till next extraction. The whole sample preparation procedure took about 30 min per sample including conditioning, sampling, drying, washing, elution and activation. At low sample flow-rate (10 mL min^{-1}), the mean of the total recoveries was %99±8 indicating a high capacity for acesulfame. In comparison with conventional SPE format (due to high back-pressure during sample load, elution and activation steps, total sample preparation of 50 mL water sample using MIP loaded SPE cartridge, took about 200 min which is unattractive for high-throughput workflows), obtained preliminary results proved the applicability of proposed method for field application.

Allowing high sample flow-rates without any loss of analytes during the extraction process is increasingly important when large series of samples have to be prepared. Particularly, onsite sampling and sample preparation require simple, fast, and robust methods. In order to evaluate the influence of the sample flow-rate on the extraction efficiency, 100 mL of distilled water containing 100 ng of acesulfame-K was loaded to a filter paper (contained 200 mg of MIP particles) at 10 and 30 mL min^{-1}. Increasing the sample flow-rate caused the total recovery reduction of just 97% to 83%.

In addition to the sample flow-rate which can restrict the reliable extraction of large sample volumes due to long extraction times, a low breakthrough volume (BV) can also restrain it, too. BV is a mutual characteristic of the adsorbent and target compound and is defined as the maximum amount of sample volume from which a target chemical compound can be extracted by a recovery of 100% [22]. Negatively charged acesulfame is highly water-soluble and very hydrophilic at a broad range of pH values. Twelve different paper filters were prepared using MIP particles synthesized in 7 various batches using the same procedure. 25, 50, 100, 200, 1000, 2500 and 5000 ng of acesulfame potassium were dissolved in 100, 200, 500 and 1000 mL of water-samples and extracted. Obtained results are shown in figure 4-19.

Using the suggested selective filter paper, the extraction time of larger sample-volumes (max. 1 L), never exceeded than 35 min. However, for the sample volumes larger than 200 mL, the recoveries were clearly reduced below 50%. Increasing the amount of MIP particles in the filter disk from 200 mg to 400 mg can compensate this effect (e.g. the percent recovery obtained for 500 mL water sample was increased from about 50% to more than 80%).

As shown in figure 4-19, the possibility of increasing the embedded MIP particles can help to compensate this recovery reduction for higher flow-rates and larger volumes, when is needed.

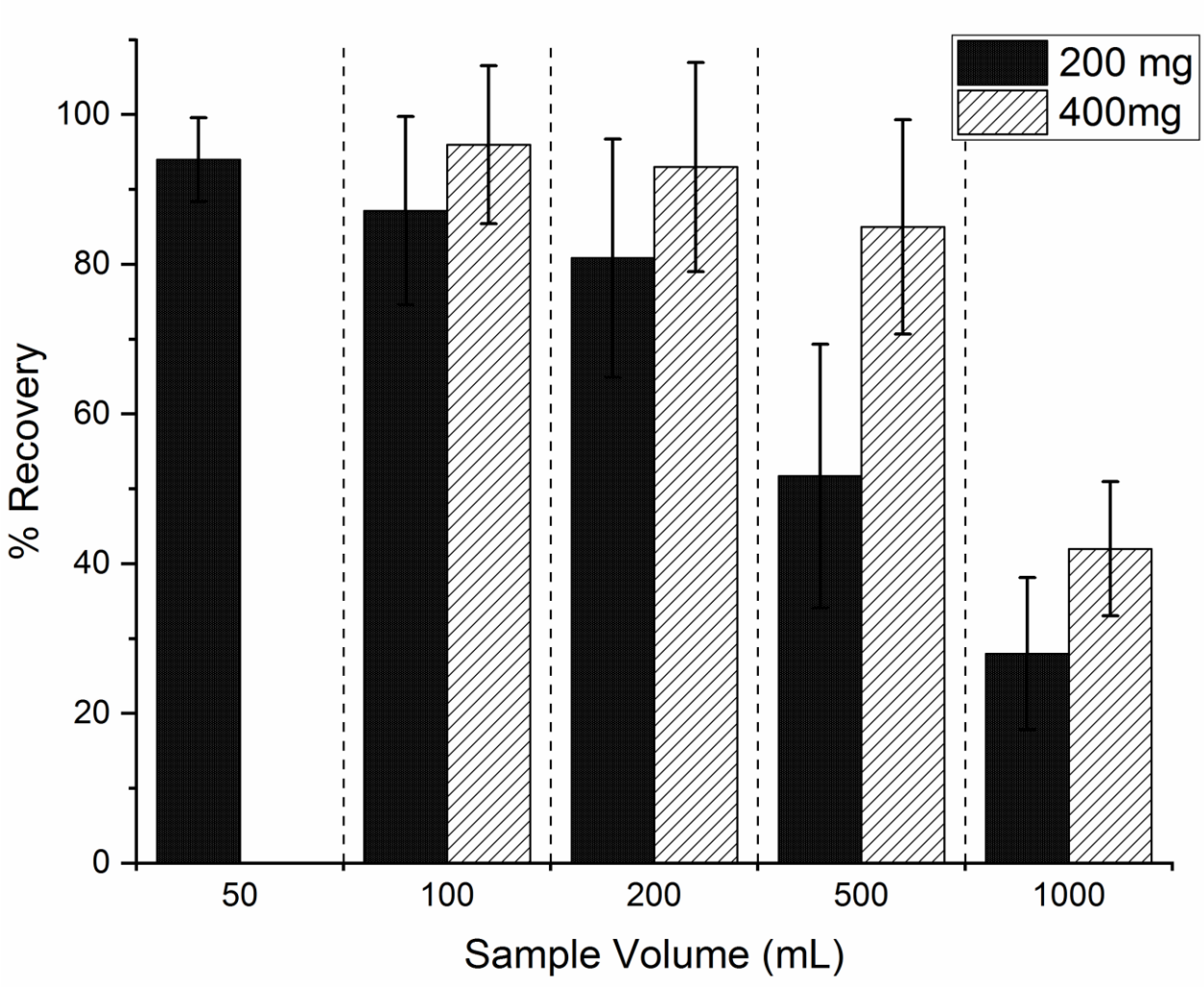


Figure 4-19: The effect of different sample-volumes on the extraction of 25, 50, 100, 200, 1000, 2500 and 5000 ng of acesulfame potassium with selective filter-papers

Contrary to the traditional SPE format, the new filter-paper disk allowed the efficient extraction of sample volumes between 100 and 200 mL within 3 min and 7 min, respectively, and achieved recoveries ranged from 81% to 96% in dependence on the MIP amount incorporated in the extraction disk.

In field sampling campaigns, the temperature of water samples can vary between groundwater and surface water and might influence the extraction results. To check the temperature effect on the extraction yields, 100 ng of different chemical compounds including acesulfame potassium, saccharin, ibuprofen sodium salt, atrazine and caffeine were dissolved in 100 mL distilled water. The temperature of water samples was adjusted at 4°C, 25°C and 35°C and were extracted by selective filter papers containing 200 mg acesulfame imprinted polymer. The total

recovery for all evaluated chemicals remained quite stable with relative standard deviations ≤ 5% for the whole set of extractions performed at different temperatures. Thus, a significant influence of the water temperature was not observed.

We have discussed already [16, 134], that imprinted cavities in the polymer matrix can be affected by milieu variations. In MISPE, a washing process after sample loading is an important step to emerge the affinity of the implemented sorbents. Due to the high affinity of MIP sorbents towards the imprinted target molecules, co-extracted non-target molecules (e.g. matrix compounds) which are less tightly adsorbed due to non-specific interaction with the polymer can be removed during the washing process with proper solvents. Before washing, the polymer sorbent needs to be dried completely to avoid interferences with residual water remained from sample loading. As a result of the higher surface area of the prepared filter-papers, the drying process took less time (≈10 min) compared to its relative SPE cartridges (≈45 min). After that, 10 mL of methanol was used as the washing solvent which was evaporated and reconstituted with the elution solvent for further HPLC-MS-MS analysis.

For the complete elution of the retained acesulfame ions, our previous experiments indicated that 10 mL of methanol/NH4OH 25% (99.5:0.5, v/v) is needed for 200 mg MIP particles in an 8 mL SPE cartridge. In MISPE format, we experienced that the increasing the amount of ammonia caused higher back pressure during the elution step which resulted in a very low flow-rate. On the other hand, reduction of ammonia concentration, reduced the cleaning efficiency of the elution process and therefore, we forced to use more elute volumes. Using the new filter-paper format, the elution step could be accelerated and an increase of ammonia content was not an obstacle. Therefore, two successive fractions of 6 mL methanol/NH4OH 25% (80:20, v/v) were used as the elution steps. The results indicated that the adsorbed chemical targets were washed out completely during the first washing step. Finally, 6 mL methanol/NH4OH 25% (80:20, v/v) was found to be the best for complete elution of the acesulfame ions.

Figure 4-20 shows that special imprinted cavity together with ammonium functional groups (from (vinylbenzyl)trimethylammonium functional monomer) placed in the backbone of the synthesized polymer play an important role towards the adsorption of anionic compounds such as acesulfame. Furthermore, cross selectivity for the extraction of compounds with negatively

charged sulfonamide and carboxyl functional groups (saccharin and ibuprofen) were observed while substances with quite different functionalities such as caffeine and atrazine were not retained by MIP-loaded filter-paper. Although the non-imprinted polymer (NIP, Figure 4-20) is able to interact in non-selective mode with atrazine and caffeine, these analytes can be easily washed out during washing process from the sorbent and were not analyzed anymore in the MIP eluates. This effect had been verified for caffeine using a SPE cartridge filled with 200 mg of acesulfame imprinted polymer [134]. In case of ibuprofen, a mixed mode of specific and non-specific adsorption leads to a noticeable retention on the MIP paper disk. It has to be emphasized that the NIP-loaded filter paper shows very low or even no affinity towards acesulfame and saccharin suggesting that specific interaction with the imprinted centers of polymer are needed to catch these negatively-charged molecules from water.

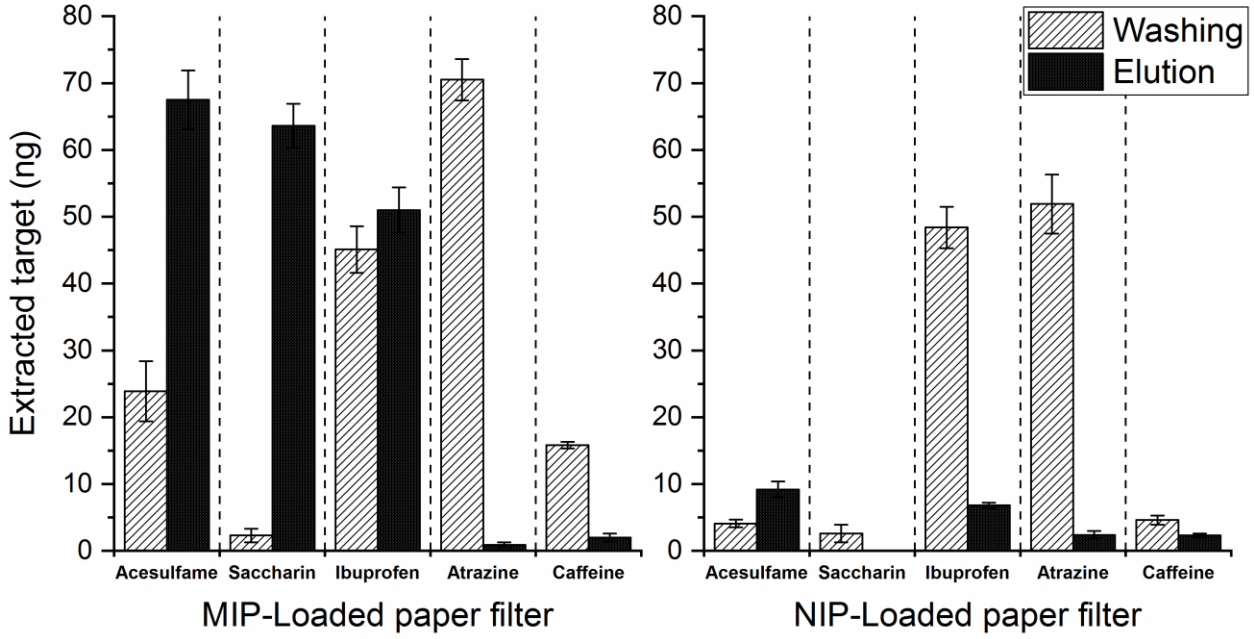


Figure 4-20: Filter-papers performance containing 200 mg MIP and NIP polymer particles for the extraction of the 100 ng of each compound from 100 mL water sample

4.4.3. Analytical evaluation and real samples

Acesulfame potassium solution in distilled water at a concentration range of 0.1-2.5 µg L^{-1} were prepared and analyzed using the developed selective paper-HPLC/MS-MS method. The calibration was linear with coefficient of determination of R^2=0.9985 and relative standard

deviations were lower than 6.8% (n=5). The limit of detection (LOD) and the limit of quantification (LOQ) at signal-to-noise ratios of 3 and10 were calculated at 0.015 µg L^{-1} and 0.05 µg L^{-1}, respectively. Along with samples, the instrument was calibrated with standard solutions to compensate the instrumental signal variability.

