CHARLES UNIVERSITY
FACULTY OF PHARMACY IN HRADEC KRÁLOVÉ
DEPARTMENT OF ANALYTICAL CHEMISTRY

DEVELOPMENT OF NOVEL APPROACHES TO AUTOMATED SAMPLE PREPARATION FOR PHARMACEUTICAL AND ENVIRONMENTAL ANALYSIS

DISSERTATION THESIS

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2020 *Mgr. Kateřina Fikarová*

UNIVERZITA KARLOVA
FARMACEUTICKÁ FAKULTA V HRADCI KRÁLVÉ
KATEDRA ANALYTICKÉ CHEMIE

VÝVOJ NOVÝCH METOD PRO AUTOMATIZACI PŘÍPRAVY VZORKU S POUŽITÍM VE FARMACEUTICKÉ A ENVIRONMENTÁLNÍ ANALÝZE

DIZERTAČNÍ PRÁCE

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STATEMENT OF ORIGINALITY

I declare that this thesis is my original work, which I developed independently under the supervision of my supervisor and consultant. All literature and other sources, used during the work processing, are listed in the list of references and cited properly in the thesis. This work has not been used to obtain another or the same title.

In Hradec Králové to the date of 24.7.2020 Kateřina Fikarová

PROHLÁŠENÍ O DÍLE

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V Hradci Králové dne 24.7.2020 Kateřina Fikarová

ABSTRACT

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Title of the Dissertation Thesis:

Development of novel approaches to automated sample preparation for pharmaceutical and environmental analysis

Since their introduction 60 years ago, flow techniques have become a popular tool for automation of various analytical processes and were applied in environmental, pharmaceutical, or food analysis. Above all, they are recognized for the small consumption of reagents, fast analysis in an automated manner, the possibility to study kinetics of the reactions, and their versatility of the operation.

This dissertation contributes to the field of automation of sample preparation and leaching studies of the environmental pollutants using flow techniques, mainly the technique Lab-In-Syringe. The theoretical part gives the reader some insight into the possibilities and advantages of automation, with emphasis to flow techniques. This section is divided into three main chapters with the first one dedicated to automation. The four subchapters are focused mostly on the flow techniques and their general principles, different instrumentation, and hyphenation with detectors. The second chapter describes the most frequently used sample preparation techniques and the possibilities of their automation. The third chapter reviews bioaccessibility studies of environmental contaminants and the ways how to automate them.

The experimental part consists of six publications and brief comments describing the novelty, the main and outstanding characteristics, and the results of each work. The first and second publication were focused on automation of in-syringe dispersive liquid-liquid microextraction for trace metal analysis in various matrices with and without back-extraction to the aqueous phase prior to inductive coupled plasma-atomic emission spectrometry. The third project was dealing with the development of direct-immersion single drop microextraction by Lab-In-Syringe technique in two different configurations of the syringe. The method was applied to the determination of lead in tap water. The fourth work described Lab-In-Syringe dispersive liquid-liquid microextraction with a continuous flow of the sample. This new method was applied to the determination of nitrophenols in the surface water using multivariate spectral analysis. The fifth project was dealing with the automation of homogeneous liquid-liquid extraction using the salting-out phenomenon with posterior preconcentration on the anion-exchange resin in one on-line system with liquid chromatography. The method was applied for to determination of sulphonamides in urine. The last work introduced a flow-based platform for automation of the dynamic leaching study of the plastic additives from microplastic to the seawater.

ABSTRAKT

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Název dizertační práce:

Vývoj nových metod pro automatizaci přípravy vzorku s použitím ve farmaceutické a environmentální analýze

Moderní průtokové techniky se od jejich prvního představení před 60 lety staly populárním nástrojem pro automatizaci různých analytických procesů a byly aplikovány v environmentální, farmaceutické analýze a analýze potravin. Jsou oblíbené hlavně pro malou spotřebu činidel, možnost rychlé automatizované analýzy, možnost sledování kinetiky reakcí a univerzálnost přístrojového vybavení.

