New Techniques for Sample Preparation in Analytical Chemistry

Microextraction in Packed Syringe (MEPS) and Methacrylate Based Monolithic Pipette Tips

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DISSERTATION

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Abstract

Sample preparation is often a bottleneck in systems for chemical analysis. The aim of this work was to investigate and develop new techniques to address some of the shortcomings of current sample preparation methods. The goal has been to provide full automation, on-line coupling to detection systems, short sample preparation times and high-throughput.

In this work a new technique for sample preparation that can be connected on-line to liquid chromatography (LC) and gas chromatography (GC) has been developed. Microextraction in packed syringe (MEPS) is a new solid-phase extraction (SPE) technique that is miniaturized and can be fully automated. In MEPS approximately 1 mg of sorbent material is inserted into a gas tight syringe (100-250 µL) as a plug. Sample preparation takes place on the packed bed. Evaluation of the technique was done by the determination of local anaesthetics in human plasma samples using MEPS on-line with LC and tandem mass spectrometry (MS-MS). MEPS connected to an autosampler was fully automated and clean-up of the samples took about one minute. In addition, in the case of plasma samples the same plug of sorbent could be used for about 100 extractions before it was discarded.

A further aim of this work was to increase sample preparation throughput. To do that disposable pipette tips were packed with a plug of porous polymer monoliths as sample adsorbent and were then used in connection with 96-well plates and LC-MS-MS. The evaluation of the methods was done by the analysis of local anaesthetics lidocaine and ropivacaine, and anti-cancer drug roscovitine in plasma samples. When roscovitine and lidocaine in human plasma and water samples were used as model substances, a 96-plate was handled in about two minutes. Further, disposable pipette tips may be produced at low cost and because they are used only once, carry-over is eliminated.
To my Parents: “scholarship and books are the building blocks of a better world”.
Abbreviations

γ-MAPS  tri((methoxysilyl)propyl)methacrylate
ACN  Acetonitrile
AIBN  2,2'-Azobis(2-methylpropionitrile)
ATP  Adenosine Triphosphate
BMA  Butyl Methacrylate
BP  Benzophenone
BPO  Benzoyl Peroxide
C$_2$  Ethyl Silica
C$_8$  Octyl Silica
C$_{18}$  Octadecyl Silica
CA  Contact Angle
Cdks  Cyclin Dependent Kinases
CEC  Capillary Electrochromatography
COC  Cyclic Olefin Copolymer
DNA  Deoxyribonucleic Acid
EGDMA  Ethylene Glycol Dimethacrylate
EMI  Electron Membrane Isolation
ESI  Electrospray Ionization
GC  Gas Chromatography
GMA  Glycidyl Methacrylate
HPLC  High Performance Liquid Chromatography
I.D.  Inner Diameter
I.S.  Internal Standard
k'  Retention Factor
LC  Liquid Chromatography
LLE  Liquid-Liquid Extraction
LPME  Liquid Phase Microextraction
MEPS  Microextraction in Packed Syringe
MeOH  Methanol
MMA  Methyl Methacrylate
MS  Mass Spectrometry
MS-MS  Tandem Mass Spectrometry
M$_r$  Molecular Weight
PC  Polycarbonate
PDMS  Polydimethylsiloxane
PMMA  Poly(methyl methacrylate)
PP  Polypropylene
PPX  Pipecoloxylidide
Q  Quadrupole
RNA  Ribonucleic Acid
RP-LC  Reversed-Phase Liquid Chromatography
SBSE  Stir Bar Sorptive Extraction
SEM  Scanning Electron Microscopy
SME  Supported Membrane Extraction
SPDE  Solid-Phase Dynamic Extraction
SPE  Solid-Phase Extraction
SPME  Solid-Phase Microextraction
SRM  Selected Reaction Monitoring
t$_0$  Retention Time Unretained Analyte
t$_r$  Retention Time Retained Analyte
UV  Ultra Violet
List of Papers Included in the Thesis

I Microextraction in Packed Syringe (MEPS) for Liquid and Gas Chromatography Applications. Part II - Determination of Ropivacaine and its Metabolites in Human Plasma Samples Using MEPS with Liquid Chromatography/Tandem Mass Spectrometry.

II New Trends in Sample Preparation: On-line Microextraction in Packed Syringe (MEPS) for LC and GC Applications. Part III - Determination and Validation of Local Anaesthetics in Human Plasma Samples Using a Cation-exchange Sorbent, and MEPS-LC-MS-MS.

III Increasing Sample Preparation Throughput Using Monolithic Methacrylate Polymer as Packing Material for 96-Tips: 2 Minutes per 96-Well Plate.

IV Surface Modified Polypropylene Pipette Tips Packed with a Monolithic Plug of Adsorbent for High Throughput Sample Preparation.

V Some Factors Effecting the Performance of the Microextraction in Packed Syringe (MEPS).

Paper I-IV: The author was responsible for the experimental work, except analysis and contact angle measurements, and for writing most of the paper.
Paper V: The author was responsible for the experimental work and for writing the paper.
Papers/manuscripts not included in this thesis

I  Use of Carbon Dioxide and Ammonia as Nebulizer Gases in Mass Spectrometry.

II  Drug Screening Using Microextraction in a Packed Syringe (MEPS) / Mass Spectrometry Utilizing Monolithic-, Polymer-, and Silica-Based Sorbents.

III  Microextraction in Packed Syringe Online with Liquid Chromatography-Tandem Mass Spectrometry: Molecularly Imprinted Polymer as Packing Material for MEPS in Selective Extraction of Ropivacaine from Plasma.

IV  Evaluation of Monolithic Packed 96-Tips for Solid-Phase Extraction of Local Anaesthetics from Human Plasma for Quantitation by Liquid Chromatography Tandem Mass Spectrometry.
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1 Introduction

1.1 Sample Preparation

Analytes of environmental or biological origin usually occur in complex matrices. Most of analytical instruments cannot handle such sample matrixes directly. Cleaning up of such samples, isolation of analytes of interest and enrichment of the analyte to a suitable concentration level may be required before instrumental analysis. Sample preparation is the series of steps required to prepare a sample in a suitable form for analysis. The faster these steps can be done, the more quickly the analysis will be completed. The procedure must be reproducible with high recovery of the analytes.

Historically, sample preparation has been considered not as a part of the analytical process, rather the “procedure” that had do be done to develop and perform analytical methods [1]. The technology has been crude and of low tech. However, the significance of the sample preparation for the total analytical performance is nowadays widely recognized. The final results of the experiments depend on the starting conditions.

An ideal sample preparation method should involve a minimum number of working steps, be easy to learn, be environmentally friendly and be economical [2]. Further, as the number of samples grows high-throughput and fully automated analytical techniques becomes required.

1.2 Commonly Used Sample Preparation Techniques

1.2.1 Liquid-Liquid Extraction (LLE)

Liquid-liquid extraction (LLE) is one of the oldest and most widely used sample preparation techniques. In LLE separation of a sample is based on distribution between two immiscible liquid phases. The classical form of LLE is using a separatory funnel for separating liquid phases from each other. LLE involves mixing an immiscible organic solvent with an aqueous solvent (e.g. plasma,
urine, serum) to extract the analyte into the organic phase. The organic phase may be transferred, evaporated to dryness and reconstituted prior to analysis. Compared to other sample preparation techniques LLE has advantages such as large sample capacity and direct analysis after concentration of the clean organic extract. Disadvantages of the classical LLE approach are that it is labor intensive, difficult to automate and uses large volume of expensive/ and environmentally harmful organic solvents [3]. To phase out these disadvantages, some modern approaches to classical LLE have appeared during the past 10 years. Examples of modern approaches to LLE are single drop-liquid phase microextraction (LPME) [4], liquid phase microextraction (LPME) [5-8] and supported membrane extraction (SME) [9-13]. Single drop-LPME is based on a drop of organic solvent hanging at the end of a syringe needle. As the organic droplet is placed in the aqueous sample, based on passive diffusion, the analytes are extracted into the droplet. After extraction, the droplet is withdrawn into the syringe for further analysis. The group of Pedersen-Bjergaard and Rasmussen developed an alternative concept based on the use of porous hollow fibers made of polypropylene. In this, the extraction phase (acceptor phase) is contained within the lumen of a porous hollow fiber. Further, the pores of the hollow fiber are filled with an immobilized organic liquid. As the fiber assembly is placed in a sample vial, the analytes are extracted through the organic liquid immobilized within the pores of the hollow fiber before they are trapped in the acceptor phase. To speed up the extraction time, the samples may be stirred or vibrated. Although, this procedure is much more robust than single drop-LPME, it suffers from disadvantages such as difficulty to automate and long extraction times (up to 60 minutes per sample). However, by utilizing an electrical potential difference across the membrane the researchers could speed up analysis time considerably (5 minutes per sample). This procedure was named electro membrane isolation (EMI) [14].

1.2.2 Solid-Phase Extraction (SPE)

Liquid-solid extraction or, as it is often called solid-phase extraction (SPE) is the method used for concentration and isolation of target analytes using a solid support. SPE is today the most commonly used sample preparation method in many areas of chemistry including clinical, environmental and pharmaceutical applications [15]. SPE was initially developed as a complement or replacement for LLE [15-17].
The first analytical application of SPE started to the best of my knowledge in the early 1950s [18]. Using an iron cylinder packed with 1200-1500 g of granular activated carbon, Braus et al. isolated organic material samples from six water plants on the Ohio River [19]. The study was performed for the determination of causes to tastes and odours of these waters. Since then an increased development of SPE has occurred with new formats and new phases with different chemistries [16]. The cartridge formats are today the most popular formats [15]. A typical construction of the cartridge device is shown in Figure 1.