The developed method was implemented for the determination of the urban wastewater marker acesulfame in the lake Auensee (Leipzig, Germany) and in river waters Neue Luppe and White Elster (Leipzig, Germany). Figure 4-21 shows the map of sampling sites in which the WWTP represents the central wastewater treatment plant of Leipzig (Germany, 500.000 inhabitants).

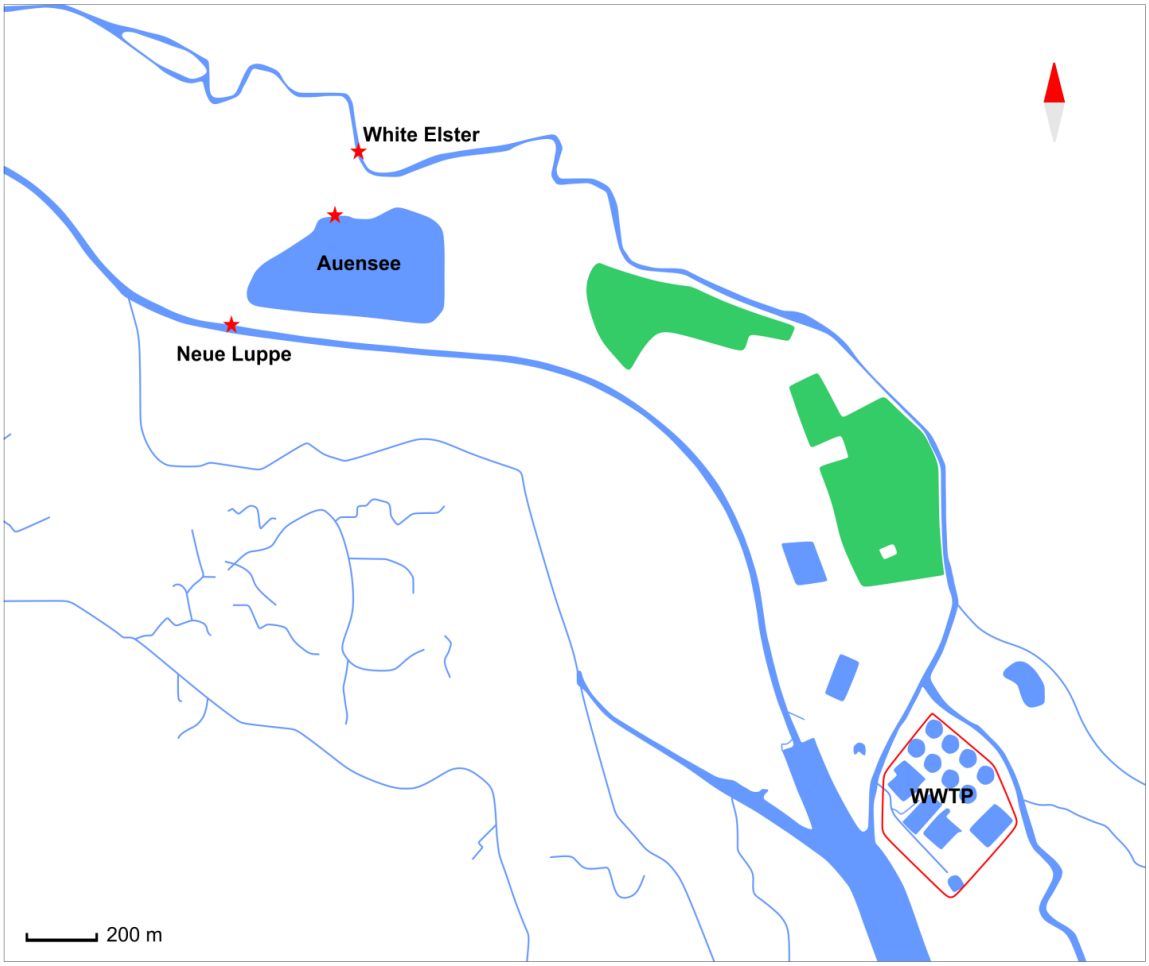


Figure 4-21: Map of the sampling sites marked with red ★at River White Elster, lake "Auensee" and River New Luppe. WWTP represents the central wastewater treatment plant of Leipzig (Germany, 500 000 inhabitants).

The research task behind the sampling site selection was identify the influence of infiltration of wastewater receiving river water into the lake “Auensee” which has a nutrient-rich water body and suffers regularly from cyanobacteria bloom. As an isolated catchment (former gravel and sand mining), it is not connected with any other surface water, thus, a permanent nutrient input via river water can be excluded. Using acesulfame as ideally accepted wastewater tracer, at least the infiltration of wastewater-loaded groundwater could be identified.

In order to detect acesulfame also at traces, an enrichment of water samples with the new selective paper disks was performed. The samples were taken in triplicate from the lake as well as from the rivers which are about 500 m away. Obtained results (Figure 4-22) showed that an infiltration of pollutants and nutrients via groundwater from the adjacent river waters into lake “Auensee” can be assumed but needs more verification.

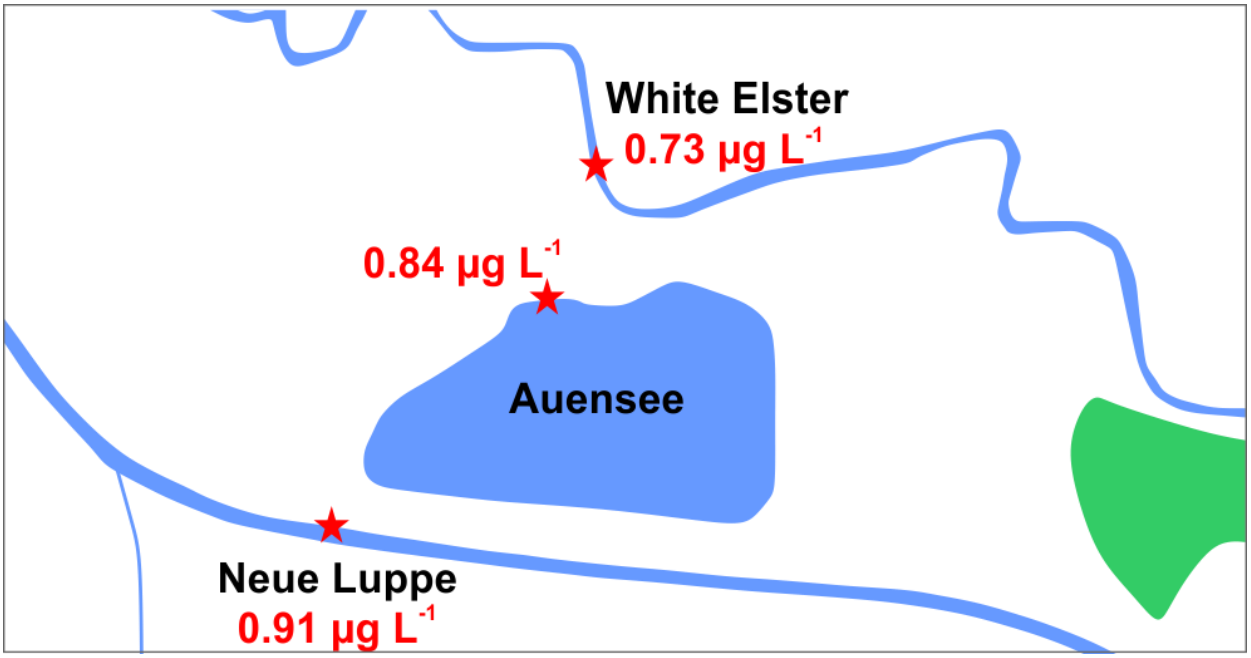


Figure 4-22: Detected acesulfame in both rivers and enclosed lake

In order to check the stability of the MIP disks loaded in field, one set of the samples were extracted directly at lake “Auensee” and stored in desiccator at 4° C. The corresponding elution step was done after four weeks from sampling and the results showed that the acesulfame concentration was in the concentration range of already measured samples (83±7.9 ng in 100

mL of water sample). However, for less persistent target substances than acesulfame, the stability of the loaded compound during transport and storage has to be proved.

In order to evaluate the matrix effects, water samples from lake "Auensee" were spiked before and after extraction with acesulfame potassium, atrazine, saccharin, ibuprofen sodium salt, naproxen sodium salt and caffeine, where the concentration of each solute was 1 µg L^{-1}. Due to the enclosed area, the water from the lake is expected to have the most severe matrix affecting the sample-preparation and detection procedures. Equations 4-1 and 4-4 were used to calculate the 'matrix effect percentage' (% ME) and the 'percent recovery accounting exclusively from the sample preparation' (% RSPE). As it is mentioned before, ammonium functional groups in the MIP materials are assumed to play an important role towards the adsorption of chemical compounds containing negatively charged groups which are often available also in environmental matrices like humic acids, amino acids, carbohydrates and fatty acids. The obtained results in table 4-9 indicated that the co-extracted matrix compounds affected the ionization process for all analyzed chemical compounds. Nevertheless, the ammonia functional groups inside the recognition sites within the MIP material were not blocked by the sample matrix and the chemicals containing sulfonamide (acesulfame and saccharin) and carboxyl (naproxen) groups showed the best recoveries. Using the same procedure, matrix effect of the two above mentioned rivers were evaluated for acesulfame and no signal reduction due to the matrix effects were observed.

Table 4-9: The matrix effect evaluation on the ionization process in HPLC-MS/MS and filter paper recovery.

	Evaluation of matrix effects					
	Naproxen	Coffeine	Atrazin	Acesulfame	Ibuprofen	Saccharin
%ME	75	115	71	91	68	75
$\%R_{(SPE)}$	80	12	35	85	55	82

4.5. Developed sample-preparation methods in summary

Figure 4-23 shows hierarchically the sample-preparation methods which developed during this study. As it shown, we started with SPME method which is known to have the inherent potential for in-filed sample-preparation applications.