Tato dizertační práce přináší nové poznatky v oboru automatizace přípravy vzorku a studie uvolňování environmentálních polutantů pomocí průtokových technik, hlavně Lab-In-Syringe techniky. Teoretická část pomůže čtenáři porozumět možnostem automatizace s důrazem na průtokové techniky. Tato část je rozdělena na tři hlavní kapitoly. První je věnována automatizaci. Ve čtyřech podkapitolách věnovaných hlavně průtokovým technikám je vysvětlen obecný princip, různé uspořádání přístrojového vybavení a spojení s různými detektory. Druhá kapitola pak představuje nejčastěji používané metody pro přípravu vzorku a možnosti jejich automatizace. Třetí kapitola shrnuje poznatky o studiích biodostupnosti environmentálních kontaminantů a způsobu automatizace těchto studií.

Experimentální část práce obsahuje souhrn šesti publikací s krátkými komentáři popisujícími originalitu projektu, hlavní charakteristiku a výsledky každé práce. První a druhá publikace byla zaměřena na automatizaci "in-syringe" disperzní kapalinové mikroextrakce pro stopovou analýzu těžkých kovů v různých matricích s použitím a bez použití zpětné extrakce do vodné fáze před emisní spektrometrií s indukčně vázaným plazmatem. Třetí projekt byl zaměřen na vývoj metody založené na extrakci do kapky rozpouštědla ponořené do vzorku s automatizací Lab-In-Syringe technikou se dvěma různými konfiguracemi instrumentace. Metoda byla použita pro stanovení hladiny olova v pitné vodě. Čtvrtá práce popisuje disperzní kapalinovou mikroextrakci automatizovanou Lab-In-Syringe technikou s kontinuálním průtokem vzorku. Metoda byla aplikována na stanovení nitrofenolů v povrchových vodách s použitím multivariantní spektrální analýzy. Pátý projekt se zabýval automatizací homogenní extrakce využívající metodu vysolování se zakoncentrováním na iontovýměnném sorbentu v rámci jednoho on-line systému spojeného s kapalinovou chromatografií. Metoda byla použita pro stanovení sulfonamidů v moči. Poslední práce představila průtokový systém pro automatizaci studie dynamického uvolňování aditiv z mikroplastů do mořské vody.