![Schematic diagram showing a typical SPE cartridge.](image)

**Figure 1.** Schematic diagram showing a typical SPE cartridge.

Generally, the device consists of an open polypropylene or polyethylene syringe barrel containing a sorbent packed between frits. SPE cartridges are available in sizes containing from 10 mg to 10 g of sorbent with the 50 mg to 500 mg sorbent cartridges being the most widely used. The most commonly used packing materials are silica-based with chemically bonded functional groups and highly cross-linked polymers such as styrene-divinylbenzene and polymethacrylate. To further eliminate causes of carry-over and memory effects the SPE cartridges are used only once, therefore the sorbent has to be cheap and thus of relatively low quality.
1.2.2.1 Sample Clean-up Procedure in SPE

A typical solid-phase extraction involves four processing steps, Figure 2. In the first step the sorbent is conditioned with 3-5 bed volumes of first an organic solvent and then by water to remove impurities and ensure reproducible retention of analytes. The second step is application of the sample solution through the extraction device. This step is followed by rinsing and cleaning of the sorbent from interferences without losing the analytes. Finally, the analytes of interest are eluted from the sorbent using a strong solvent [15, 18, 20].

![Figure 2. The four processing steps in the operation of SPE experiment.](image)

The parameters describing the processing steps in SPE are according to the theoretical principles of liquid chromatography (LC) [16, 18, 20].

In SPE, separation is based on the selective distribution of analytes between the solid packing material and the liquid mobile phase. There are different SPE selectivities available and the classification of these is based on the type of distribution applied in the extraction [15]. The dominating selectivity is reversed-phase SPE. Here, the stationary phase is usually silica spheres with chemically bonded alkyl and/or aryl functional groups onto the surface. C\textsubscript{18} silica dominates but C\textsubscript{8} silica is also used extensively. The packing material in LC (see section 1.5) and SPE are basically the same except that the spheres are larger in SPE. Most organic solvents will flow through the sorbent by gravity, but for aqueous and other viscous samples a slight vacuum is commonly employed. The retention of analytes onto these sorbents is due primarily to
hydrophobic interactions. To elute analytes from these sorbents non-polar solvents are used.

For analytes that are charged when in solution, ion exchange SPE can be used. Ionic interaction occurs between an analyte carrying a positive or negative charge and a sorbent carrying the opposite charge. The pH of the matrix must be adjusted so that both the analyte and the sorbent are ionized for retention to occur. For example, to retain weakly basic local anaesthetics with pKa of 8.5, the pH has to be at least two pH units below the pKa of the analyte (pH<8.5) and at least two pH units above the pKa of the sorbent. The elution of the analyte is achieved by adjusting the pH to suppress the charge on either the analyte or the sorbent. With strong ion-exchange sorbents, the charge on the phase cannot easily be suppressed, so elution is achieved by suppressing the charge of the analyte. In addition, elution can be performed with use of a counter-ion at high ionic strength.

1.3 Trends in Sample Preparation

Recent trends in the sample preparation area focus on how to miniaturize the process, increase the sample throughput, use selective sorbents and on-line couple the sample preparation units to separation system or detection systems [15, 17, 20-23].

The first attempts to miniaturize the process and provide high sample throughput were done with the introduction of new formats such as SPE disks [24], pipette tips [24-26], column switching systems [15, 26-29] and multi-well plates [30]. Further, miniaturization resulted in development of new extraction techniques. Some examples of emerging new techniques will be given here.

Solid-phase microextraction (SPME) is presently the most commonly used microextraction technique [1, 31-33]. SPME is used routinely with GC but can also be coupled to LC. In SPME a fused-silica fibre coated on the outside with an appropriate stationary phase is used for sampling. When the stationary phase is placed in contact with the sample matrix a partitioning of the analytes between the two phases takes place. After that the fibre is inserted in the inlet of a GC injector for direct desorption. The extraction efficiency of SPME depends on a number of factors such as extraction time, agitation, sample pH, salt concentration and temperature. SPME enables extraction and pre-concentration of analytes from gaseous, liquid and solid samples.
Solid-phase dynamic extraction (SPDE) is a technique which utilizes wall coated needles prepared from stainless steel capillary columns for sampling [34-36]. The inside wall of the needle is coated with a 7 µm thick film of polydimethylsiloxane (PDMS) and activated carbon as stationary phase. The dynamic sampling is performed by pulling and pushing a fixed volume of the sample through for an appropriate number of times. The adsorbed analytes are then recovered using carrier gas or indoor air into a GC injector. The technique can be used for vapour and liquid samples.

Another technique that is used to extract analytes from liquid samples is stir bar sorptive extraction (SBSE) [37-38]. Stir bar sorptive extraction (SBSE) was introduced in 1999 as a solventless sample preparation method for the extraction of organic compounds from aqueous matrices. The method is based on sorptive extraction, where solutes are extracted into a polymeric coating on a magnetic stirring rod [39]. The basic principles of SBSE are thus identical to SPME using polydimethylsiloxane-coated fibers, but the volume of extraction phase is 50-250 times larger [39]. Stir bars, 1 or 2 cm long coated with 0.5 or 1 mm thick film are commercially available (Twister™, Gerstel GmbH, Mülheim an der Ruhr, Germany). After the extraction, the stir bar is removed, dipped in a clean paper tissue to remove droplets, and introduced into a thermal desorption unit. Alternatively desorption can be made in an appropriate solvent. Difficulties of automation and lack of appropriate selective phases are some of the drawbacks related to SBSE.

The extraction principles of these techniques, SPME, SPDE and SBSE, are identical and they utilize the same extraction medium, PDMS, but the amounts are different.

In addition to these emerging new techniques, a large number of non-selective and selective sorbents has been developed to compensate for some of the drawbacks of silica based materials, e.g. some irreversible adsorption of basic analytes [15].
1.4 Microextraction in Packed Syringe (MEPS)

Microextraction in packed syringe (MEPS), also called Microextraction by Packed Sorbent, (Invented at AstraZeneca AB, Södertälje, Sweden, by Prof. Mohamed Abdel-Rehim) is a new sample preparation technique that uses a gas tight syringe as extraction device [40-57]. In MEPS the sorbent material, about 1 mg, is either inserted into the syringe barrel as a plug with polyethylene filters on both sides, or between the syringe barrel and the needle, Figure 3.

![Figure 3. Schematic picture of microextraction in packed syringe (MEPS).](image)

The sample processing steps in MEPS are similar to those of SPE, Figure 4. Basically, after conditioning of the sorbent with an appropriate solvent(s), the sample solution is drawn through the needle into the syringe up and down once or several times. This is followed by a washing step to remove interferences and, finally the analytes of interest are extracted directly into the LC or GC injector.
MEPS can be connected on-line to LC [41-47, 50-53, 56-57] or GC [40, 48, 49, 54] without any modification of the instrument, and connected to a robot the method can be fully automated. In MEPS any sorbent material can be used either as packing bed or as coating. Compared to above mentioned microextraction techniques which use PDMS as sorbent in most of the cases, MEPS has a big advantage, because it can be used with a wide range of available sorbents.

1.5 Pipette Tips SPE

There is a trend in analytical chemistry towards miniaturization of analytical systems. This trend has in the sample preparation area for instance prompted the development of new formats such as micropipette tips. The first commercially available micropipette tip was based on chromatographic media, micro particulates C\textsubscript{18}, embedded in the scaffold of a polymer (ZipTip, Millipore, Bedford, MA, USA). Since then, different types of pipette tips based on micro particulates, polymers and monoliths, and with different interaction
modes such as hydrophobic, ion-exchange and affinity have been introduced [24-26, 58-62]. In addition to advantages such as reduced sample- and solvent consumption, the main advantages of pipette tips based sample preparation is that it can be used with micropipettors and may be used easily with commercially available liquid-handling systems for automated high-throughput applications.

Based upon different interactions micropipette tips based SPE involves four different processing steps (see section 1.2.2). After the conditioning step with appropriate solvent(s), the sample of interest is applied through the extraction phase. Following this, excess salts and other interferences are rinsed out in the washing step. Finally, the analyte(s) of interest are eluted from the sorbent using an appropriate elution solution. In addition, different aspiring and dispersing cycles may be needed depending on sample and matrices.

1.6 Polymer Monoliths

The term monolith appeared in the chromatographic area for the first time to describe a single piece of cellulose sponge [63]. This simple term was considered handier than multi-word expressions such as continuous polymer beds or continuous polymer rods used earlier. The word “monolithos” (from Greek: “mon”, which means ‘one’ and ”lithos”, which means ’stone’) in chromatographic terms means constituting or acting as a single, often rigid and uniform whole. That is, the material fills the entire volume of the tube, and the mobile phases must flow through the pores in the stationary phase.

The first continuous support column prepared with polymethacrylate-based monomers was introduced by Kubin et al., in 1967 [64]. As an alternative to, at that time, popular crosslinked polysaccharides, they synthesized a spongy elastic gel like structure in a 22 mm inner diameter glass tube, which they used later for the separation of water soluble polymers in size-exclusion chromatographic mode. Unfortunately, the resulted flow rates were too low to make this material useful as chromatographic medium at that time.