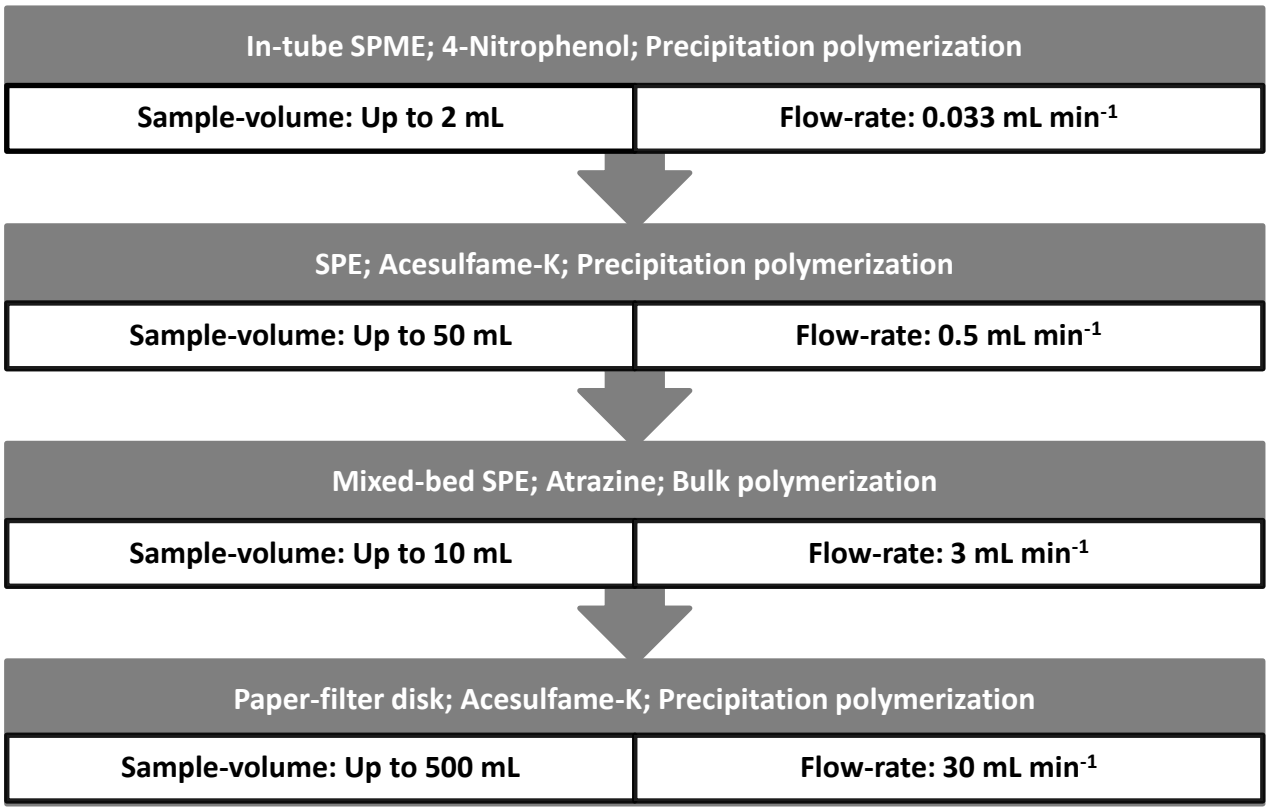


Figure 4-23: A schematic which represent hierarchically developed sample-preparation methods during this study.

The extraction method was optimized for 4-nitrophenol, the known priority pollutant. The relative MIP materials were synthesized using precipitation polymerization method, but in a capillary format. However, the optimized values for the sample-volume and flow-rate could not be used for fast sampling of relatively large volumes of water samples which are necessary factors for in-filed sample-preparation of environmental water samples. Therefore, in the next step, SPME was replaced with traditional SPE method. The new synthesized imprinted polymer materials for acesulfame potassium, the accepted anthropogenic marker, were used to pack a SPE cartridge. Due to high amount of used polymers, we could use sample volumes up to 50 mL. However, the flow-rate was restricted to low values due to swelling effect and reduced size of

synthesized polymer particles. To produce micro-channels within the packed bed of SPE cartridge, a new SPE format was developed and termed mixed-bed SPE. In this method, swellable and irregular MIP particles were mixed with regular and rigid sorbent materials. Promising results obtained when the developed mixed-bed SPE was compared with its relative SPE cartridge. However, the optimized procedure proposed to use the reduced mass of MIP particles (≈10 mg). In the final step and using the same ideology, 200-400 mg of swellable nano-size acesulfame imprinted polymer was mixed with cellulose fibers to produce selective filter-paper disks. As can be seen from the values, this method can be used for in-field sample-preparation of water samples. Furthermore, the proposed method for the preparation of selective filter-papers was simple and cost-effective. The whole equipment for in-field sample-preparation using developed selective filter-paper disks is shown in figure 4-24.

Figure 4-24: Complete equipment for field sampling using developed selective filter-paper disks

During evaluation and optimization of the selective disks, treated samples were measured using HPLC/MS-MS instrument. Despite the overall outstanding performance of the HPLC/MS-MS instrument, it is an expensive and laboratory-based large analytical instrument which needs skilled personnel. In the next step, we tried to combine our developed fast sample-preparation method with a relatively cost-effective and portable detector. Therefore, we developed an ion mobility spectrometry (IMS) instrument which is a transportable detector and can be used for high speed on-site measurements [186].

5. Ion mobility development

As mentioned in the introduction section (1.5.), ion mobility spectrometry (IMS) is operated at atmospheric pressure, the results are available within seconds and different substances can be separated. Coupling the electrospray ionization (ESI) to IMS enabled this potentially portable detector for widespread measuring of the liquid samples. Designed ESI-IMS is already described in details in section '2.2.4.' and is shown in figure 5-1.

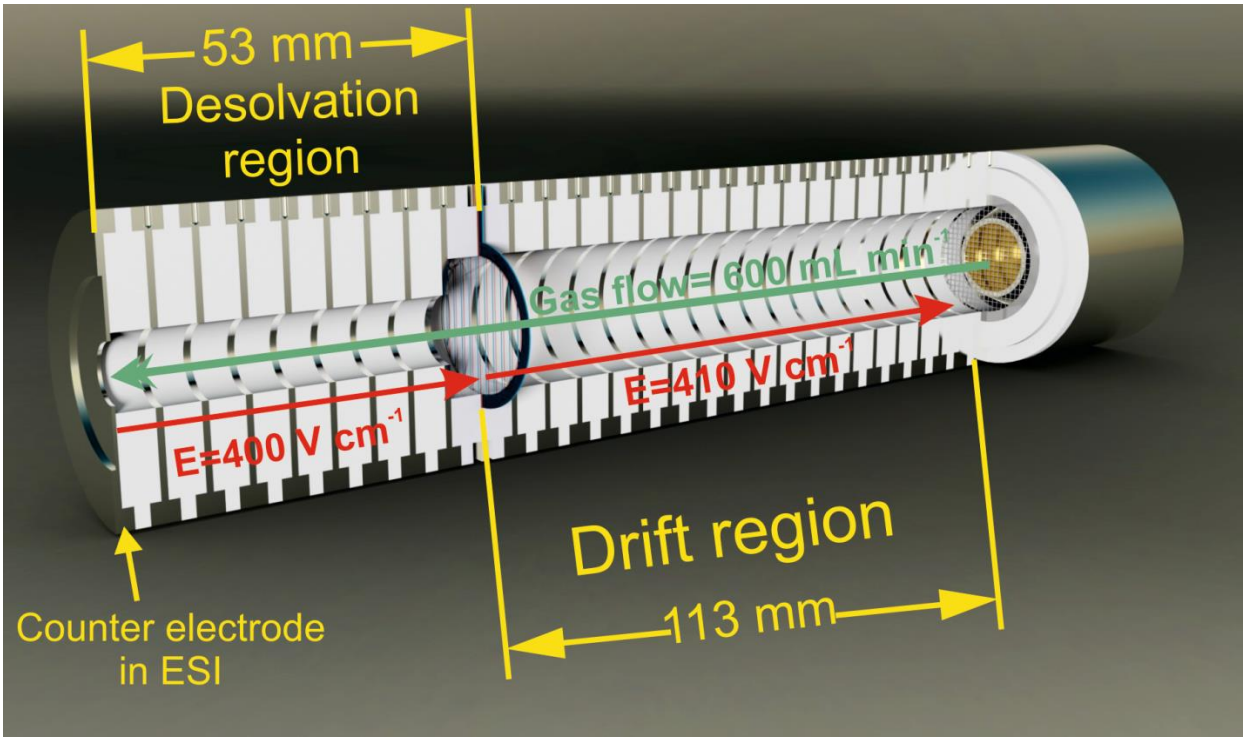


Figure 5-1: Ion mobility spectrometry instrument which was designed to couple to electrospray ionization as ion source. Electric fields in desolvation and drift regions are 400 and 410 V cm^{-1}, respectively. Nitrogen is used as drift gas with flow-rate of 600 mL min^{-1}.

An electrospray ionization source was positioned in front and to the center of the first ring of desolvation region which acts also as counter electrode in ESI. The used ESI source is shown in figure 5-2. The emitter was a fine coned silica tip which was adjusted approximately 2 mm in front of counter electrode. For the designed ESI-IMS, obtained results showed that a 2.3 kV differential potential, between emitter and counter electrode, could produce a stable electrically generated spray.

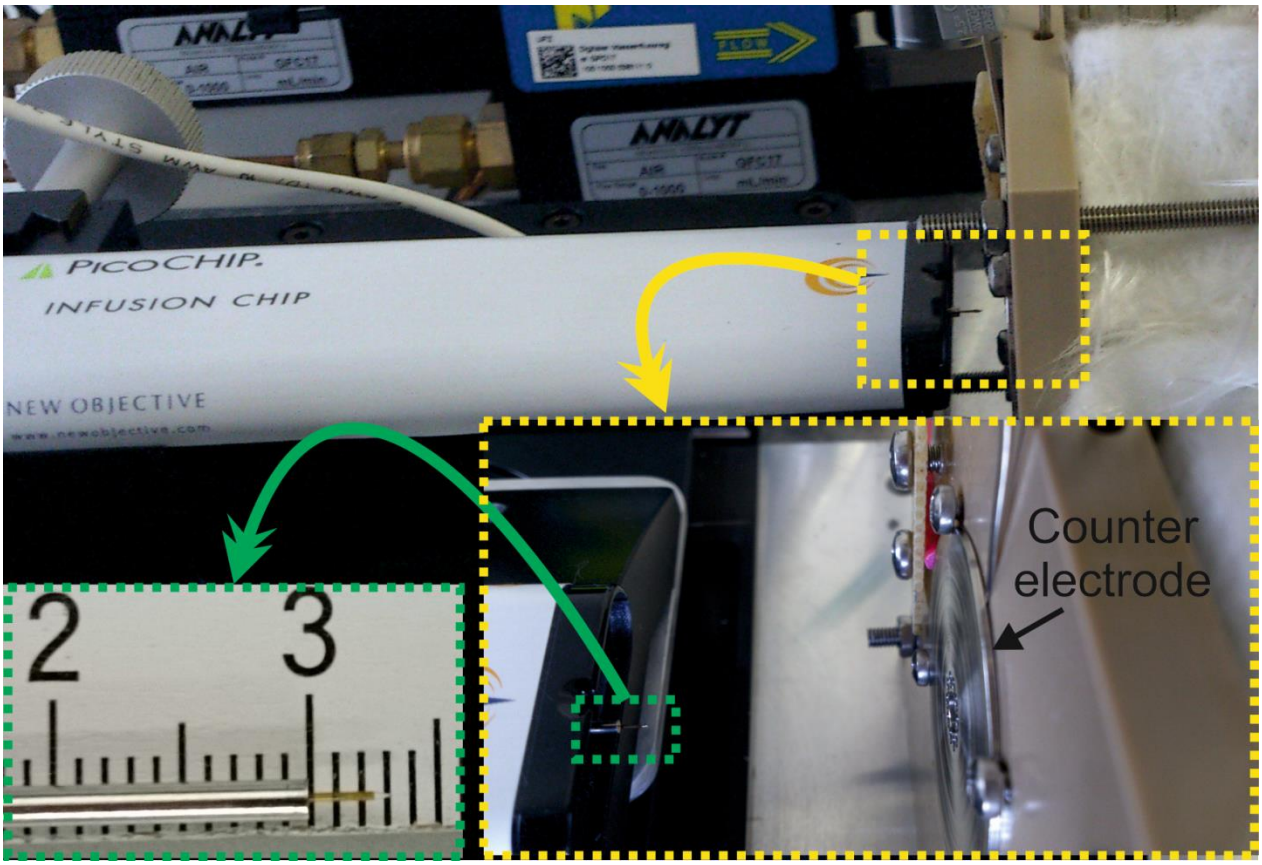


Figure 5-2: Electrospray ionization source combined with designed IMS. Inset pictures magnify the silica tip emitter and counter electrode which is also the first ring of desolvation region.