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INDEX OF ABBREVIATIONS

ACN - acetonitrile

APDC - ammonium dithiocarbamate

BBP - benzyl-butyl-phthalate

BI - bead injection

CE - capillary electrophoresis

DDTP - diethyl-dithiophosphate

DEP - diethyl-phthalate

DI-SDME - directly immersed- single drop microextraction

DLLME - dispersive liquid-liquid microextraction

DMP - dimethyl-phthalate

DnBP - di-n-butyl- phthalate

dSPE - dispersive solid-phase extraction

DV-SIA - dual-valve sequential injection analysis

FAAS - flame atomic absorption spectrometry

FBA - flow-batch analysis

FIA - flow injection analysis

FID - flame ionization detector

FT - flow technique

GC - gas chromatography

GFAAS - graphite furnace atomic absorption spectroscopy

HF-LLME - hollow fibre-liquid-liquid extraction

HLLE - homogeneous liquid-liquid extraction

HPLC-UV - high performance liquid chromatography with ultraviolet detection

HS-SDME - headspace-single drop microextraction

ICP-AES - inductively coupled plasma-atomic emission spectrometry

LC - liquid chromatography

LIS - lab-in-syringe

LLE - liquid-liquid extraction

LLLE - liquid-liquid-liquid extraction

LOD - limit of detection

LOQ - limit of quantification

LOV - lab-on-valve

MCFIA - multicommutated flow injection analysis

MeOH - methanol

MEPS - microextraction by packed sorbent

MIP - molecularly imprinted polymer

MS - mass spectrometry

MSFA - monosegmented flow injection analysis

MSFIA - multisyringe flow injection analysis

PE - polyethylene

PEEK - polyether ether ketone

PMDS - polydimethylsiloxane

PTFE - polytetrafluoroethylene

PVC - polyvinylchloride

QuEChERS - quick, easy, cheap, effective, rugged, and safe

RAM - restricted access material

SALLE - salting-out liquid-liquid extraction

SBSE - stir bar sorptive extraction

SDME - single drop microextraction

SFA - segmented flow analysis

SIA - sequential injection a

SIC - sequential injection analysis

SP - syringe pump

SPE - solid-phase extraction

SPME - solid-phase microextraction

UV-Vis - Ultraviolet-Visible spectroscopy

1. INTRODUCTION

Automation of analytical tasks such as colorimetric reactions, the study of the kinetics of enzymatic and chemiluminescence reactions, liberation and permeation studies or analyte extraction can be carried out by flow techniques (FT). The techniques are based on the handling of solutions in flow of a carrier stream inside a closed system. The basic instrumentation consists of a pump, flexible tubing, valves for sample introduction, and a detector. Since the introduction of flow segmented analysis in the 1950s [1] they have undergone a massive development of the instrumentation increasing robustness and versatility of FT. The first modification based on unsegmented flow denoted as Flow Injection Analysis (FIA) was introduced in the 1970s [2]. By the reproducible injection and mixing of the sample, and repeatable timing, the obtained response is also reproducible before the reaction reaches equilibrium. Thus, the time of the analysis is reduced. The introduction of a computer-controlled bi-directional syringe pump brought the advantage of precise aspiration of solution volumes. By flow reversal, efficient mixing of the aspirated zones is achieved. Introducing a multiposition selection valve into a system increased the versatility of the system operation. The technique combining a pump (typically the syringe pump) with the multiposition selection valve is denoted as Sequential Injection Analysis (SIA) [3]. More than one analytical task can be then performed in one SIA system without changes in a configuration. From this technique, many others derived, which differ from each other by the way of sample handling and analytical performance.

One of the areas where FT can be advantageously utilized is also the automation of sample preparation. In general, this step is the most time-consuming in the analysis and might suffer from poor repeatability if it is carried out manually, therefore the development of automated procedures is of high importance.

Lab-In-Syringe (LIS) [4] is a flow-batch technique using SIA instrumentation but the void of the syringe itself is used as a size-adaptable reaction chamber suitable for the automation of e.g. mixing and extraction. Thus, it combines the advantages of both flow and batch processing. Among others, LIS technique can be used for the automation of single drop microextraction in headspace [5] and directly-immersed format [6], dispersive liquid-liquid microextraction [7,8], or homogeneous liquid-liquid extraction [9].

This thesis is mostly focused on the automation of the extraction procedures using the LIS technique in different configurations and coupled with various detection principles to prove its versatility in the field of sample pretreatment. One work is also focused on the automation of dynamic leaching study of emerging contaminants by SIA. The thesis is written in cumulative form and comprises of 6 publications and the theoretical background related to the topic.

2. OBJECTIVES

The main objective of this thesis was to explore the possibilities of non-separation flow techniques in sample preparation in environmental and pharmaceutical analysis and for the study of the dynamic leaching of emerging pollutants.

The aim was to develop automated methods using mostly Lab-In-Syringe technique in various configurations and coupling the system on-line with advanced detection techniques such as inductively coupled plasma-atomic emission spectrometry (ICP-AES) and high-performance liquid chromatography with ultraviolet detector (HPLC-UV). The thesis is mostly focused on the development of liquid-phase extraction and its possible combination with solid-phase extraction within one manifold. The developed methods were intended to be applied to different sample matrices such as surface water, tap water, seawater or urine, surrogate digestive fluids, or soil leachates.

The specific objectives were as follow:

1. Development of Lab-In-Syringe automated dispersive liquid-liquid microextraction for trace metal analysis in different matrices.
2. Study of the on-line coupling with ICP-AES for direct measurement of extract in an organic solvent and secondary purified extract in the aqueous phase.
3. Comparison of two different configurations of Lab-in-Syringe for automation of direct-immersed single drop microextraction with solvent lighter and denser than water.
4. Development of automated dispersive liquid-liquid microextraction with a continuous flow of the sample through the syringe acting as a flow-through extraction chamber. Application of the multivariate spectral analysis approach for analysis of a mixture of nitrophenols in the extract omitting their separation by HPLC.
5. Development of dual-stage procedure combination of homogeneous liquid-liquid extraction with solid-phase extraction in an automated system
6. Study of the dynamic leaching of the plastic additives from microplastics to the seawater and evaluation of their bioaccessibility in an automated manner.

3. THEORETICAL PART

3.1. Approaches for automation of analytical processes

3.1.1. Importance of automation of laboratory procedures

Up to this date, the number of samples for the analysis has constantly increased considering a steadily rising number of considered contaminants, higher awareness in the population, and more strict regulations. Therefore, a demand for faster and more reliable analytical methods arises. An important way how to fulfil this demand is the automation of the implicated procedures.