About two decades later, columns based on this type of material, however designed according to a different principle was introduced by Svec et al. [65]. These, “continuous polymer rods” which the columns were called, were based on poly(glycidyl methacrylate-co-ethylene dimethacrylate). After functionalization
with diethylamine in the second step of polymerization, the columns were used for the separation of proteins in anion-exchange mode. Since then, materials based on this principle have been used in many chromatographic modes such as HPLC [65-70], capillary electrochromatography (CEC) [71-73], gas chromatography (GC) [74] and solid-phase extraction SPE [75-78], but also as carriers for catalysts and in enzyme reactors [79-81].

At about the same time Stellan Hjértén at Uppsala University and co-workers prepared “continuous polymer beds” as chromatographic media [82]. Using N’N-methylenebis(acrylamide), acrylic acid and phosphate buffer they prepared hydrophilic polymer beds which they afterwards compressed to about 20% of original size. Upon compression, the distance between the pores was decreased which in chromatographic terms means less zone broadening. Using this approach but without compressing Hjértén and coworkers later prepared continuous polymer beds in fused silica capillaries [83] for different applications such as reversed-phase [84], ion-exchange [85] and affinity chromatography [86-87].

Monolithic stationary phases can be classified into two main categories silica-based monoliths and polymer-based monoliths [88]. Generally, monolithic silica columns are prepared by sol-gel technology [89]. The sol-gel process usually involves hydrolysis of sol-gel precursors, such as alkoxysilanes (tetraethoxy- or tetramethoxysilane), and catalytic polycondensation of the hydrolyzed products to form a macromolecular network structure of the material. Different parameters have been investigated to optimize the final structure of the network. The most important factor affecting the resulting network structure is the reaction starting conditions. To obtain monolithic structure with desired properties different ligands and catalysts such as acids, bases and ions have been investigated [90-93].

The majority of current polymer-based monoliths are based upon styrene-divinylbenzene copolymers [94], but monoliths based upon other technologies such as methacrylate *quin al ne*, acrylate [66-67, 71-72], acrylamide [82-87] and nonbornene [69-70, 93] have also been successfully synthesized. Because of the large number of commercially available monomers providing different functionalities, and ease of fabrication, the methacrylate-based polymer monoliths have attracted most attention among organic monoliths.
Compared to conventional packing with silica particles, the technology for the preparation of monolithic packing is simple, cheap and easier to transfer to micro-fluidic devices. Due to the highly porous structure and flow-through pores, the monoliths are ideal for high throughput applications, and more packing material can be used to obtain efficient separations without excessive flow backpressure. Further, because monolithic supports are synthesized in situ and may be covalently attached to the walls of the chromatographic device, there is no need for frits to keep them inside the device. Wide pH-tolerance (pH 2-12) is another advantage of monolithic materials when compared to conventional silica particles [94]. However, problems with inadequate reproducibility in monolith synthesis are still a major obstacle for their commercialization breakthrough.

1.6.1 Methacrylate Based Porous Polymer Monoliths

As mentioned above, the preparation of methacrylate porous polymer matrixes and their use in chromatographic separation was published for the first time by Kubin et al [64]. Since then, such monolithic supports have been used in many chromatographic areas including GC, LC, CEC, SPE and microfluidic devices.

The polymerization mixture of methacrylate based monoliths consists of the monomer(s), a cross-linker and an initiator in the presence of a combination of porogenic solvents. The preparation procedure is simple and straightforward. Basically, after mixing, the polymerization mixture is degassed using nitrogen gas in order to remove oxygen and poured into a surface modified chromatographic device for polymerization in situ, thermally or under UV-light. Surface modification may be necessary in order to covalently attach the monolithic polymer to the tubing. This results in a mechanically stable chromatographic device without voids forming between the monolith and the tubing walls. Before use, the monolithic material is washed with an organic solvent to remove possible unreacted compounds. Figures 5-6 show the structure of monomers and initiators used in this work.
There are a number of factors affecting the porous properties of the monoliths. The factors to be considered are the composition and amount of the porogenic solvents, the cross-linker, the type and amount of initiator, and the polymerization temperature or the intensity of the UV-light [95-98]. To obtain monoliths with desired pores properties, most often, the amount and composition of the porogenic solvents in the polymerization mixture are optimized.
1.7 Separation Techniques

1.7.1 Liquid Chromatography (LC)

Chromatography was invented by Michail Semenovich Tswett (1872-1919) in his investigation of plant extracts [99]. Tswett named the technique chromatography which literally means “writing in colours”. This refers to the coloured band separated such that they were visible to the eye. Liquid chromatography (LC) is presently the dominating separation technique in analytical chemistry [100]. The separation mechanisms and classification principles in LC and SPE are essentially the same (section 1.2.2). Modern high-performance liquid chromatography (HPLC) arose in the 1960s [101-103]. HPLC is a suitable technique for the analysis of compounds having different polarities, molecular weights, thermal instability or tendency to ionize in solution. This flexibility has resulted in different separation modes such as reversed-phase, normal-phase, ion-pairing, ion-exchange and size exclusion.

Nowadays the dominating LC mode is reversed-phase liquid chromatography (RP-LC). In this technique the stationary phase is usually silica spheres with hydrophobic surfaces, and the mobile phase is hydrophilic and is often prepared as a buffer for stable pH. The commonly used silica spheres have particle sizes of 3-10 µm, depending on the application. The most common analytical columns are made of stainless steel tubing of 2-5 mm internal diameter and 50-200 mm length. A basic LC apparatus is shown in Figure 7.

![Figure 7. Schematic setup of an LC system. 1. Pump, 2. Mobile phase mixer, 3. Injector Valve, 4. Column and 5. Detector.](image)

The sample is introduced at the top of the column by the injector. The pump(s) create a flow of mobile phase through the column. The analytes move with
different rates through the column, elute one after another from the column and finally they are measured by the detector.

The average rate, at which the analytes migrate through the column, depends on the differences of distribution of analytes between the stationary and mobile phases. The separation is thus based on the affinity of analyte to the stationary phase. Analytes with higher affinity to the stationary phase will have longer retention time in the column than analytes with lower affinity. This principle is common for all types of chromatography.

The degree of retention, expressed by the retention or capacity factor $k'$, can be calculated from the following equation:

$$k' = \frac{t_r - t_0}{t_0}$$  \hspace{1cm} (1)

where $t_0$ is the retention time for an unretained analyte and $t_r$ is the retention time for a retained analyte from point of injection to the apex of its peak.

### 1.8 Mass Spectrometry (MS)

Mass spectrometry (MS) was discovered by the physicist J. J. Thomson (1856-1940) [104]. MS is today one of the most important analytical techniques for molecular analysis. The basis in MS instruments is the production of ions and separation or filtration according to their mass-to-charge (m/z) ratios under vacuum [105]. Presently mass spectrometry has the best compound selectivity, sensitivity and specificity among chromatographic detectors. From a mass spectrum qualitative as well as quantitative information can be obtained.

Combining an LC instrument with MS was considered as an “unnatural marriage” [106]. LC operates in condensed phase while MS operates under vacuum. Because of this difference, care must be taken when integrating these two techniques. In order to maintain this intactness, the solvent molecules must be evaporated and targeted analytes must be transferred into the gas phase. The ionization technique used in this study was electrospray ionization (ESI), operated in positive ion mode. The solvent from the LC was passed along a stainless steel capillary tube, to the end of which a positive electrical potential
(3-5 kV) was applied. The electrical field causes the solution to be vaporized into a spray of fine droplets. To assist this, drying gas, nitrogen (N₂), flows along and past the capillary. Before entering the mass spectrometer probe these droplets pass through a slightly heated vacuum tube where the solvent evaporates and droplets become smaller. As the droplets become smaller the electrical charge density increases until such that neutral molecules are released from the surface. Finally, the remained sample ions enter the analyser where their mass to charge ratios can be determined [106-107].

In this study a triple quadrupole mass spectrometer (MS-MS) was used to obtain the desired information about targeted analytes. As the targeted analytes from the chromatographic source entered the MS-MS, the first quadrupole (Q1) was focused on parent ions of the selected target analytes, Figure 8. In the second quadrupole (Q2), using argon as collision gas, these ions were fragmented into lower (m/z) ratio product ions. These product ions were then accelerated into the third quadrupole (Q3), where only one characteristic product ion from each targeted analyte was monitored. This technique is called selected reaction monitoring (SRM). Because each analyte has unique SRM pattern, each analyte can in principle be quantitatively analysed without chromatographically being separated from each other [107-108]. This technique was necessary in quantitative analysis in this study, when no column was used (Paper I-II).

![Figure 8. Schematic presentation of how SRM ion experiments in MS-MS are carried out.](image)

1.9 Surface Modification of Polypropylene (PP)

Most often chromatographic devices for microanalysis are fabricated from inorganic materials such as glass, quartz and silicone. The well established
surface pretreatment methods using the silane 3-tri((methoxysilyl)propyl)methacrylate (γ-MAPS) [109-112] may be one of the primary reasons for this. However, plastic materials such as polypropylene are becoming increasingly popular due to their low cost of production in large quantities. This facilitates their one time disposable use.