Due to the fact that mass spectrometry (MS) instruments work in vacuum, produced ions in most ESI-MS instruments are transferred using pinholes or capillaries with a diameter <500 µm as orifice to MS reducing the transmission of the ion plume. In contrary to MS, the IMS works at atmospheric pressure and a transition from atmospheric pressure to the first vacuum stage is not necessary. Therefore, the inner diameter of the transfer stage (counter electrode) is between 0.5 and 1 cm and nearly 100% of the ion plume is therefore transferred into the ion mobility spectrometer [132]. However, desolvation is not effectively completed before the droplets enter the drift tube [187]. While in a MS, the residues of solvent can be readily evaporated under vacuum conditions, an additional desolvation region is integrated into IMS before the ions enter the drift tube.

Based on these considerations, a 1.0 cm entrance electrode (which act as counter electrode in ESI) without additional grid inside was used to obtain the best ion transmission efficiency and highest signal intensities (Figure 5-1). By reducing the inner diameter of the desolvation region

from 2 cm (drift region) to 1 cm, and therefore increasing the velocity of the preheated gas, complete desolvation of the ions was achieved which lead to stable and reproducible results. The aim of this instrumental development was to combine it as detector with the previously developed selective filter-paper sample-preparation method. As aceulfame imprinted polymer materials were used to develop the filter-paper disks, ESI-IMS was optimized for anthropogenic marker acesulfame. Due to the fact that artificial sweetener acesulfame can be detected in negative mod of ESI-IMS, our detector was adjusted in negative mode. As there are relatively few examples for negative ESI-IMS in the literature, it is therefore important to understand the optimal values of the operational and instrumental parameters.

In the first step, standard solutions for acesulfame, saccharin, bentazon, ibuprofen, naproxen, atrazine and caffeine was prepared and analyzed using ESI-IMS which operated in negative mode. Besides, a standard mixture solution contained all mentioned targets was prepared and analyzed with ESI-IMS with the same operational conditions. Obtained spectrums for the pure targets are shown in figure 5-3 (A-G) and figure 5-3 (H) represents the obtained signal for the mixture solution. The spectra obtained from the same sample on different days showed comparable intensities suggesting a long-term stable response. As can be seen in figure 5-3 (A-C) and also in figure 5-3 (H), three sharp and symmetric peaks can be seen for acesulfame, saccharin and bentazon. Figure 5-3 (D-E) shows that ibuprofen and naproxen could be detected with small and relatively asymmetric peaks. In the obtained spectrum for mixture solution, these two targets can be hardly detected with two overlapped small peaks. However, as shown in the spectrums of figure 5-3 (F-G), no peaks were detected for atrazine and caffeine suggesting that these two targets cannot be detected in negative mode ESI-IMS.

The obtained spectrums in figure 5-3 can be explained using the known basic of ESI described already in section ‘1.5.1.’. To produce ions using ESI, the target molecules should be first ionized inside the liquid phase (A^+B^-). In negative mode, A^+ will be electrochemically reduced ($A^+ + e \rightarrow A$) and its negative counter B^- ion will be released to produce final negative ions in an ESI procedure. As can be seen from the molecular structure of acesulfame, saccharin and bentazon in figure 5-3, the sulfonyl group can significantly stabilize the produced negative charge on the neighboring amine group.

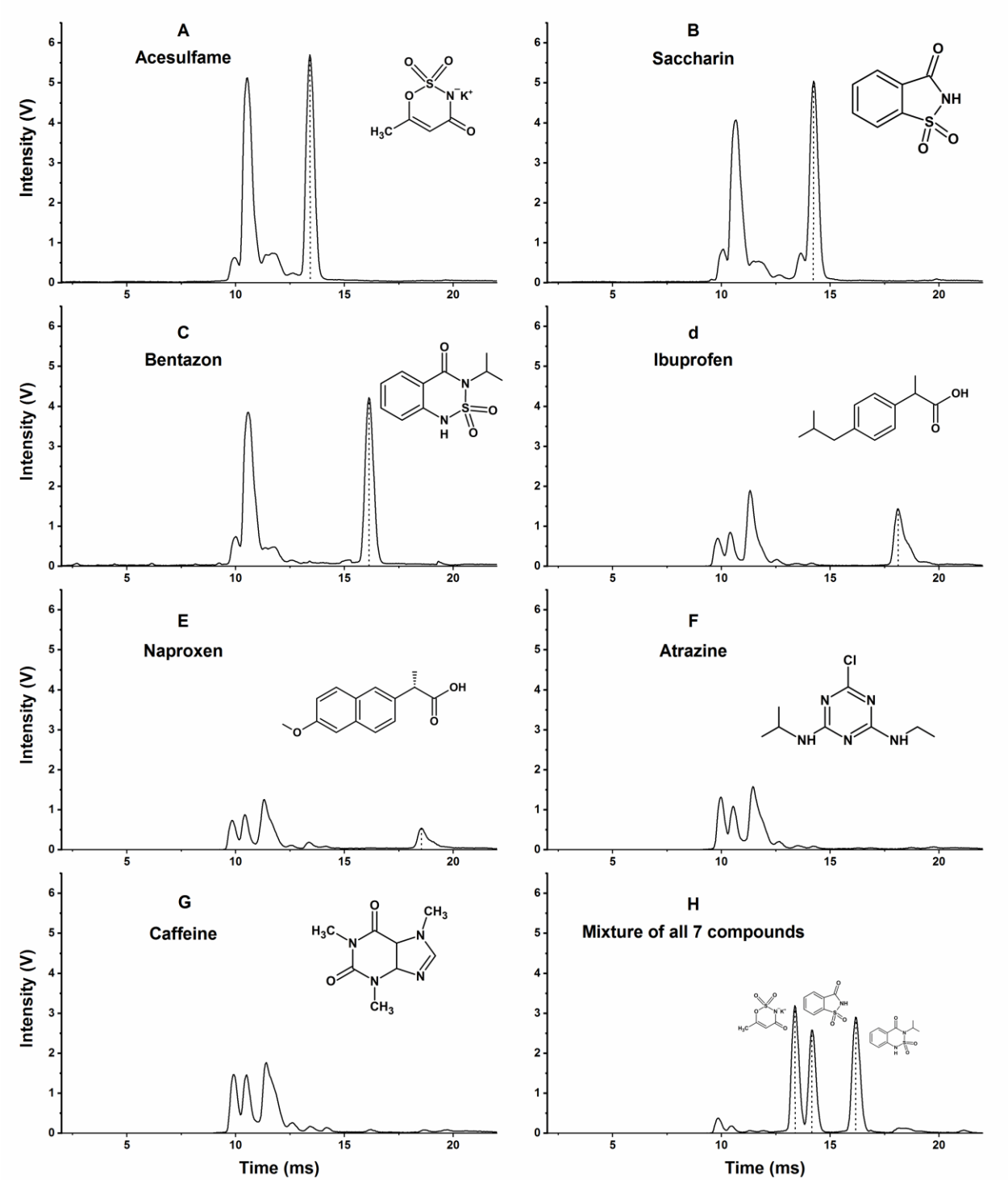


Figure 5-3: A-G shows the obtained ESI-IMS spectrums for acesulfame, saccharin, bentazon, ibuprofen, naproxen, atrazine and caffeine and H shows the spectrum for a mixture of all at 10 ng µL^{-1}.

Therefore, the B^- can be easily produced in the liquid phase which accordingly can produce abundance ions in gas phase. Carboxylic acid group in both ibuprofen and naproxen is a weak

acid which could support the production of B^- in liquid phase but weaker than those other three targets containing sulfonamide group. Atrazine and caffeine have basic character to produce A^+ and therefore can be detected in positive mode ESI. After obtaining results in the first step of our experiments; acesulfame, Saccharin and Bentazon were selected for further optimization of operational parameters.

It has been shown [188] that for the determination of small organic molecules with negative ESI indicated, methanol provides higher responses when it is compared to pure water or acetonitrile. Due to the high water solubility of our target analytes, we added increasing amounts of water to methanol. The influence of these solvent compositions is shown in figure 5-4.

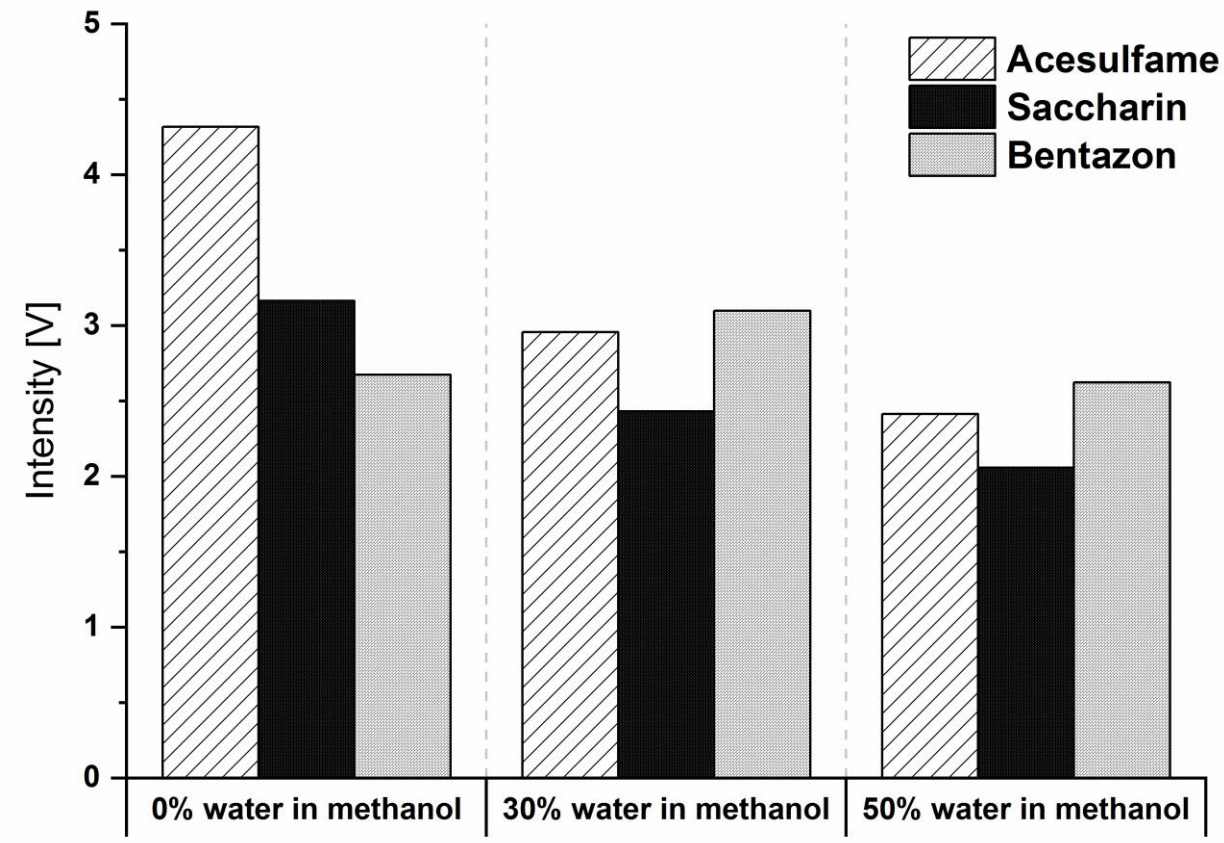


Figure 5-4: Influence of solvent composition on signal intensity (measurements with 10 ng µL^{-1} per substance)

In contrast to literature, where a general decrease in signal intensities was observed with increasing water content of methanol [189], our results vary depending on the properties of analytes. The addition of water to the methanol causes a clear decrease in response for

acesulfame. The same effect can be observed for Saccharin. However, the decrease in signal intensities is not as significant as detected for acesulfame. The response of Bentazon is less affected by the water content and the signal intensities are nearly comparable. These results show that the ionization efficiency depends on both the properties of analytes and the experimental conditions. For further optimization of operational parameters, we used pure methanol as solvent due to the highest signal response for acesulfame.