Classical laboratory procedures involve glass flasks and beakers of millilitre to litre volumes. Typical processes that are carried out manually include weighing, sample fractionation, solution preparation, digestion, or filtration. These tasks are labour-intensive and time-consuming requiring several minutes up to several hours. At first, an afford was made to simplify some tasks such as pipetting, centrifugation, or mixing and make them faster by simple mechanization [10]. With the introduction of computers, more sophisticated systems were set up enabling automation of more complex procedures such as analyte extraction.

The difference between mechanization and automation is not clearly defined. Nevertheless, according to the International Union of Pure and Applied Chemistry [11], the mechanization can be understood as the usage of some simple device to supplement human effort. On the other hand, automation is mechanization with process control usually by using a computer. Groover defined automation as a "technology by which a process or procedure is performed with minimal human assistance" [12]. Fully automated systems should be able to make decisions according to the obtained results [13]. Automation of the procedural steps of the sample handling and treatment then potentially enables, besides of higher throughput of the analysed samples, also higher repeatability and accuracy of the measurements, reduction of errors and sample contaminations, and prevention of unnecessary handling with the hazardous chemicals by the operator (e.g. in radiochemical analysis) [10].

In addition to the previously addressed advantages, automation is often linked to procedural miniaturization, which is the minimization of volumes of samples, reagents, and other consumables required for the analysis. This includes a reduction in the cost of the analysis and of the production of hazardous waste. Miniaturization will be further discussed in the chapter describing sample preparation. Moreover, portability of the instrumentation used for procedural automation is one of the key features for in-field work such as environmental analysis or medical diagnostics [10]. Automation brings many of the above-mentioned advantages, on the other hand, it demands investments in advanced instrumentation and training of the operator. Therefore, it is advantageous mostly for the laboratories aiming for a high sample throughput of tedious analytical procedures [14]. Moreover, there is no universal methodology for all types of samples. In other words, for each kind of analyte and sample, a suitable sample preparation technique and procedure must be chosen and optimized, so that automation is most interesting where large sample numbers can be expected [15].

Automation/mechanization of analytical processes can be carried out by many approaches. One approach is using centrifugal analysers that are based on simultaneous mixing of samples and reagents

in microfluidic channels of a rotary disk by centrifugal force so that timing is equal for all samples and read-out can be done at once. Therefore, the reaction kinetics can be also studied. Most widely used are autosampler systems or more sophisticated robotic devices denominated as “discrete analysers”, which are briefly described in chapter 3.1.2. Another approach is based on using in-flow treatment of the sample, e.g. mixing of sample and reagent. Related so-denoted flow techniques will be described profoundly in chapter 3.1.3 as this dissertation is based primarily on their use.

3.1.2. Automation by robotic systems and autosamplers

Automation of the wide range of analytical processes including sample preparation procedures can be achieved using robotic systems. These instruments are particularly advantageous for analysis “in-batch”, that is inside a vessel, beaker, or vial. In general, they allow the treatment of multiple samples at the same time while the processing of the individual sample can last a significant time. This treatment in parallel can result in a highly effective sample throughput. Typically, the performed tasks are pick or place something, pipetting liquids, shaking, mixing, and heating or cooling but it is also possible to perform different types of extraction procedures. Robotic systems also allow emulating of the human labour and handling of the samples. On the other hand, FT are more appropriate for the automation of processes of fast reaction kinetics, which is explained in section 3.1.3.

Generally, three different instrumentations are used for batch-wise sample analysis that were compared in a recent review [16]:

1. Cartesian robots can move an injector (single-probe or multi-probe) in x,y,z-axis. Simple versions are used as autosamplers or fraction collectors in HPLC systems, ICP-AES, or other instrumental techniques including FT. They can be combined with a multi-probe injector equipped with disposable pipette tips in combination with 96-well plate [17], which is moving around the fixed 96-tips head, enables in-parallel colorimetric reactions using spectrophotometric, fluorometric or chemiluminescence detection, or semi-automated LLE. Also, some autosamplers possess positions for heating, shaking, or vacuum application of vials for automated extraction procedures.
2. A cylindrical robot is a mechanical arm that can rotate around a fixed axis and is equipped with an exchangeable gripper or pipettor [18]. Such a system is able to lift or position objects such as vials or pipettes or to pour solvents. The robot can be coupled on-line with detection techniques or other external devices such as stirrer ensuring fully automated procedure. Simple versions can be found in many instruments such as gas chromatographs (GC) or graphite furnace atomic absorption spectrometry (GFAAS) systems where the “arm” is a tube connector to an automatic pump. In discrete analysers, it is often the vials that rotate around an axis to move between active positions on which spectrophotometric measurements or addition or withdrawal of reagents can be performed.
3. An anthropomorphic robot is a mechanical arm imitating the human arm [19]. It has several axes and can include even a hand with gripping fingers. Additional joints improve the stage of the motion (5-6 axes). Automation of liquid-liquid (LLE) extraction can be carried out by this robot with high accuracy, precision, and robustness as well as practically all other tasks performed by humans, however visual recognition, artificial intelligence, or at least positioning of all objects on the steady same position related to the robot might be required.

It should be pointed out, that this classification is according to mechanical functioning while different levels of versatility can be found for each item.

More specifically, an autosampler can simply be used to change the sample solution as well as include active positions for e.g. sample vortexing, detection, vacuum application, etc. that leads to what is called discrete analysers.

Among others, in-batch liquid phase microextraction can be automated by autosamplers directly or with the combination with FT [20]. Autosamplers are used for in-batch processes and enable homogeneous mixing of the solutions thus suitable for reaction required equilibrium. On the other hand, autosamplers are limited by the volume of the used vials and are quite expensive. FT based on dispersion and gradient formation are less predictable and require an experienced operator. The sample is handled sequentially one by one. However, large volume samples can be handled easily in the continuous flow. Thus, it's suitable for continuous sampling purposes in the detection instruments such as ICP-AES or monitoring of the reaction kinetics, which is scarcely possible in autosamplers [14]. By combining both approaches as done in the experimental work 5 of this thesis, we can exploit advantages and partly overcome above mentioned disadvantages. The Flow-Batch approach, described in chapter 3.2.5.3, is based on combining principles from both automation modalities using FT for sample handling but with incorporated mixing chambers in the tubing manifold.

3.1.3. Automation by flow techniques

3.1.3.1. General principles of flow techniques

Flow techniques are a group of various approaches enabling the automation of the sample treatment and, most often, detection in one closed system that generally consists of flexible tubing, pumps, valves, and a detector. The sample is injected or introduced into the flow system and propelled towards the detector by a liquid carrier. The sample is generally modified before it reached the detector via a flow-through detection cell. Such modifications include different analytical processes such as mixing with reagents, e.g. chromogenic to yield a coloured, easy to detect product, enzymatic reactions, analyte extraction including liquid-liquid extraction, solid-phase extraction or membrane-based extractions, dialysis, gas diffusion, or simple sample dilution.

Flow techniques are popular tools for laboratory automation due to their high reproducibility, low cost compared to robotics systems, easy operation, and low consumption of the sample and reagents or solvents. Moreover, manual handling of possible toxic liquids is reduced. These features of FT meet the requirements of green analytical chemistry for the minimization of the toxic waste generation [21].

The fundamental principles of FT are precise metering of the sample and reagents, e.g. by injection, a controlled dispersion of the solutions by steady mixing patterns, and reproducible timing. Generally, this allows the detection of a reaction product of sample and reagents before reaching the reaction equilibrium, which enables very fast sample analysis [22]. These principles enabled highly reproducible measurement and automation/mechanization of laboratory tasks already in pre-computer times, the robustness of such method, in particular, the mixing patterns in the tubing manifold (and thus reaction yield) can be affected by bubbles or changing temperature and viscosity. In contrast, if

homogenous mixing and steady-state reaction are the basic principles, robustness is increased but the analysis speed and kinetic information are lost.