Polyolefins such as polypropylene (PP) are based on carbon and hydrogen originating from monomers containing a double bond in the 1-position, Figure 9. The performance of polymeric material relies largely upon the properties of their surfaces. Polyolefins have hydrophobic inert surface properties which often limit their further applicability. To improve the wettability and adhesion properties of these polymers, a surface modification may be necessary. Ideally, the modified layer should be a surface layer such that the bulk properties beneath the polymer surfaces are not affected. Plasma treatment using gases such as oxygen, nitrogen, argon or carbon dioxide is commonly used as a surface modification technique [113-114]. The change of surface wettability after plasma treatment seems to depend largely on the gas that has been used. This technique has been further explained [115-116]. However, such modification techniques seem to be difficult to control, they often cause problems with respect to reproducibility and are therefore not suitable for the modification of micro-devices intended for chromatography.

![Propylene Polypropylene Structure](image)

*Figure 9. The structure and reaction of polypropylene.*

The application of UV initiated grafting is known to be a simple method for the modification of polymeric surfaces [117-118]. Here UV-light and initiators that can abstract protons, thereby generating surface radicals, are being used. This technique has been applied for the modification of various types of polymers e.g. COC, PDMS, PC and PMMA [119-125].

A slightly modified sequential photoinduced living graft polymerization method developed by Bowman and coworkers was used in this work for the modification of polypropylene [126]. This process consists of two steps. In the first step, a surface initiator is covalently attached to the surface of the PP
substrate under UV irradiation. The initiator abstracts hydrogen from the substrate to generate surface initiator sites. In the second step, the monomer solution is added to the modified substrate and attached initiators initiate the graft polymerization under UV irradiation. The effects of wetting and adhesion behaviour of modified PP were examined using contact angle measurements. Further, the mechanical stability and attachment of the monoliths to the plastic walls of PP tips were studied using scanning electron microscopy (SEM) and by using the modified pipette tips for the sample preparation of drugs in plasma samples.

1.9.1 Contact Angle (CA) Measurement

When a liquid droplet is placed on a solid surface, a three-phase equilibrium exists between the liquid molecules, solid surface molecules and vapor phase. The shape of the liquid droplet is determined by forces existing between liquid molecules, and between liquid and solid surface molecules. The liquid droplet spreads out if the attractive (adhesion) force between liquid and solid is stronger than, in our case, hydrogen forces that exist between the aqueous liquid molecules. If the hydrogen forces are stronger, the opposite happens and the liquid droplet beads up. The angle formed between liquid-solid interface and the tangent to the droplet profile at the liquid-solid-vapor contact point is referred to as contact angle (θ), Figure 10. CA is usually measured to qualitatively and quantitatively represent wettability or surface energy of a solid. Wettability is the degree to which a solid may be wetted by a liquid. Usually, the higher the contact angle, the lower is the surface energy, the lower the wettability and the liquid droplet beads up [127-128].

![Figure 10. Sideview of aqueous liquid droplet on a solid surface. Where SV, LV and SL are solid-vapor, liquid-vapor and solid-liquid interfaces.](image)

The hydrophilic properties of polymer surfaces may be improved by surface modification. Commonly used modification techniques are flame treatment and plasma treatment such as corona discharge [127-128]. In such, the surface
wettability is changed by introduction of polar groups on the surface. A common way to characterize the surface wettability is by the drop shape method. Using a microsyringe, one liquid drop (~2 µL) is applied at the solid surface. The adhesion character is then evaluated by following the shape of the liquid drop using a high-speed camera. As the droplet is released from the syringe, images are recorded until equilibrium is established. The contact angle is then measured using goniometry.

1.9.2 Scanning Electron Microscopy (SEM)

Scanning electron microscopy (SEM) is one of the oldest and one of the most widely used techniques for surface analysis [129-130]. Scanning electron microscopy (SEM) creates three dimensional visual images by using electrons instead of light. The SEM images are formed by scanning a focused electron beam across the sample of interest. Electrons thus hit the conducting sample and knock out secondary electrons from the sample surface. These secondary electrons are counted by a detector and sent to an amplifier. Thus, the shape of final image is dependent on the number of secondary electrons knocked out from the sample surface. In this work before imaging, the nonconductive polypropylene polymer samples were placed on conductive carbon cement and sputtered under vacuum with a thin layer of gold coating in order to make them conductive.

1.10 Analytes

1.10.1 Local Anaesthetics

All local anaesthetics have three characteristic components: an aromatic head, an intermediate portion and an amino group tail. The intermediate portions of the anaesthetics in this work are an amide (CONH-). Ropivacaine, lidocaine and bupivacaine are basic, amide type local anaesthetic drugs, Figure 11. Ropivacaine and bupivacaine are mainly used for surgery and for postoperative pain relief [131]. Unlike bupivacaine, which is used as a racemic mixture, ropivacaine is exclusively the S-(−)-enantiomer. Ropivacaine has a lower central nervous and cardiotoxic potential than bupivacaine [132]. Lidocaine has antiarrhythmic effects and is used in the treatment of cardiac disorders [131].
Ropivacaine is metabolized before being excreted, mainly in the liver [133]. The metabolic pathways include aromatic hydroxylation and N-dealkylation [134]. The major metabolites of ropivacaine are PPX and 3-OH-ropivacaine, Figure 11.

![Structures of local anaesthetics](image)

**Figure 11.** Structures and molecular weights of local anaesthetics utilized in this work.

### 1.10.2 Roscovitine and Olomoucine

Roscovitine and olomoucine, Figure 12, are purine derivatives considered as possible new anti-cancer drugs [135-136]. The drugs selectively inhibit cyclin-dependent kinases (Cdks), which are enzymes that play a crucial role in cell cycle regulation and several vital cell processes. The cellular effects of these
drugs include inhibition of cell proliferation, induction of DNA fragmentation, inhibition of RNA and DNA synthesis, cell cycle arrest in S-phase, induction of apoptosis and as competitive inhibitor for ATP [136-141].

Figure 12. The structures and molecular weights of roscovitine and olomoucine (I.S.).
2 The Aim of This Work

Short total analysis time is desired in chemical analysis. Most often the total analysis time is limited by the sample preparation step in the analytical procedure. Thus, sample preparation is often considered as a bottleneck in a system for chemical analysis. The general aim of this work has been to investigate and develop new techniques to address this problem. The importance of these new techniques has been to provide full automation, miniaturization, on-line coupling to detection systems, short sample preparation time and high-throughput.

More specific aims were to:

- apply and evaluate microextraction in packed syringe (MEPS) as a new sample preparation method.

- prepare and evaluate disposable plastic pipette tips packed with porous monolithic polymers in connection with 96-well sample plates.

- evaluate the methods by the analysis of local anaesthetics in biological matrices such as plasma.
3 Results and Discussions

3.1 MEPS Considerations (Papers I, II and V)

In Papers I, II and V, MEPS was performed using a 250 µL gas tight syringe. The sorbent in the form of particles was weighted and inserted into the syringe barrel as a plug and tightened with a polyethylene filter (20 µm pore sizes) from both sides. Before using for the first time, the sorbent was manually conditioned with 50-100 µL methanol followed by 50-100 µL of water. After that, when automated (Papers I and II), the syringe was connected to an autosampler and spiked plasma sample (25 µL) was drawn onto the syringe by the autosampler. It is important that plasma samples are drawn slowly (20 µLs⁻¹) to obtain good percolation between sample and solid support. The flow rate also can affect the retention of the analytes. The sorbent was then flushed with washing solvent to remove interferences and the targeted analytes were after that desorbed by an elution solvent directly into the analytical system liquid chromatography-tandem mass spectrometry.

In Paper V, above mentioned processing steps were performed manually. Before using for the first time, the sorbent was manually conditioned as mentioned above. After that, manually spiked plasma sample (100 µL) was drawn onto the syringe and then washed once with 100 µL of water (0.1% formic acid) to remove interferences. Finally, the analytes were desorbed by 100 µL methanol/water 95:5 (v/v) (0.25% ammonium hydroxide) directly into polypropylene vials for analysis with liquid scintillation counter.

In MEPS, many extractions were performed with the same plug of sorbent. To be able to do that, the sorbent was flushed between every extraction, first with elution solvent then by the washing solvent. This step decreased memory effects, but also functioned as conditioning step before the next extraction. In the case of plasma samples the same plug of sorbent was used for about 100 extractions. Then the extraction efficiency was reduced, and therefore the sorbent was exchanged.

To measure analyte response when using MEPS the recovery was defined. The recovery was measured as response of a processed spiked plasma sample as percentage of pure standard solution.
3.2 Method Development (Papers I, II and V)

To optimize the recovery for the selected analytes, some parameters affecting the recovery were determined. These were: type and amount of sorbent, the composition and volume of the washing solution, composition and amount of the elution solution, selectivity and carry-over. Further, good understanding of the interactions between the analyte, the sample matrix and the sorbent was necessary for optimization of the extraction process.

3.2.1 The Sorbent

In Papers I, II and V the studied substances are amide type local anaesthetics, weakly basic, and with pK_a-values between 7.0 and 8.1. When these drugs are in plasma samples they may be protein-bound which reduces the recovery. To disrupt these bindings the pH of the samples was shifted (pH ~3) using 0.1% formic acid. At this pH the studied analytes are positively charged. Thus, when the type of sorbent was selected both ion-exchange and hydrophobic interactions had to be considered. Sorbents containing strong cation-exchange and non-polar functional groups was chosen for the extraction of these analytes, Papers I-II and V.