The inner diameter and the tip size of spray emitter are important hardware factors for controlling the ESI flow rate. Figure 5-5A shows the comparison between two emitters (a 15 µm tip size and a flow rate of 20 µL h^{-1} and a 30 µm tip size and a flow rate of 60 µL h^{-1}). The results clearly confirm the higher sensitivity of acesulfame signal intensities for the 30 µm emitter tip with higher ESI flow-rate, as described above. Therefore the spray emitter with 30 µm tip dimeter was selected for further optimization.

Using the selected emitter, a standard mixture solution, containing acesulfame, bentazon and saccharin each at 10 ng μL^{-1} in methanol, was analyzed at different ESI flow-rates. Obtained results in figure 5-5B shows that increasing the flow-rates cause to increase slightly the signals for acesulfame and saccharin. However, that decreases obtained signal for bentazon. The maximum intensity for acesulfame, which is our target of interest, was obtained at 60 µL h^{-1}. Therefore, 60 µL h^{-1} was selected as optimized flow-rate for further evaluation.

In the next step, the mixture solution was sprayed at 60 µL h^{-1} using the 30 µm emitter tip and the temperature of desolvation region and drift region was changed as shown in figure 5-5C. The same optimum at 55°C was found which is also reported in recent ion mobility measurements in positive mode [133] with ESI operating at atmospheric pressure and methanol as solvent. The microspray ESI needle in our set-up is positioned only few millimeters from the first drift ring, out of which the heated gas flows. While higher temperatures lead to improved desolvation, temperatures near the boiling point of methanol (64.8°C) and higher can cause an instable spray with decreasing intensities [128]. Therefore, the temperature of ion mobility spectrometer including desolvation region was adjusted to 55°C for further optimization and measurements.

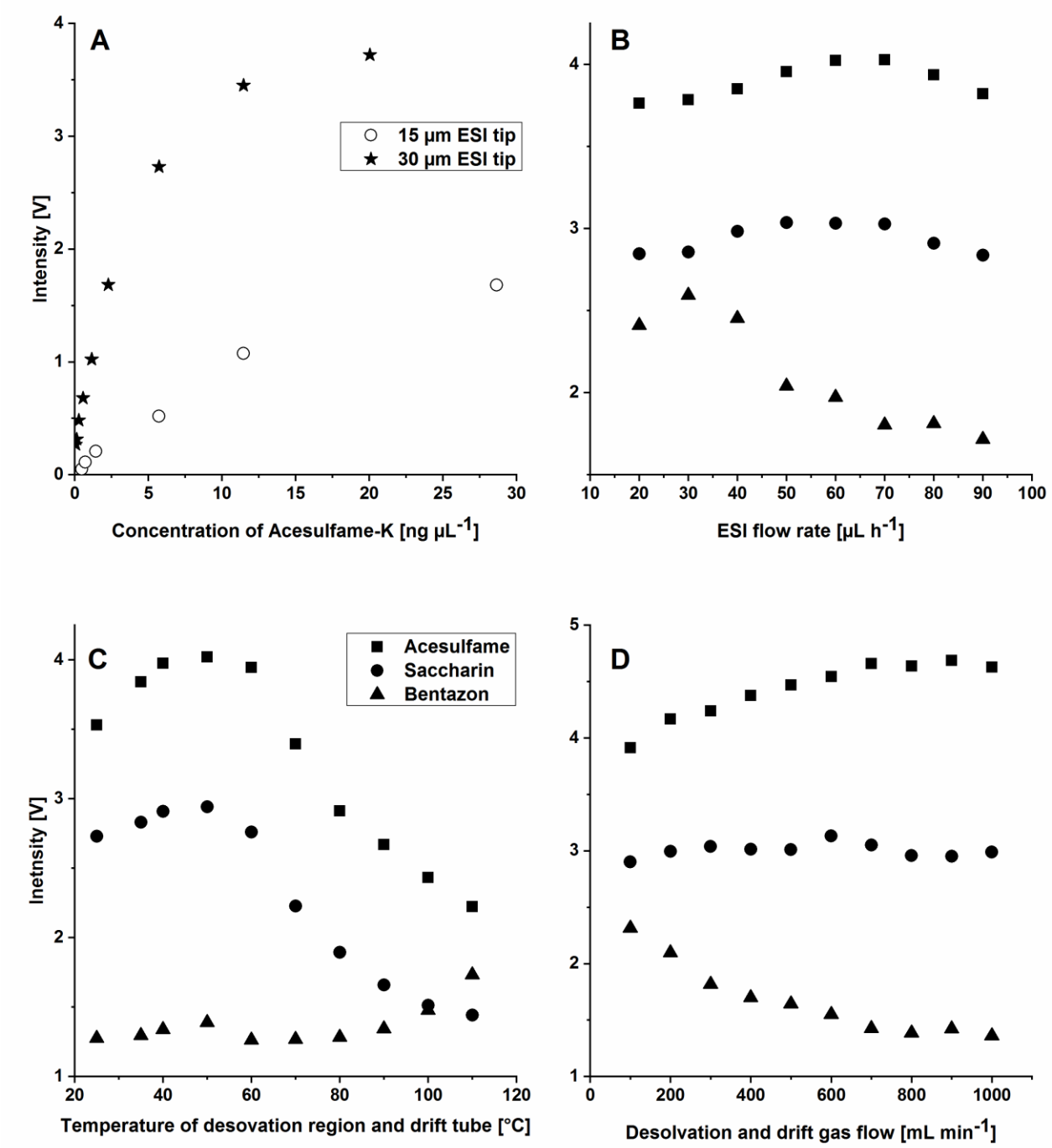


Figure 5-5: Influence of experimental conditions on signal intensity, A: inner diameter of emitter tip, B: solvent flow-rate (30 µm emitter), C: temperature of ion mobility spectrometer (30 µm emitter, 60 µL min^{-1}), D: gas flow through the ion mobility spectrometer (30 µm emitter, 60 µL min^{-1}, 55°C). The concentrations in B, C, and D were approximately 10 ng µL^{-1} per substance)

The influence of drift gas flow, which also acts as desolvation gas, is shown in figure 5-5D. An increasing gas flow obviously leads to an improved desolvation for acesulfame and therefore to

enhanced signal intensities. The increasing was leveled off at about 600 ml min^{-1} which was selected as optimum gas flow in our ESI-IMS.

In addition to parameters which influence the signal intensity, different experimental conditions can also affect the drift times for the different ions, in particular the temperature of ion mobility spectrometer and the drift gas flow. These results are summarized in figure 5-6.

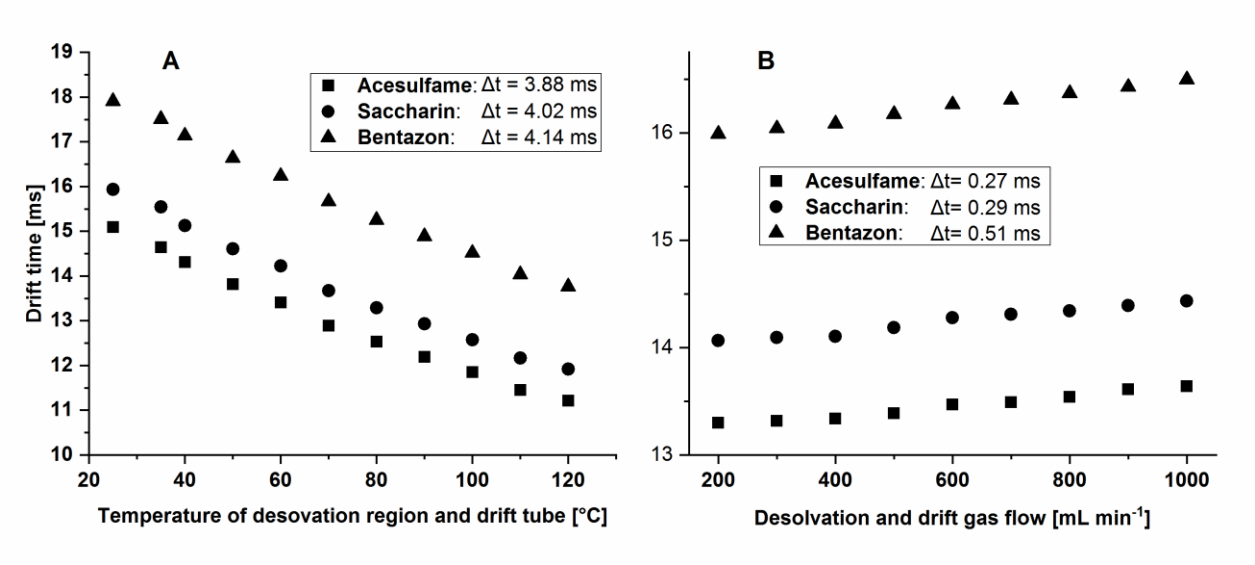


Figure 5-6: Influence of ESI-IMS temperature and gas-flow on drift times. A: temperature of ion mobility spectrometer and B: gas flow through the ion mobility spectrometer. The concentrations were approximately 10 ng μL^{-1} per substance)

The temperature can generally affect ion mobility in different ways. On the one hand, the temperature has influence on the nature of ions formed, on their degree of clustering and in the case of ESI-IMS on desolvation processes. Therefore, changes in temperature can cause changes in the collisional cross section and the ionic mass which are inversely proportional to ion mobility coefficient. Therefore, changes of the temperature can influence the mobility of ions and accordingly their drift times. Due to the combination of these effects, temperature often affects mobility coefficients in a non-linear manner. As can be seen from figure 5-6A, all compounds show negative temperature dependence and have a similar behavior. As the temperature is increased from 25°C to 120°C, the drift times for all substances reduce by approximately 4 ms. This negative, nearly linear temperature dependence indicates that changes in ionic composition or clustering are negligible.

Drift time changes of evaluated targets in dependence with the amount of gas flows through the drift and desolvation regions were evaluated and obtained results are shown in figure 5-6B. In IMS, an enhanced drift gas flow causes a slight increase in the pressure inside the drift tube. This can cause drift times increase linearly with drift gas flow rate due to the higher gas density and the resulting higher collision frequency between ions and drift gas molecules during the transport through the drift tube. However, as can be seen from the results these differences are below one millisecond and much less significant in comparison to the influence of temperature. Therefore, the drift times of all three compounds show a very similar dependence on temperature and drift gas flow. Changes in these parameters have therefore no influence on the peak-to-peak resolution.

In summary, our ESI-IMS was optimized to measure acesulfame with highest sensitivity and obtained results for operational parameters are as followes: 1) ESI-IMS works in negative mode, 2) targets is dissolved in methanol, 3) silica tip emitter with 30 µm tip diameter was selected, 4) sample-flow was adjusted at 60 µl h^{-1}, 5) temperature of desolvation and drift regions were adjusted at 55°C and finally 6) the gas-flow was adjusted at 600 mL min^{-1}.

As mentioned before in sections ‘1.5.1’ and ‘4.2.’, matrix effect is an important factor which affect the ionization process in an ESI. This can cause a negative or even a positive effect on the quantitation measurements with detectors which are equipped with an ESI. Due to the lack of a separation step in our developed ESI-IMS, this factor is even more critical.