The applied injection volume depends on the used flow system and the aimed procedure and varies from a few microliters, e.g. if high sample dispersion and dilution are favoured, up to several millilitres in case of flow-batch analysis [23], where a mixing chamber is integrated into the flow manifold or if aiming for the automation of analyte preconcentration. In the earlier FT, the sample was injected via injection septum by a rotary injection valve using continuously operating peristaltic pumps. Later systems used individually actuated pumps for sample introduction, or sample aspiration from one port of a multiposition selection valve into the flow system, generally employing a syringe pump [22]. The closed manifold system prevents, in contrast, to open vials, any sample contamination from outside during the analytical process, and minimizes the contact of the user with harmful reagents [24]. A liquid carrier is used in most FT to drive the sample through the tubing system that acts further as a cleaning solvent so that carry-over effects are neglectable despite using one single apparatus for all samples [25].

The inner diameter of the tubes ranges generally from 0.3 to 1.0 mm. The low pressure applied and typical flow velocities of less than one millilitre up to a few millilitres per minute means that the flow inside the tubing manifold is mostly laminar. This dictates a parabolic velocity profile of the introduced sample/reagent where the streamlines are parallel but with a difference in velocity of the streamlines close to the wall and the ones in the centre of the tubing cross-section due to friction.

The flowing sample is mixed with the carrier and reagent by a process called dispersion. The dispersion means redistribution of the mass from the sample zone to the carrier zone during the sample transport to the detector. The sample dispersion is mostly influenced by axial convection given by the laminar flow. The diffusion of the sample given by the concentration gradient between the sample and carrier also contributes. The dispersion can be furthermore promoted by secondary convection flows when using coiled or knotted tubing. The degree of dispersion of the injected sample is expressed by the dispersion coefficient defined as

$$D = \frac{c_0}{c_{max}}$$

where c_0 stands for concentration of the injected sample and c_{max} is the maximum concentration of the sample zone after undergoing dispersion [22].

Timing as well as the sample dispersion is given by the injection volume, tubing dimensions and geometry, and the flow velocity. This is crucial for achieving reproducible measurements depending solely on the analyte concentration. The sample viscosity, however, changing with the temperature, as well as the presence of bubbles also determinate mixing patterns so that control of these parameters is important for reliable analysis. A variable degree of dispersion is required for different modes of measurement, e.g. aiming for sample dilution or, vice-versa, maximization of sensitivity. All reactions are time-dependent, therefore reaction time is one of the main parameters that is imperative to be controlled. If reproducible, it is possible to measure a reaction product without the need to wait for the reaction equilibrium (not valid for all the processes) or evaluate the presence of a catalyst of such reaction [24].

In FT, generally, a transient analytical signal in space is obtained as a result of the processed sample flowing through the detector. This signal has the shape of an asymmetric peak with a significant tailing due to the parabolic flow profile where the peak height, area, and width depend on the analyte concentration as well as the injection volume of sample and dispersion [13]. Due to the high reproducibility of timing between injection and detection, the peak height can be used for quantification in the FT, which is in contrast to HPLC or capillary electrophoresis (CE) where the analyte migration or retention can slightly vary between samples. Then, the chosen time of measurement does not have to coincide with the peak maximum but also another time for signal evaluation can be chosen if the timing is highly reproducible. This will yield a significantly lower value but always in a constant ratio with respect to the peak maximum. This operation is denoted as "electronic dilution". Finally, it is also possible to achieve a transient signal in time, i.e. to observe signal change with the mixture of sample and reagent stopped inside the detection cell that enables background correction and kinetic evaluations [22].

3.1.3.2. History of flow techniques

Flow techniques are going back to the 1950s when the number of samples for clinical analyses reached a level where the automation of the analysis started to be inevitable [26]. One of the first technique for mechanical automation was proposed by Skeggs in 1957 [1] and was called segmented flow analysis (SFA, Figure 1 a, g). The technique is still used up to this date, e.g., in clinical analysis and oceanography. It is based on a continuous flow of the sample propelled by a peristaltic pump. Additional flow channels on the peristaltic pump are for the continuous pumping and addition of reagents by confluence. Using another channel that continuously injects air into the main flow channel, liquid segments are generated. The bubbles prevent a carry-over between the different segments and their dispersion and cause turbulent convection inside each segment that achieves homogenous mixing of the sample and the added reagents. The bubbles also enable that a new sample can be introduced before the previous one has passed the detector so that multiple samples can run in the same flow line and high sample injection throughputs can be achieved. On the other hand, the continuous flow of the sample and reagents results in high consumptions (millilitres per minute) and, due to the compressibility of the air segments, the flow velocity of each segment is not smooth, steady, and reproducible but moves instead forwards with certain pulsation. This requires that any reaction must reach its equilibrium state before detection to enable reliable quantifications. Moreover, the segmentation bubbles must be often eliminated before the detection cell [13,26].