In Paper I different silica based sorbents (C_2, C_8 and C_18) and a hydroxylated polystyrene-divinylbenzene polymer (ENV+) were investigated, see Figure 13. The silica sorbents had particles with an average size of 50 µm and polymer particles were 90 µm [142]. In LC, it is well known that the hydrophobic retention depends on the length of the carbon chain as well as the number of carbon chains bonded at the surface of the silica spheres. An increase of both these factors increases the hydrophobicity. A large number of carbon chains bonded at the silica surface also increases the specific surface area of the sorbent. In addition, there are residual silanols at the surface of the silicas used in this work. Because these silanol groups were not totally shielded from the analytes, in addition to hydrophobic interactions, ionic interactions could occur when analytes were positively charged. As can be seen from Figure 13, different silicas (C_2, C_8 and C_18) provided higher sample recovery than the polymer (ENV+). Further, the recovery increased with decreased length of the carbon chain at the silica surface. The highest recovery was obtained when silica based C_2 was used as sorbent material. This, probably because the targeted analytes
were slightly polar and were made positively charged in plasma matrices and/or because the C₂ was less hydrophobic the analytes could more easily be desorbed.

![Figure 13. Effect of type of sorbents on the recovery (%) of ropivacaine, Paper I.](image)

In Paper V when ENV+ and C₁₈ were used as sorbent, ENV+ showed slightly higher recovery than C₁₈. This may be due to the fact that the sorbents in Paper I and V were from different batches and may have had different average particle sizes. However, it has been reported [142] by the manufacturer of the sorbent that ENV+ has generally higher surface area and thereby higher sample capacity than silica based sorbents. In addition, polymeric sorbents such as ENV+ do not have problems associated with silanol residual (hydroxyl groups attached to the silicas) interaction, and may be used under wider pH ranges. However, a slight swelling of the polymer may lead to increased backpressure.

In Paper II, a silica based benzenesulphonic acid was utilized as sorbent material. This sorbent is strongly acidic (pKa~1) and thereby charged over the entire pH range. The primary retention on this sorbent is due to strong cation exchange, but there are also other interactions such as non-polar interactions. When the pH of the sample matrix was low, the targeted local anaesthetics were positively charged and the sorbent material was negatively charged. In such case the analytes were adsorbed to the sulphonic functional groups at the surface of the sorbent material mainly because of ionic interactions. To break these
interactions the analytes were made neutral by using an elution solvent with high pH-value (pH~11).

To achieve acceptable recovery and eliminate carry-over (section 3.3), the amount of sorbent was optimized in relation to the nature and amount of sample, the washing solvent and elution solvent. As can be seen from Figures 14 (Paper I) and 15 (Paper II), using an amount of 0.5 mg sorbent material, recovery was lower than with 1 mg of sorbent. This was probably due to insufficient adsorption capacity of the sorbent. Also when 2 mg of packing bed was used the recovery decreased. The reason for this could be that a larger volume of the elution solvent was needed for desorption of the analytes. The smallest amount of sorbent which resulted in about 50 % recovery was 1 mg when 25 µL plasma sample was extracted, Figures 14 and 15. This amount of sorbent was suitable for a concentration range of 2-5000 nM of the test analytes. For higher concentrations the amount of sorbent should be increased.

---

**Figure 14.** Effect of amount of sorbent, C₂, on the recovery of ropivacaine and its metabolites PPX and 3-OH-ropivacaine compared to direct injection of pure standard solutions, Paper I.
3.2.2 The Washing Solvent

The purpose of the washing solvent in the MEPS process is to selectively remove unwanted compounds from the sorbent without losing the analytes. As mentioned above, the analyzed local anaesthetics are weakly basic and their extraction from the plasma samples is based on ionic and non-polar interactions. In such a case, both the pH and the concentration of organic solvent will have effect on desired washing performance.

In Paper I, water containing different concentrations of organic solvents was tested to optimize the washing solvent. The volume of the washing solvent was 50 µL. As can be seen from Figure 16, increasing concentration of organic solvents in the washing solution decreased the analyte response. The reason for this could be that the sorbent was silica based C<sub>18</sub>, where the interactions are primarily hydrophobic. The use of 10% methanol decreased the recovery by about 10% compared to water alone.
In Paper II, when benzenesulphonic acid cation exchange sorbent was used the isolation of the analytes from plasma matrices was primarily due to ionic interactions. To prevent too high analyte losses, control of the pH, ionic strength of the washing solution as well as the concentration of organic solvent was necessary. Using a solution at low pH, the analytes remained positively charged and their interaction with the sorbent material was not interrupted. Different mixtures of water and methanol containing 0.1% formic acid were tested as washing solution. The lowest amount leakage (<0.2), with no detectable interferences and highest recovery was obtained when using 100 µL water containing 0.1% formic acid as washing solution.

Volume washing also has significant effect on resulted extract. Although cleaner extract may be obtained, higher washing volumes result in more leakage of analyte, Figure 17.
Figure 17. Effect of volume washing solvent, water (0.1% formic acid), on analyte leakage. 1 mg of ENV+ was used as sorbent and 1970 nM [3H]-bupivacaine was used as sample, Paper V.

3.2.3 The Elution Solvent

The elution solvent should be one which is able to displace targeted analytes from the sorbent in a minimum volume, a quantity that is directly injected into the analysis instrument, LC or GC. If the retention is based on hydrophobic interactions only, a non-polar solvent would be enough to disrupt the forces that bind analytes to the sorbent. Further, in cases when there are ion exchange interactions, the pH of the elution solvent should be 2 pH units above \( \text{pK}_a \) values of the targeted analytes for their elution. In addition, in Paper II the targeted analytes can be eluted by neutralization of the sorbent material or using a solution with high ionic strength. However, because ESI-MS-MS was used the latter options were avoided because of the risk for source contamination and interferences with targeted analytes when using high salt concentrations [143-144].

In Paper I, when silica based C2 was used as sorbent material, besides the hydrophobic forces between the targeted analytes and the packing bed, there were also ionic interactions. Residual silanols were probably the reason for the latter interactions. In Paper II, the primary retention mechanism was ionic
interactions and secondary interactions were hydrophobic. This means that the retention mechanisms in both cases were quite similar. To elute the targeted analytes a solvent capable of breaking both hydrophobic and ionic forces was needed. As can be seen from Figure 18, to optimize the recovery different mixtures of water and methanol containing 0.25% ammonium hydroxide were tested as elution solvents. Keeping the concentration of ammonium hydroxide constant the recovery of ropivacaine increased as the concentration of methanol increased, Figure 18. The percentage recovery was about 50% when methanol/water 95:5 (v/v) containing 0.25% ammonium hydroxide was used. This elution solvent was used in both papers.

![Figure 18](image_url)

**Figure 18.** Effect of different elution solvents on the recovery of ropivacaine using 1 mg of C₂ and C₈ as sorbents.

In paper V, to further investigate the effect of elution solvent, different elution volumes were tested for optimizing amount extracted. As can be seen in Figure 19 the analyte response increases as elution volume increases up to 75 µL. This breakthrough volume is significant for this particular application.
As has been pointed out above, sample solution may be drawn through the needle into the syringe up and down once or several times. Figure 20 shows the effect of such procedure using 1 mg of ENV+ and C18 respectively as sorbent. As can be seen from Figure 20, sample response increases as applied sample volume increases up to examined 750 µL sample. This corresponds to three times, 250 µL, up and down.

**Figure 20.** Response of 1970 nM [3H]-bupivacaine as function of applied sample volume. Elution volume and washing volume were 100 µL respectively. The same syringe and sorbent was used for all runs. Between the extractions the sorbent was washed with 2*250 µL of elution- and washing solution respectively, Paper V.
3.3 Carry-over

One of the limitations of automated systems is analyte carry-over. This effect depends on many factors including adsorption properties of the analytes, apparatus being employed and sensitivity of the method. In a worse case, carry-over can severely affect the precision and accuracy of the method. The smaller the carry-over the better the performance of the method will be [145].

In Papers I and II carry-over was tested by injecting the elution solvent after the highest standard concentration (2000 nM) had been run. To eliminate carry-over the sorbent was washed between every extraction as described in section 3.1. Using this procedure less than 0.5% carry-over was observed and after an additional blank no carry-over could be observed. According to Rossi et al. carry-over ranging from 0.01 to 0.5% is typical for automated systems [145].

Performance of MEPS regarding carry-over for ENV+ and C_{18} was further investigated in Paper V. According to this, ENV+ results in higher carry-over than C_{18} when same conditions were applied. As can be seen from Figure 21, the percentage carry-over increases with volume sample extracted up to applied 750 µL of sample. Percentage carry-over for ENV+ is, in general, higher than for C_{18} sorbent, Figure 21. This may be due to the higher interactions that exist for ENV+ polymer.

![Figure 21](image)

**Figure 21.** Carry-over of C_{18} and ENV+ sorbents using different sample volumes. [^H]-bupivacaine with the concentration 1970 nM, Paper V. Before analysis of carry-over the sorbent was cleaned first with 250 µL of elution solution and then 250 µL washing solution.
To reduce carry-over the sorbent may be cleaned up more intensively between extractions. As can be seen from Figure 22 the response of carry-over was decreased from about 0.7 to 0.1 when the cleaning up volumes were increased from 250 µL to the tested 3x250 µL between extractions. That is, first with 3x250 µL elution solvent and then with 3x250 µL of washing solvent.