Therefore, we evaluated the signal intensity of acesulfame in dependence on the occurrence of different additional trace organic compounds as follows: saccharin, bentazon, ibuprofen, naproxen, caffeine and atrazine. While Figure 5-7A shows the obtained spectrum for standard acesulfame, figure 5-7B shows that the signal intensity for acesulfam in the presence of six other chemicals mentioned above each at 10 ng μL^{-1}. Obtained results show that the signal intensity could be decreased up to 50% when it is measured in the presence of other chemicals. Further evaluation showed that bentazon has the most significant influence on the signal intensity of acesulfame. Figure 5-7C, D shows the reduction of acesulfame signal in the presence of bentazon. In summary, obtained results confirmed that the selective separation of acesulfame from the sample matrix before the analysis using ESI-IMS has a particular importance.

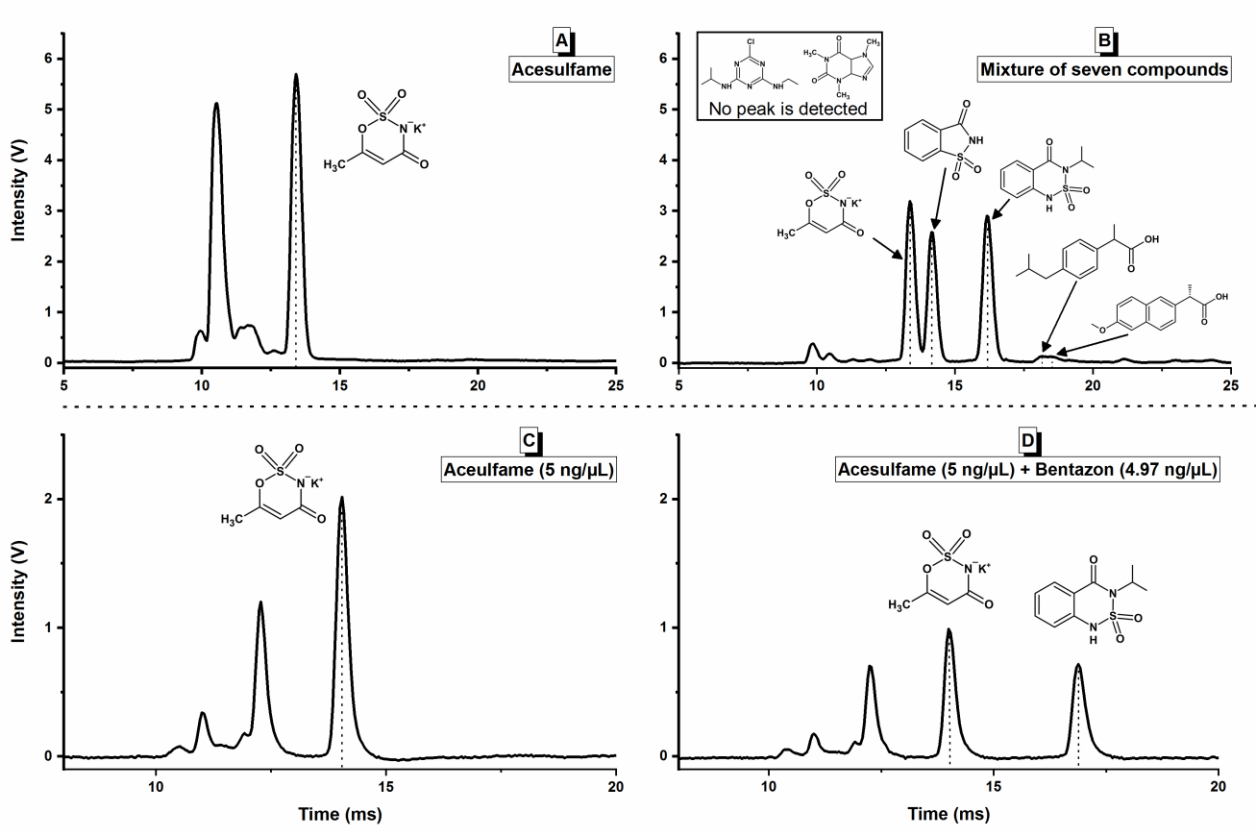


Figure 5-7: Obtained ESI-IMS spectrums for acesulfame alone and in the presence of others chemicals. A and B show acesulfame signal, alone and in the presence of six other chemicals each at 10 ng µL^{-1}, respectively. C and D show acesulfame signal, alone and in the presence of bentazon, respectively.

In addition to the trace organic compounds, natural aquatic systems contain also dissolved organic matter (DOM), a fraction of a broad variety of soluble organic materials with very different physicochemical properties.

The DOM content of natural waters is typically in the range of up to 10 ng µL^{-1}. Using a sample-preparation method, which concentrates the DOM content of a large volume of water sample (e.g. 300 mL water sample), can provide an eluate solvent with the DOM content much more than 10 ng µL^{-1}. Therefore, we evaluated the influence of DOM on the determination of acesulfame using ESI-IMS (Figure 5-8).

For this purpose, soluble humic acid sodium salt was dissolved in methanol in concentrations up to 100 ng µL^{-1}. Furthermore, a constant concentration of acesulfame (0.975 ng µL^{-1}) was added to each methanolic DOM sample.

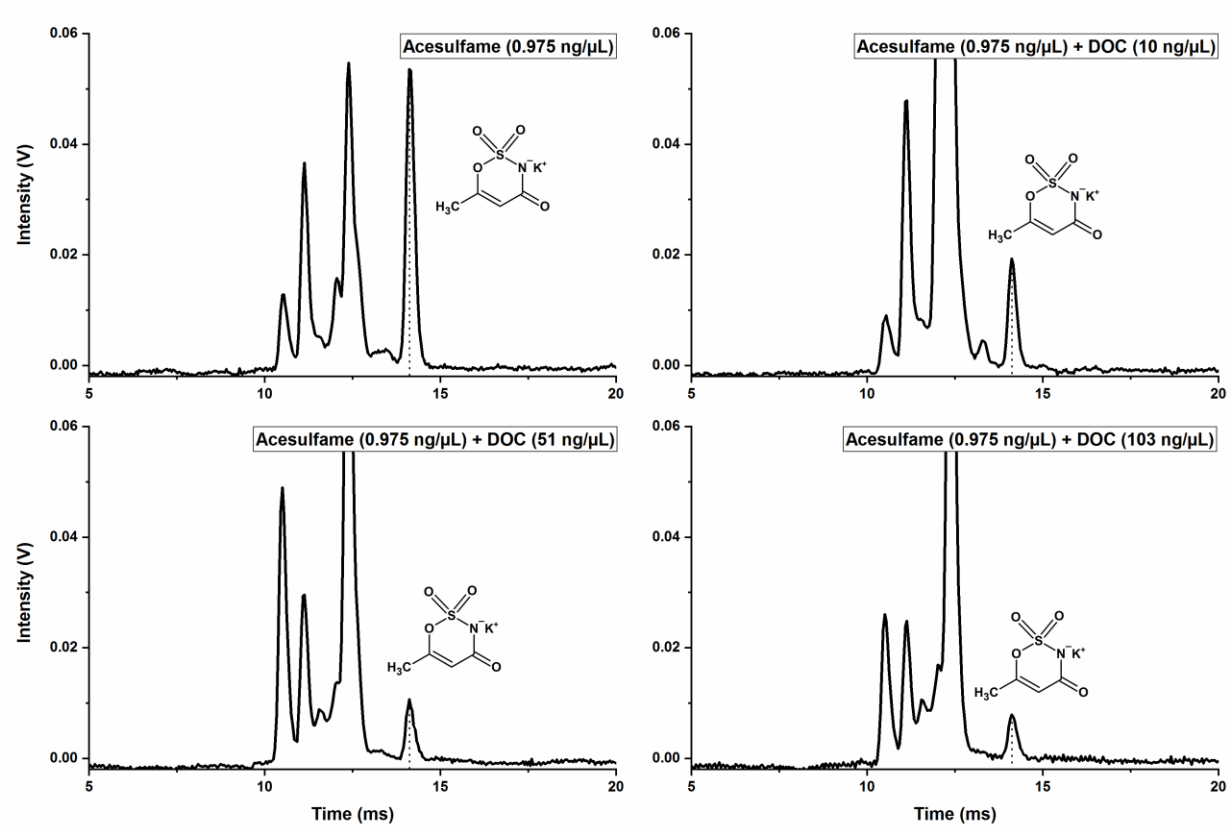


Figure 5-8: Influence of dissolved organic matter (DOM) on the determination of acesulfame.

This solution was directly injected to the ESI source of the IMS. As can be seen from the obtained results in figure 5-8, humic substances do not show a signal in negative ion mobility spectrum, but the quantitative determination of acesulfame is considerably affected. Even comparative low concentrations of DOM (10 ng μL^{-1}) reduce the signal intensity of acesulfame by 65%. Higher DOM concentrations lead to a further decrease. Consequently, using developed filter paper disks as selective sample-preparation method, DOM has to be eliminated as much as possible prior ESI-IMS analysis.

6. Selective filter-paper combined with developed detection method

Despite the inherent advantages of IMS detector, we showed that ESI-IMS suffers severely from matrix effect when a mixture of chemicals is injected directly and without any pre-separation. Therefore, we tried to use our previously developed selective paper-based SPE disk to remove unwanted chemicals from sample matrix. Using this methodology, we would be able to mitigate matrix effect and therefore measure the accepted marker acesulfame using our developed ESI-IMS detector. Acesulfame concentrations in river waters are in the range of few μg L^{-1} [179]. Therefore, we had to use more sample volumes up to 300 mL due to the lower sensitivity of ESI-IMS in comparison to HPLC/MS-MS. For this reason, new filter-paper disks were prepared which contained 400 mg acesulfame imprinted polymer particles using with the preparation procedure described in section '4.4.1.' with slight modification.

In summary, the 400 mg polymer was first mixed with 20 mL of MeOH and 40 mL distilled water. This mixture was sonicated to achieve highly dispersed polymer particles. 60 mL of pure paper-pulp was added to this polymer/methanol/water mixture and thoroughly mixed. The final prepared mixture containing 120 mL methanol/water solution was used to construct the selective middle layer which was coated on both sides with a thin protecting layer containing pure cellulose fibers as described in section '4.4.1.'.

It was shown in the ESI-IMS development section that the signal intensity of acesulfame was negatively affected when it was measured in the presence of other organic chemical compounds and dissolved organic matter (DOM). In comparison to the HPLC/MS-MS, this matrix effect is more critical using our developed ESI-IMS instrument due to the lack of a separation method. Therefore, we needed to evaluate and develop the washing step to increase the selectivity of the pre-separation method.

It is known that the synthesized MIP particles show the highest selectivity when they are loaded with chemicals in a solvent in which the polymer is synthesized. Our polymer was synthesized in chloroform but loaded with water samples. Therefore, chemicals are adsorbed via selective and non-selective sits available on the surface and within polymer structure. Chapuis et al. [183] showed that using a washing step with a weakly polar and aprotic solvent after loading the water sample could incredibly highlight the selectivity of used polymer. As mentioned, during

loading the water samples, chemicals could be adsorbed via selective and non-selective sites. However, using an extra washing step with an organic solvent like dichloromethane can cause to remove co-extracted contaminates and provoke the selective interaction of template molecules and selective sites. In our previous studies, after loading the polymer was washed just with 10 mL methanol. To use the filter-paper disks as a selective sample-preparation method before ESI-IMS analysis, we developed the used washing procedure whereupon the loaded polymer particles were washed with dichloromethane and methanol, successively. For this reason, the filter paper was loaded with 300 mL water sample containing acesulfame-K, bentazon, saccharin, ibuprofen sodium salt, naproxen sodium salt, atrazine and caffeine (where the concentration of each solute was 3.3 µg L^{-1}). After sample loading, the loaded disk was dried completely due to immiscibility of water and dichloromethane. After that, dried disk was washed with 10 mL dichloromethane, dried again and washed with 10 mL methanol. In this study, obtained extracts were measured with standard HPLC/MS-MS method in parallel with our developed ESI-IMS. Obtained results with HPLC/MS-MS showed that even with this successive washing process, acesulfame could be extracted with 65% percent recovery. On the other hand, filter paper embedded with imprinted polymer sorbents showed the highest affinity towards negatively-charged acesulfame. Caffeine and atrazine were removed completely. Two structural analogues, bentazon and saccharin which have negatively-charged sulfonamide group at environmental pH values, were extracted with recoveries lower than 24%. Naproxen and ibuprofen with respective recoveries of 7 % and 3 % did not indicate any interference with the acesulfame response. The obtained selectivity permitted the determination of acesulfame with ESI-IMS without being influenced by the other evaluated chemicals.

DOM content of natural waters is another important factor which can severely affect the quantitation measurements of acesulfame using ESI-IMS. To evaluate the DOM removal efficiency of selective disks, different water samples were prepared each contained acesulfame and DOM. The volumes of the water samples were 300 mL and the concentrations of acesulfame were kept constant at 5.8 µg L^{-1}. The concentrations of humic acids were increased from 0 to 4 and 6 mg L^{-1}. Figure 6-1 shows the influence of DOM content on the quantitative results for acesulfame using our selective extraction procedure with MIP loaded filte-papers.

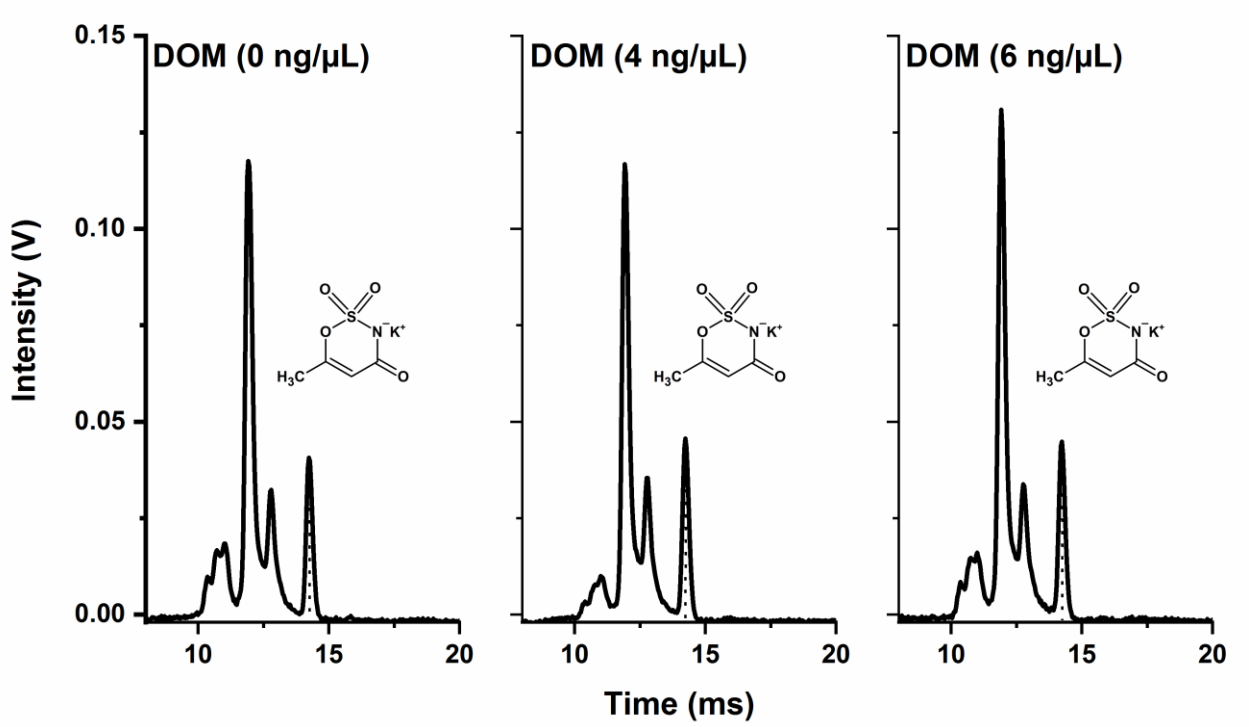


Figure 6-1: Influence of dissolved organic matter on the determination acesulfame after complete sample-preparation procedure. Three samples, each has acesulfame at 5.8 µg L^{-1} and DOM at different concentrations in 300 mL water.

As can be seen from the Figure 6-1, the peak intensities of acesulfame are not affected by DOM concentrations up to 6 mg L^{-1}. Obtained results proved that developed selective filter papers can mitigate the matrix effect which could arise from DOM content of natural water samples. Therefore, the influence of DOM can be neglected for the water samples with average DOM content using our paper based MIP disks.

After matrix evaluation using selective sample-preparation, standard concentrations of acesulfame were prepared and analyzed with ESI-IMS and selective filter-paper/ESI-IMS methods. The optimized parameters for ion mobility measurements permit the sensitive determination of acesulfame. Without sample preparation, 93 µg L^{-1} was calculated as instrumental LOD and 287 µg L^{-1} as instrumental LOQ for ESI-IMS instrument. For the validation of the extraction of acesulfame from water samples using our sample preparation procedure, standard solutions were prepared within a concentration range of 0.24 and 31 µg L^{-1} and analyzed using optimized selective filter-paper/ESI-IMS method.. The calibration curve can be described with the function ($y=0.0001x^3-0.012x^2+0.3998x+0.0406$) with the coefficient of

determination R^2=0.9982 (Figure 6-2). The LOD for the samples analyzed with IMS after preparation using the presented procedure was 0.19 µg L^{-1} and the LOQ was 0.63 µg L^{-1}. The RSDs at different concentrations were between 0.49 and 9.53%.

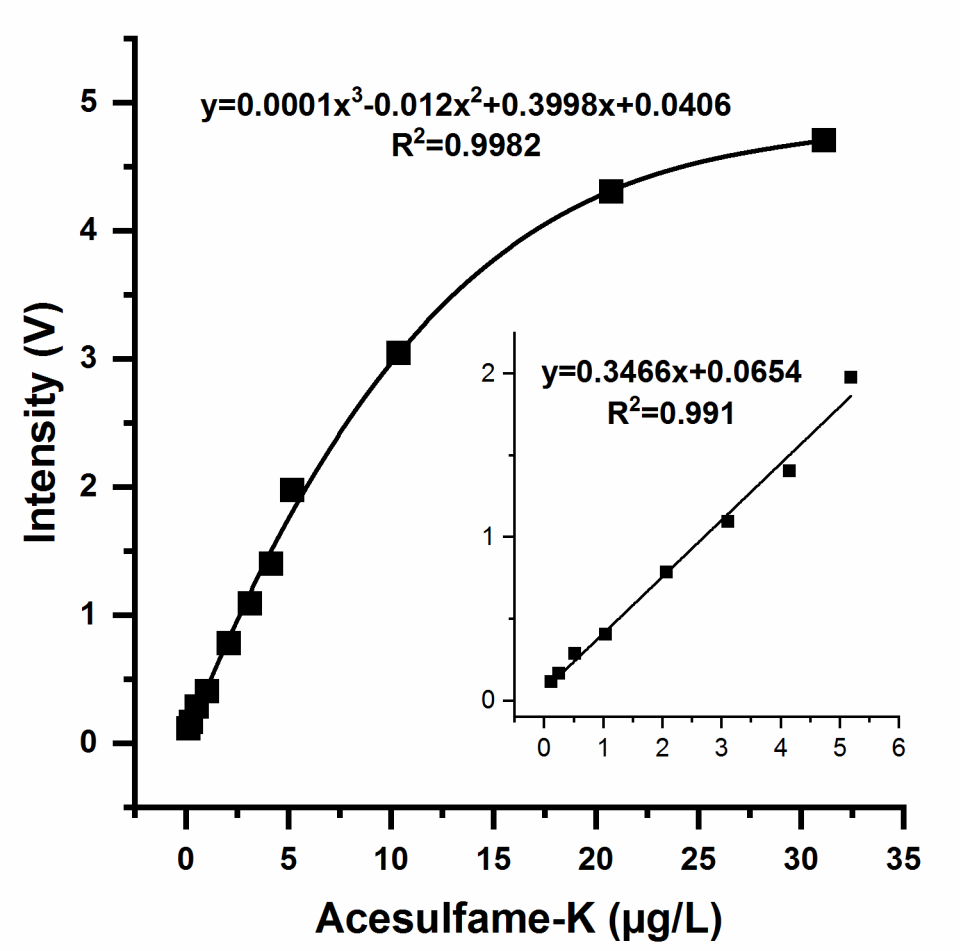


Figure 6-2: Calibration curve obtained with optimized selective filter-paper/ESI-IMS method.

The objective of our analytical procedure was to demonstrate that it was suitable for its intended purpose for environmental water samples. In order to validate our sample-preparation procedure, we compared three different analytical approaches for the determination of acesulfame in samples from a river. A standard procedure using commercial styrene-divinylbenzene (SDB) extraction column containing 200 mg of sorbent was used to compare the selectivity levels of the developed MIP-embedded filter paper (SDB procedure was obtaibed from [179] and described already in section 4.2.). The eluates of the presented procedure were analyzed with HPLC/ -MS-MS and ESI-IMS. The samples were taken from the river White Elster

(Leipzig, Germany) at locations upstream and downstream of the effluent of the central municipal waste water treatment plant (WWTP, approximately 500,000 population equivalents; Figure 6-3). The whole equipment for sampling is shown in figure 4-24 and sampling procedure was done in-field. The disks loaded in-field were transported to the laboratory and stored in desiccator at 4°C till further analysis.

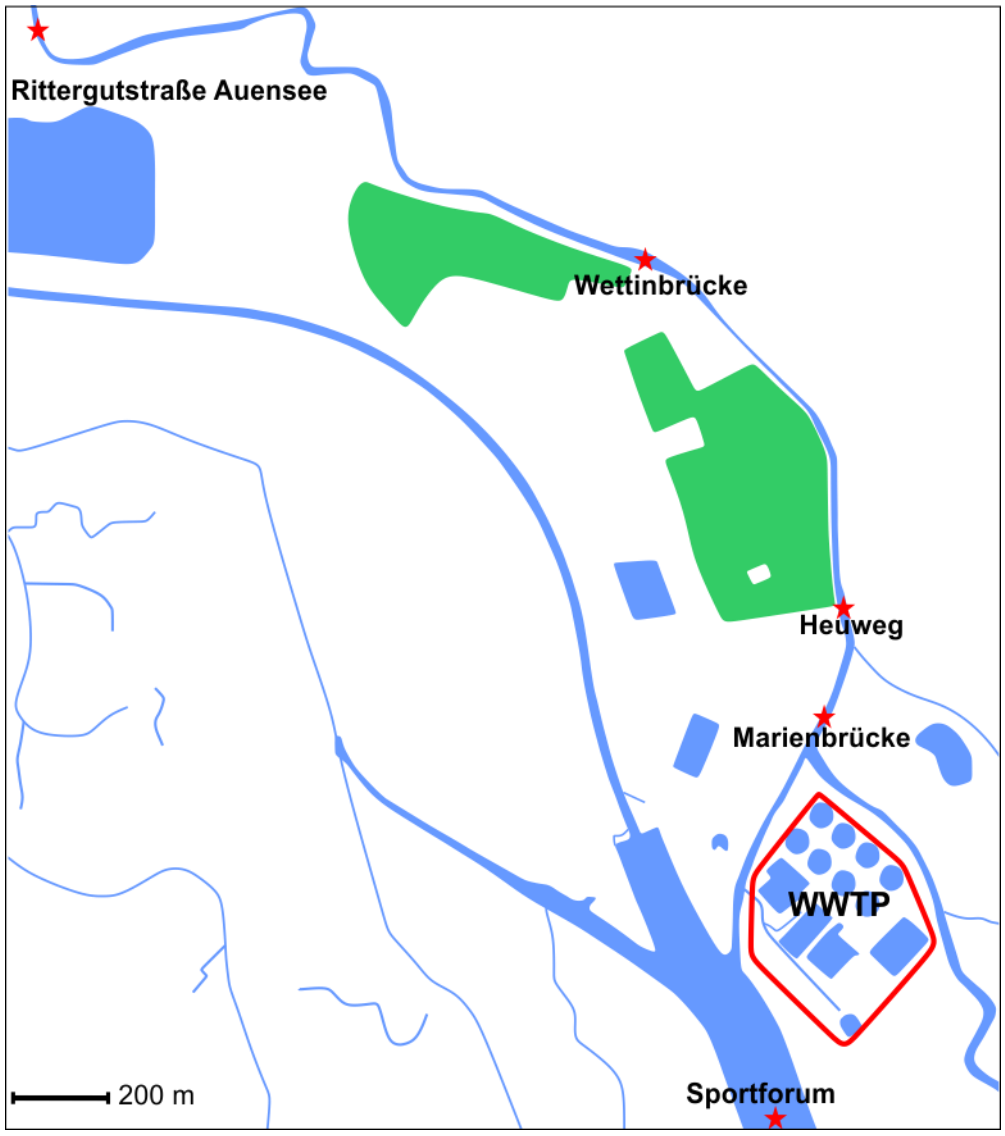


Figure 6-3: Map of the sampling sites marked with red ★. WWTP represents the central wastewater treatment plant of Leipzig (Germany, 500 000 inhabitants).