Figure 22. Effect of cleaning up of sorbent between the sample extractions on response of carry-over. 1970 nM [³H]-bupivacaine was used as test sample and 1 mg of ENV+ as stationary phase. Carry-over was examined with 100 µL of elution solution after the cleaning up procedure where the sorbent was first cleaned with elution- and then with the washing solution, Paper V.

3.4 MEPS Reproducibility (Paper V)

For the reproducibility measurements in Paper V, three different syringes packed with about 1 mg of C₁₈ sorbent each were compared. Reproducibility reflects the between-syringes precision of the method. To evaluate reproducibility different plasma samples were spiked with [³H]-bupivacaine (1970 nM and 10 nM) and analyzed under the same experimental conditions. Results from such evaluation of recovery and relative standard deviation (RSD %) are shown in Table I-II. As can be seen from Table I-II, an average recovery of about 44% and 35% was obtained for the concentrations 1970 nM and 10 nM of [³H]-bupivacaine respectively. Further, most of the adsorbed analyte was desorbed with the first elution step, elution 1. Considering washing and carry-over, less than 3% analyte leakage and 0.3% carry-over were obtained.
MEPS is also intended to be used for automated multiple pushing and pulling of sample through the sorbent. The results of applied sample 3x100 µL show an average recovery of about 60% (Table I). This is an increase in recovery of about 16% compared to applied sample volume 100 µL. A similar trend is seen for [3H]-bupivacaine (10 nM) although with somewhat lower recovery (Table II).

**Table I.** Sample recovery and RSD% of [3H]-bupivacaine (1970 nM) in plasma samples. RSD was measured as percentage standard deviation divided by mean value. Applied sample was washed with 100 µL water (0.1 formic acid) before desorbing with 100 µL MeOH/H2O 95:5 (v/v) (0.25% ammonium hydroxide) directly into liquid scintillation bottles for measurements, Paper V.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Recovery (%)</th>
<th>Average (%)</th>
<th>RSD%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Syringe 1</td>
<td>Syringe 2</td>
<td>Syringe 3</td>
</tr>
<tr>
<td>Applied sample 100 µL</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wash</td>
<td>2.1</td>
<td>2.1</td>
<td>1.9</td>
</tr>
<tr>
<td>Elution 1</td>
<td>51.4</td>
<td>44.1</td>
<td>38.6</td>
</tr>
<tr>
<td>Elution 2</td>
<td>2.2</td>
<td>4.8</td>
<td>3.4</td>
</tr>
<tr>
<td>Elution 3</td>
<td>0.2</td>
<td>0.6</td>
<td>0.8</td>
</tr>
<tr>
<td>Carry-over</td>
<td>0.0</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>Applied sample 100 µL x 3</td>
<td>30.5</td>
<td>30.8</td>
<td>30.7</td>
</tr>
<tr>
<td>Wash</td>
<td>2.5</td>
<td>2.7</td>
<td>3.1</td>
</tr>
<tr>
<td>Elution 1</td>
<td>63.1</td>
<td>59.9</td>
<td>59.3</td>
</tr>
<tr>
<td>Elution 2</td>
<td>2.9</td>
<td>5.6</td>
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<tr>
<td>Elution 3</td>
<td>0.8</td>
<td>0.9</td>
<td>0.9</td>
</tr>
<tr>
<td>Carry-over</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
</tr>
</tbody>
</table>

**Table II.** Sample recovery of [3H]-bupivacaine (10 nM) in plasma samples, Paper V. Other experimental conditions see Table I.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Recovery (%)</th>
<th>Average (%)</th>
<th>RSD%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Syringe 1</td>
<td>Syringe 2</td>
<td>Syringe 3</td>
</tr>
<tr>
<td>Applied sample 100 µL</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wash</td>
<td>2.3</td>
<td>1.7</td>
<td>2.2</td>
</tr>
<tr>
<td>Elution 1</td>
<td>28.1</td>
<td>36.2</td>
<td>41.2</td>
</tr>
<tr>
<td>Elution 2</td>
<td>10.8</td>
<td>14.0</td>
<td>10.6</td>
</tr>
<tr>
<td>Elution 3</td>
<td>7.5</td>
<td>8.0</td>
<td>6.6</td>
</tr>
<tr>
<td>Carry-over</td>
<td>6.7</td>
<td>5.4</td>
<td>6.1</td>
</tr>
<tr>
<td>Applied sample 100 µL x 3</td>
<td>29.2</td>
<td>30.6</td>
<td>31.1</td>
</tr>
<tr>
<td>Wash</td>
<td>2.3</td>
<td>3.5</td>
<td>3.6</td>
</tr>
<tr>
<td>Elution 1</td>
<td>39.8</td>
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<td>7.4</td>
<td>10.2</td>
<td>8.3</td>
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<tr>
<td>Elution 3</td>
<td>18.0</td>
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<td>5.8</td>
</tr>
<tr>
<td>Carry-over</td>
<td>3.2</td>
<td>5.3</td>
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</tr>
</tbody>
</table>
3.5 High Sample Throughput Using Monolithic Packed Tips (Paper III-IV)

Pipette tips solid-phase extraction is getting increased attention for analytical applications. As highly efficient, inexpensive and disposable, pipette tips are commonly used in analysis for desalting, concentrating samples or removing interferences. In Paper III and IV disposable pipette tips (550 µL) packed with methacrylate based monolithic sorbent in connection with 96-well plates were used for sampling. The 96-well plates were placed underneath the tips and the samples were eluted into them for further analysis by LC-MS-MS. In this way a 96-plate could be handled in about two minutes.

Most often the sorbent material used for sample cleaning up in pipette tips is silica spheres with chemically bonded functional groups, or a combination of silica spheres and a polymer. For instance, the first commercially available pipette tips ZipTip from Millipore (Bedford, MA, USA) contained silica spheres embedded in a polymeric scaffold.

Monoliths as sorbent materials for high-throughput and on-line SPE have been utilized by Xie et al. [146]. Further, several groups have recently used different approaches for the fabrication of monolithic packed pipette tips. For example, Hsu et al. [59] used photografting for the fabrication of disposable plastic pipette tips which they called EasyTip. The bed contained silica spheres (C18) blended with an acrylate polymer mixture. In order to physically stabilize the adsorbent plug they inserted a 1 mm thick ring obtained from the sharp end of a pipette tip into another pipette tip in which the monolith was prepared. In this case, the polymer was not chemically bonded to the tip wall. Stachowiak et al. [122] used photografting to fabricate monoliths covalently attached to the walls of micropipette tips. The approach was made in two steps, where the first step was to modify the surface of the tip.

Also monolithic silica based pipette tips have been developed [60]. Monolithic silica modified with C18 or coated with a titania based phase was e.g. used for analysis of proteins. The packing was fixed into 200 µL pipette tips by supersonic adhesion.

In this work (Papers III and IV) we developed a simple synthesis strategy for the fabrication of photopolymerized methacrylate based monolithic polymers attached to the walls of polypropylene pipette tips. The pipette tips were then
used for the sample preparation of some local anaesthetics and roscovitine in human plasma samples.

### 3.5.1 The Preparation of Porous Polymer Monoliths

Polypropylene based pipette tips with total volume of 550 µL, suitable for our liquid handling system from Apricot Designs, Inc (Monrovia, CA, USA) were used for sampling. The monolithic mixture was prepared using a modified method originally suggested by Svec and Fréchet [65]. As can be seen from Table III the relation monomers to porogenic solvent in the polymerization mixture is 40:60 in both our papers. This is according to the optimized ratio presented by Svec and coworkers. Before polymerization, the prepared mixture was vortexed for 10 min to dissolve the initiator and purged with nitrogen for 10 min in order to remove oxygen.

**Table III.** Composition of the polymerization mixture used for the preparation of monolithic plug in Paper III and IV respectively.

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Paper III</th>
<th>Paper IV</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>wt%</td>
<td>wt%</td>
</tr>
<tr>
<td>BMA</td>
<td>3,5%</td>
<td>30,0%</td>
</tr>
<tr>
<td>EGDMA</td>
<td>15,5%</td>
<td>70,0%</td>
</tr>
<tr>
<td>GMA</td>
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<td></td>
</tr>
<tr>
<td>1-dodecanol</td>
<td>30,0%</td>
<td></td>
</tr>
<tr>
<td>Cyclohexanol</td>
<td>30,0%</td>
<td></td>
</tr>
<tr>
<td>1-propanol</td>
<td></td>
<td>65,0%</td>
</tr>
<tr>
<td>1,4-butanediol</td>
<td></td>
<td>25,0%</td>
</tr>
<tr>
<td>Water</td>
<td></td>
<td>10,0%</td>
</tr>
<tr>
<td>AIBN</td>
<td>1,0%</td>
<td></td>
</tr>
<tr>
<td>BPO</td>
<td></td>
<td>2,0%</td>
</tr>
</tbody>
</table>

The final properties of the pores of a monolith depend on the composition of the polymerization mixture as well as polymerization temperature or radiation power used for the initiation. Of these factors, the type and composition of the porogenic mixture seem to be the key factors for fine tuning of the final properties of the polymer monoliths. A dissertation dealing with e.g. optimization of the ternary porogenic mixture 1,4-butanediol/1-propanol/water and its effect on the pore sizes distribution and surface area of the final monolith was published by Eeltink [147]. According to this, median
pore sizes of the monolith increases as percentage 1,4-butanediol in the polymerization mixture increases up to 25%. The pore sizes seem to decrease as the percentage 1,4-butanediol in the polymerization mixture is above 25%. In addition, as was pointed out by Eeltink, small changes in the content of the polymerization mixture have large effect on the pores properties of the final monolith. On the basis of this, a polymerization mixture containing about 25% 1,4-butanediol was used for preparation of monolithic plug in pipette tips, Table III (Paper IV). This resulted in a monolith with large pores properties and thereby low backpressure necessary for sample cleanup by the robot.