The results are summarized in figure 6-4. Due to the low removal efficiency in conventional WWTPs, the concentration of acesulfame increases downstream the wastewater discharge.

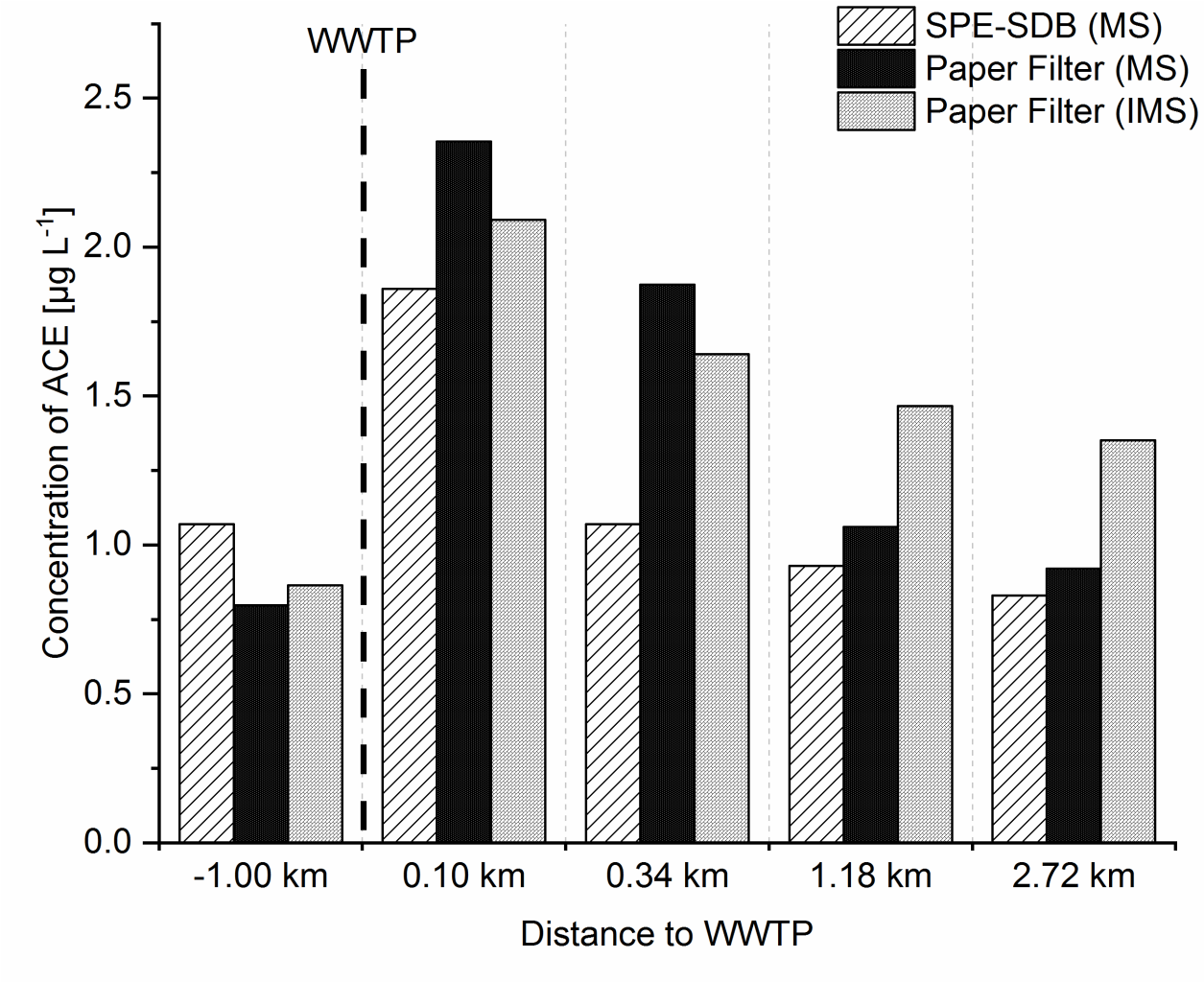


Figure 6-4: Concentration of acesulfame along a river at locations upstream and downstream of the effluent of a waste water treatment plant (WWTP)

Further dilution along the flow path decreases the concentrations of acesulfame to a comparable level before wastewater input. A very good agreement between the quantitative results for acesulfame was observed. The SPE (SDB)-MS procedure provided slightly lower concentrations for the samples after the influent of WWTP where the samples are loaded with a complex matrix. The measurements with IMS and MS after the extraction with MIP loaded paper filters provided comparable results. It is notable that the presented ESI-IMS procedure with paper filters is the simplest and fastest method. The paper filters can be loaded in the field

and can be used for transportation to the lab instead to transport the water bottles. We investigated the long-term stability over 4 days without loss of signal intensity for acesulfame. The measurement with ESI-IMS requires few minutes with a less complex instrumentation.

7. Conclusion

Different sample-preparation methods were developed using molecularly imprinted polymers (MIPs) as sorbent materials. MIPs as artificial selective polymers function based on the Lock and Key principle. Despite the lower selectivity of these polymers in comparison to specific antibodies or selective aptamer materials, their synthesis is relatively easy and has easy-engineering procedure. More importantly, they show high resistivity towards harsh environmental conditions. Combination of the selectivity feature of MIP materials to the developed sample-preparation methods can help to mitigate the matrix effect.

Fast, efficient and precise sample-preparation is the main goals during each development. For environmental water samples, treatment of the large sample volumes is another important factor due to the relatively low concentrations of the target analytes in the environmental water samples.

During this study, the developments were orientated towards the fast and selective sample-preparation of relatively large sample volumes. The known sample preparation formats like SPME and SPE were used in combination with synthesized MIPs. Despite the interesting results, these traditional formats could not satisfactorily support our aim. Therefore, new extraction formats, e.g. mixed-bed SPE and selective filter-paper disks, were developed using selective polymers. Among all evaluated and optimized methods, developed selective filter-paper disks enabled the fast and selective sample-preparation of relatively large sample volumes. It needs a simple and easy-to-handle extraction procedure which requires little equipment for the sample-preparation of trace organic compounds in water samples. Therefore, no transportation of water samples is required.

Furthermore, we constructed an IMS detector using ESI as ionization source. The whole instrumental parameters for ESI-IMS instrument were evaluated and optimized. Combination of selective filter-paper disks as relatively fast sample-preparation method with ESI-IMS detector could improve the feasibility for field applications. The whole protocol can become a mobile, sensor-like instrumentation, fast and robust for on-site monitoring.

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Declaration

I hereby declare that this thesis is entirely my own work and has been written without any help of others and that are used the mentioned sources and indicated all kind of citations correctly. This dissertation has not been submitted before.

Leipzig, 01.12.2018

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2004-2007	M.Sc. study in Analytical Chemistry in a Discrete Master's degree program, University of Tehran, Tehran, Iran (Total G.P.A.: 18.60 out of 20). Oral defense 08.07.13; Thesis: "Construction of a portable ion mobility analyzer with membrane inlet system" (Score: 19.25 out of 20).

2000-2004	B.Sc. study in Applied Chemistry in a full-time degree program, Isfahan University and Technology (IUT), Isfahan, Iran (Total G.P.A.: 16.91 out of 20).

Prizes and Awards

- Best Student Award (1996-2000)
- Ranked 3th, Chemistry Department, Isfahan University and Technology (IUT) (2004)
- Ranked 25th (among approximately 200,000 entrants) in the Nationwide University Entrance Exam for M.Sc. Program in Iran (2004)

Research Experiences; Activities

- Development of new analytical devices
- Synthesis, evaluation and application of selective molecularly imprinted polymer (MIP) for different chemical targets
- Sampling, sample preparation and extraction techniques
- Basic knowledge and practical experiences with different electrochemical methods (e.g. Voltammetry, Amperometry and Potentiometry) and chromatographic separation techniques (e.g. GC-MS, HPLC and HPLC/MS-MS).

International Conference

- 15th EuCheMS International Conference on Chemistry and the Environment; 20 - 24 September 2015; Leipzig, Germany (Poster Communication).

Publications

- **Mashaalah Zarejousheghani**, Steffi Schrader, Monika Möder, Thomas Mayer, Helko Borsdorf; Negative electrospray ionization ion mobility spectrometry combined with paper-based molecular imprinted polymer disks: A novel approach for rapid target screening of trace organic compounds in water samples; **Talanta**; 190: 47–54 **(2018)**
- **Mashaalah Zarejousheghani**, Andreas Walte, Helko Borsdorf; Sprayed liquid-gas extraction of semi-volatile organophosphate Malathion from air and contaminated surfaces; **Analytical Methods**; 10: 2503–2511**(2018)**
- **Mashaalah Zarejousheghani**, Malcolm Cämmerer, Thomas Mayer, Andreas Walte, Helko Borsdorf; Sprayed liquid-gas extraction in combination with ion mobility spectrometry: A novel approach for the fast determination of semi-volatile compounds in air and from contaminated surfaces; **International Journal for Ion Mobility Spectrometry**; 21:33–41; **(2018)**
- **Mashaalah Zarejousheghani**, Steffi Schrader, Monika Möder, Matthias Schmidt, Helko Borsdorf; A new strategy for accelerated extraction of target compounds using molecularly

imprinted polymer particles embedded in a paper-based disk; **Journal of Molecular Recognition**; 31:e2629 (**2018**)

- **Mashaalah Zarejousheghani,** Steffi Schrader, Monika Möder, Pierre Lorenz, Helko Borsdorf; Ion-exchange molecularly imprinted polymer for the extraction of negatively-charged acesulfame from wastewater samples; **Journal of Chromatography A**; 1411: 23-33 **(2015)**
- **Mashaalah Zarejousheghani,** Petra Fiedler, Monika Möder, Helko Borsdorf; Selective mixed-bed solid phase extraction of atrazine herbicide from environmental water samples using molecularly imprinted polymer; **Talanta**; 129: 132-138 **(2014)**
- **Mashaalah Zarejousheghani,** Monika Möder, Helko Borsdorf; A new strategy for synthesis of an in-tube molecularly imprinted polymer-solid phase microextraction device: Selective off-line extraction of 4-nitrophenol as an example of priority pollutants from environmental water samples; **Analytica chimica acta**; 798: 48-55 **(2013)**
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