Another commonly used porogenic mixture for the preparation of methacrylate based monoliths is cyclohexanol and 1-dodecanol [148]. In such a porogenic mixture the pore sizes seem to increase as the percentage dodecanol in the polymeric mixture increases. For the preparation of the monolithic plug in Paper III the porogenic solvent contained 30% 1-dodecanol and 30% cyclohexanol. This resulted in monoliths with pores properties useful for low backpressure sample cleanup by the robot, Figure 23.

Figure 23. Influence of the wt% 1-dodecanol in the polymerization mixture on the backpressure. Test solvent was methanol at the flow rate 100 µL/min.
3.5.2 Surface Modification of Polypropylene (PP)

In Paper IV, the plastic walls of the pipette tip were modified prior to *in situ* preparation of the monolith. The aim of the surface modification was to create reactive groups and improve the wetting ability of the PP substrate that may help to covalently attach a plug of monolithic polymer. To demonstrate surface modification of pipette tips, contact angle measurements of easily handled rectangular PP sheets, about 4 cm x 1 cm x 1 mm, were utilized as models. A flat surface is required for the correct measurements of contact angles. Two different surface modification approaches were tested. The first modification approach was developed by Bowman et al. [126] and is referred to as photoinduced grafting. The second modification approach was developed by Svec and coworkers [122] and is referred to as surface modification by grafting.

3.5.2.1 Photoinduced Grafting

A hydrophilic character of the PP is important for the anchoring of a monolithic plug to the PP polymer. To compare the hydrophilicity of the original PP surface and the modified PP surface, contact angle measurements of water were performed. A comparison was made between modified and non-modified PP sheets after different irradiation times. As shown in Table IV the original surface of PP is very hydrophobic with a contact angle of about 98° (n=10), the manufacturer reports 92° to 100° [149]. This contact angle value is in line with values between 84.6° and 103° which have been reported by other research groups [126, 150]. Contact angle measurements are very sensitive to impurities and irregularities at the surface. These in combination with slight differences in manufacturing procedures and temperature may result in somewhat different contact angle values. This may be the reason for the relatively wide differences in results in contact angle values. The precision of our measurements for non-modified PP, inter-day and intra-day values were between 2% and 4%. Impurities at the PP surface, temperature changes or surface unevenness may be the cause of this. In addition, small changes in camera angle may have an impact on the result.
Table IV. Contact angles average and precision, intra- and inter-day assays for the modification of polypropylene sheets with 5% benzophenone in methanol, Paper IV.

<table>
<thead>
<tr>
<th>Polymerization Time (s)</th>
<th>Contact Angle (θ) Average (n=10)</th>
<th>Precision (RSD) Intra-day (n=10)</th>
<th>(%)</th>
<th>Inter-day (n=2)</th>
<th>(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>98.0</td>
<td>2.8</td>
<td>3.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>270</td>
<td>84.8</td>
<td>10.2</td>
<td>10.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>500</td>
<td>82.3</td>
<td>3.8</td>
<td>3.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1500</td>
<td>82.4</td>
<td>6.6</td>
<td>5.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2700</td>
<td>81.2</td>
<td>7.2</td>
<td>6.3</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

n= number of measurements.

A significant decrease in contact angle values was observed with increasing irradiation time, Figure 24A. Compared to unmodified PP the contact angle values of water decreased from 97° to about 85° after only 270 seconds of irradiation. Appearance of more hydrophilic groups at the surface of the PP-sheets or grafting of initiators at the PP surfaces may result in the decrease of contact angle values. Further increases in irradiation time to 2700 seconds resulted in a decrease of the contact angle of water, to about 82°. This indicates that most of the modification of the PP surface and thereby changes of the contact angle values occur during the first 500 seconds of the irradiation. After 500 seconds the value remained almost constant. These results were in line with results presented by Lee and coworkers [151]. The intra- and inter-day variations were between 2.8% and 10.2%, and 3.2% and 10.2% respectively, Table IV.
Figure 24. Contact angle (θ) values of water on polypropylene (PP) sheets after different irradiation times. Modification mixture composed of (A) 5% BP in methanol and (B) methanol/1,4-butanediol 1:1, Paper IV.

Different solvents were tested to optimize the surface modification method, Figure 24B. Methanol may evaporate quickly and it is difficult to apply as an even layer on the hydrophobic PP surface. To investigate the effect of the solvent, a more viscous mixture with higher boiling point composed of methanol/1,4-butanediol 50:50 (wt%/wt%) with 5% BP as initiator was tested. Compared to the use of methanol as solvent this more viscous solution led to slightly higher contact angle values at the PP surface. This result may be due to the confining effect of solvent molecules, the cage effect, and it may lead to secondary reactions including recombination of the radicals to regenerate the
This effect is assumed to increase as the viscosity increases [152]. The energy gained by radicals from BP is partly lost in their collision with the surrounding large liquid molecules of 1,4-butanediol and the radicals are therefore in part stopped before reaching the PP surface.

To further evaluate the surface modification procedure, SEM images of non-modified pipette tips and tips modified with 5% BP in methanol were compared. The surface of a non-modified tip looks smooth without any observable irregularities, Figure 25A. However, in Figure 25B showing a surface modified tip, some irregularities can be seen. The cause of this may be the traces of initiator remaining after the modification and the subsequent washing procedure. Domains having different degrees of irregularity are visible and such irregularities may be the cause of the lower precision of the contact angle measurements as mentioned above.

3.5.2.2 Surface Modification by Grafting

Figure 26 shows SEM images of surface grafted PP tips using BP and AIBN as initiators respectively. To optimize the modification approach, amount of initiator as well as initiation time were optimized so that polymerization resulted in a thin layer on the walls of the pipette tips. In cases where high amounts of initiator and long irradiation times were applied the polymer gel formed inside the tips could not be forced out. In contrast, when low amounts of initiator and short initiation times were applied, the degree of modification was not enough. Sufficient degree of modification usually resulted in that the almost transparent pipette tips became grayish and cloudy. When BP was used as initiator, an
initiation time of 10 minutes with 4% initiator was chosen as optimal. In cases, when AIBN was used as initiator even longer irradiation time did not result in changes that could be visually observed. However, as can be seen from Figure 26A these changes were visible when investigated with SEM.

3.5.3 Synthesis of Monoliths for Pipette Tips

The porous monolith was synthesized directly inside disposable pipette tips by in situ UV-light initiated polymerization at 254 nm. The pipette tips were placed vertically inside the polymerization apparatus and polymerization was allowed to proceed for 60-80 min with the sharp end of the tip down and at a distance to the UV lamp of 15 cm, and then for 25-50 min with the sharp end up and at a distance of 5 cm to the UV lamp.

After completion of polymerization the tips were removed, inspected under microscope for bubbles, and washed with methanol to remove the porogenic solvents and other compounds remaining in the monolith. Before use, the tips were washed with elution solvent.

Figure 27A-B displays images of the monolithic structure inside a pipette tip indicating complete filling of the polymer across the tube. No significant differences and no voids between the polymer matrix and the tip can be seen.
Figure 27. (A) Photographic images of UV-polymerized monolith inside a disposable micro pipette tip, Paper III. (B) SEM image of poly(ethylene glycol dimethacrylate-butyl methacrylate) monolith inside polypropylene based pipette tip, Paper IV. The tip was modified with 5 wt% BP in MeOH. No voids between plastic walls of the tip and monolith are visible. Porous monolith inside pipette tip modified with (C) MMA/EGDMA 1:1 (wt%/wt%) with BP (5 wt%) and (D) 5 wt% BP in methanol, Paper IV. The selected area indicates the gap between the plastic walls of the tip and the monolith.

In Figure 27C, the SEM image of a monolith inside a pipette tip modified with MMA/EGDMA 1:1 with 5% BP shows binding between the monolith and the pipette wall. The monolith inside the tip surface modified with 5% BP in MeOH shows binding between monolith and wall in the upper part of the image, Figure 27D. The lower part of the image shows a gap between the monolith and the pipette wall. The surface of the pipette wall in this part appears to be much smoother compared to the surface in the upper part of the image. Hence, the surface modified part seems to have obtained a more adhesive surface. However, because of the experimental simplicity and less sensitizing chemicals needed this modification approach was used for further studies of packed tips intended for sample preparation in Paper IV.
Another property important for pipette tips useful for sample preparation is good mechanical stability to withstand a complete sample preparation run cycle (conditioning, sample application, washing of the interferences and elution of analyte). Further, monoliths should have good hydrodynamic properties for easy and uniform pumping of solvent through the bed. In spite of the relatively high backpressure that results when working with plasma samples and a large number of aspirating/dispensing cycles, the monolith did not collapse or slip out of the tips. In fact, even under the application of pressure up to 10 bars (tested from both sides) the monolith did not slip out of the tip. This indicates a sufficient mechanical stability as well as suitable hydrodynamic properties of the tips. The same tip could be used for more than five complete run cycles as described above without any visually observable damages on the monolith.

3.5.4 Sampling

To evaluate the performance of the monolithic tips, roscovitine and lidocaine in human plasma were used as model substances in Paper III. In Paper IV ropivacaine in human plasma was used as model substance.

Before use, the tips were thoroughly washed with methanol and then conditioned with the wetting solution, water, to ensure optimum adsorption of the analytes. The manually prepared 96 pipette tips were handled by a robot, a Personal Pipettor (PP-550N-MS) obtained from Apricot Designs, Inc. (Monrovia, CA, USA), Figure 28.

The spiked plasma samples were aspirated and expired by the robot from the 96-well plate. When the samples passed through the monolithic bed the analytes were adsorbed to it. The monolithic bed was then washed once by water (100 µL) to remove hydrophilic interferences such as proteins and salts. To remove traces of washing solution left, the sorbent was dried with a stream of air. Finally, the analytes were eluted by methanol (100 µL) or the LC mobile phase directly into the 96-well plate for further analysis by LC-MS-MS. Using this procedure, the cleaning of the samples from possible interferences in a 96-well plate was performed in about two minutes; the speed of this part of the analytical procedure was thus greatly increased.
3.5.5 Selectivity

The method selectivity is defined as non-interference with the endogenous substances in the regions of interest. Figures 29A and 29B show representative mass chromatograms of blank human plasma and human plasma spiked with roscovitine and lidocaine, Paper III. According to these chromatograms, in the LC-MS-MS analysis of roscovitine in human plasma using monolithic pipette tips, no interfering compounds were detected at the same retention time as the studied compounds. This indicates a good selectivity for the application of monolith containing tips as sample preparation method in the analysis of roscovitine and lidocaine.
Figure 29. Mass chromatograms of (A) blank plasma and plasma spiked with 125 nM roscovitine and (B) blank plasma and plasma spiked with lidocaine (LLOQ), Paper III. Chromatographic conditions: column Zorbax 50 x 2.1 mm I.D., SB-C8, 3.5 µm connected to a guard column, an Optiguard 10 x 1 mm I.D., C8; mobile phase (A) 0.1% formic acid in ACN-Water (10:90, v/v), (B) 0.1% formic acid in ACN-Water (80-20, v/v); linear gradient 0% B for 1 min, 0-80% B in 4 min, 80% B in 1 min; flow rate 150 µL/min.

Figure 30 shows the extraction performance of pipette tips using ropivacaine in plasma samples at three different concentration levels, Paper IV. The results indicate the good binding capacity for ropivacaine under current conditions. By comparing the response factor (substance area/area internal standard) the extraction performance of different tips was compared. The relative standard deviation of quantitative determination using tips was 9%, 6% and 10% for concentration levels 30 nM, 300 nM and 3000 nM respectively (n=12).
These results indicate that both recovery and reproducibility of our tips were well suitable for qualitative and quantitative analysis.

![Graph showing response factors of monolithic pipette tips for ropivacaine extraction in human plasma samples](image)

**Figure 30.** Response factors of monolithic pipette tips for the extraction of ropivacaine in human plasma samples, Paper IV. The RSD% of the extraction performance of tips was 9%, 6% and 10% for concentration levels 30 nM, 300 nM and 3000 nM respectively (n=12).

In addition, we noticed a better flow through when the tips were kept in methanol prior their first use. The reason for this may be the relatively high amount of crosslinker in our polymerization mixture, which results in highly crosslinked globules with higher surface area [153]. Such monoliths are useful for sample preparation purposes. Higher degree of crosslinking may also result in monoliths with decreased pore sizes as a result of limited swelling of crosslinked nuclei during the polymerization [154]. However, laboratory results show a better flow through and that the degree of swelling and thereby the permeability may increase if these monoliths are kept in methanol prior to their use. An alternative to using pipette tips packed with a plug of monoliths as sample adsorbent where a certain backpressure is inevitable, micro-pipette tips with small internal diameter coated with the monolithic adsorbent may be used [155]. In such, the monolithic adsorbent does not completely fill the cross section of the tip rather the internal walls of the tips are coated with a porous layer of monolithic polymer. The liquid flow through the tips with negligible backpressure in combination with sensitive detector such as MS makes these pipette tips an interesting alternative to above mentioned packed pipette tips.
4 Conclusions and Future Aspects

A new method for sample preparation named Microextraction in Packed Syringe (MEPS) has been developed. In this thesis MEPS as a new miniaturized, on-line and fully automated solid-phase extraction (SPE) technique has been described. In addition, pipette tips packed with a plug of methacrylate monolith was used for high throughput sample preparation of some local anaesthetics and an anti-cancer drug, roscovitine, in human plasma samples.

Before conclusions from this thesis, some personal remarks about future of sample preparation:

Sample preparation has for long been a neglected area of research. Although there has been a fast development of other parts of the analytical instrumentation such as detectors, pumps, columns, etc during the past 30 years, which facilitates shorter analysis times, the total time spent on sample preparation is almost the same. Still about 80% of total analysis time is spent on sample preparation in some cases. This is a huge hurdle on the way of high throughput and fully miniaturized analytical systems. However, the importance of sample preparation for the total analytical procedure is nowadays realized [74, 156-159]. The quality of the sample preparation step is in many cases a key factor for success of analysis. The international symposium series International Symposium on Advances in Extraction Technologies (ExTech) which is fully dedicated to sample preparation has shown that.

Development of sample preparation with improvements in miniaturization, automation, formats and introduction of new selective solid phases such as immunoaffinity will continue. Lab-on-a-chip technique is an excellent idea in this development [160]. This is an attractive idea of a small chip with integrated complete analysis capability. The development of such devices could revolutionize analytical chemistry by making chemical measurements cheaper and more accessible [161]. In this development, polymeric phases, monoliths, may have an important role dependent on their reproducible preparation.

Among sample preparation techniques SPE has become the most popular. One reason for this is to decrease organic solvent usage in the lab. Another reason is the availability of large number of sorbents with different functionalities. I believe the development of more selective phases may be the key factor to separation free analysis. That is analysis without separation column. This, of
course, in combination with a selective detector such as the mass spectrometer. The direct analysis of samples, such as biological samples, by MS without a sample clean up step as have been suggested by some researchers may be difficult. The reason for this is reduced ionization efficiency and ion suppression because of matrix effect. Thus sample preparation will have an important role in the future of chemical analysis.

…MEPS conclusions

- Microextraction in packed syringe (MEPS) on-line with LC-MS-MS is an excellent sample preparation technique which was demonstrated for the determination of local anaesthetics in human plasma samples.
- Connected to an autosampler MEPS was fully automated and each sample took only about one minute to extract.
- In application to plasma samples the same plug of sorbent could be used for about 100 extractions before its extraction efficiency and the recovery was reduced.
- MEPS can provide suitable selectivities, require small sample volumes, consume low solvent volumes and can be used with different sorbent materials.

…Pipette tips conclusions

- Disposable pipette tips were packed with a plug of methacrylate based porous polymeric monoliths as sample adsorbent.
- Manually packed 96-tips were used in connection with a 96-well plate for the analysis of roscovitine and some local anaesthetics in human plasma samples.
- Using this system 96 samples were prepared for analysis in about two minutes.
- The surface of PP based pipette tips were modified using different experimentally simple approaches.
- Contact angle (CA) measurements and scanning electron microscopy (SEM) were performed to visualize the degree of grafting.
- The degree of grafting seems to vary with irradiation time, type- and amount of initiator, and the viscosity of the solvent.
- The SEM images indicate covalent attachment of the monolith to the modified surface of the tips.
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- All friends and relatives for support and providing a nice social environment.

- **Baw û De, Xweh n Bira**, sipas jibo destekawa û go win bi mira idaratikin. Bey destekawa av kar na mumkunbu.

- **Bêğên û Malin**, sipas ku win hana.
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New Techniques for Sample Preparation in Analytical Chemistry

Sample preparation is often a bottleneck in systems for chemical analysis. The aim of this work was to investigate and develop new techniques to address some of the shortcomings of current sample preparation methods. The goal has been to provide full automation, on-line coupling to detection systems, short sample preparation times and high-throughput.

In this work a new technique for sample preparation that can be connected on-line to liquid chromatography (LC) and gas chromatography (GC) has been developed. Microextraction in packed syringe (MEPS) is a new solid-phase extraction (SPE) technique that is miniaturized and can be fully automated. In MEPS approximately 1 mg of sorbent material is inserted into a gas tight syringe (100-250 μL) as a plug. Sample preparation takes place on the packed bed. Evaluation of the technique was done by the determination of local anaesthetics in human plasma samples using MEPS on-line with LC and tandem mass spectrometry (MS-MS). MEPS connected to an autosampler was fully automated and clean-up of the samples took about one minute. In addition, in the case of plasma samples the same plug of sorbent could be used for about 100 extractions before it was discarded.

A further aim of this work was to increase sample preparation throughput. To do that disposable pipette tips were packed with a plug of porous polymer monoliths as sample adsorbent and were then used in connection with 96-well plates and LC-MS-MS. The evaluation of the methods was done by the analysis of local anaesthetics lidocaine and ropivacaine, and anti-cancer drug roscovitine in plasma samples. When roscovitine and lidocaine in human plasma and water samples were used as model substances, a 96-plate was handled in about two minutes. Further, disposable pipette tips may be produced at low cost and because they are used only once, carry-over is eliminated.