A different approach based on a very similar manifold assembly was introduced in 1975 by Růžička and Hansen as Flow Injection Analysis FIA (Figure 1b) [2]. Compared to the earlier described discrete analysers, which are based on the measurement in-batch/vial or SFA after reaching the reaction steady-state, FIA is based on a different concept. This involves the injection of a precise sample volume into a carrier stream that often merges with more reagent streams and transports the dispersing sample to and through the detector. The time of the reaction is given solely by the geometry and dimensions of the tubing and the flowrates involved in the absence of bubble segmentation, are very reproducible. Therefore, there is no need for reaching the reaction equilibrium (Figure 1 h). While the sample throughput is similar to SFA or higher (around 100 inj/h) [26]. Generally, only one sample is run at the time through the flow system to keep the timing reproducible, but the treating time itself is, taking

advantage of pre-equilibrium detection, is far lower. FIA is one of the most-often used FT in commercial laboratories due to the low cost of the instrumentation and its facile implementation, short time of analysis, and no need for computer control/software which was an advantage mostly in the past [26]. Many operations and manifold modifications leading to new approaches to FIA have been invented. This includes the combination of FIA with solenoid valves leading to multicommutated flow injection analysis [27] which enabled increasing operational flexibility and reduction of reagent consumption. On the other hand, mono-(air)segmented FIA [28] combines the working principles as well as advantages of both SFA and FIA using only 1-2 bubbles to reduce sample dispersion without impairing reproducibility of timing. To describe them here would be out of the scope of this introduction and comprehensive reviews and monographs are recommended to the reader [13,22,26,29].

Sequential injection analysis (SIA, Figure 1e), i)) was developed as another approach of FT in 1990 by Růžička and Marshall [3] and will be described in detail in chapter 3.1.3.2. Unlike FIA, this technique is based on using a bidirectional syringe pump and selection valve and on using sequential aspirating and dispensing steps of the required solutions instead of a continuous flow. Noteworthy differences towards FIA are that SIA requires computer-control of the system components and, typically, does not comprise mixing by confluent addition but by dispersion and penetration of stacked solution zones. Multisyringe flow injection analysis [30] can be understood as hyphenation of FIA and SIA principles, instrumentation, and operation and should be listed here only for the reason of completeness.

Efforts toward miniaturization of analytical assays resulted in the creation of Lab-On-Valve (LOV) [31] and lab-on-a-chip platforms [32], which enable handling of micro- to nanolitre volumes of liquids. The combination of the principles of FT with batch-wise analysis using vials resulted in flow-batch techniques, where a mixing chamber is included in the flow manifold. These include the most recent FT known as Lab-In-Syringe (LIS, Figure 1 f) that uses the syringe void as such a mixing chamber. It was introduced by Maya et al. in 2012 [4] and is explained in chapter 3.1.3.3. as FT that was used mostly in the experimental works belonging to this dissertation.

FT are generally a non-separative. Nevertheless, the attempt to connect a monolithic column, originally intended for fast-flow HPLC, to a lateral port of the multiposition selection valve of an SIA system resulted in another hybrid technique presented by Šatínský et al. as Sequential Injection Chromatography [33]. With the typical low-pressure syringe pumps, there are some limitations in the features of the usable columns (in most cases monolithic columns or core shell sorbents), volumes, and flow rate, while other advantages such as flexibility for pre-separation sample modification are noteworthy. Today, there is a trend towards using FT for on-line sample preparation that is coupled online to conventional separation techniques HPLC, GC, or CE [34].

Looking on the history of FT it can be observed very clearly how technological developments have restricted and allowed the stepwise development of more and more versatile FT and approaches over time as well as how FT development has promoted the developments of instrumentation that are also used in other analytical techniques. Only by inventing monolithic columns, SIC was imaginable while syringe pumps were developed on the demand for bidirectional pumps, that were not available for the first works on SIA. Some important milestones of FT inventions are displayed on the timeline in Figure 2.

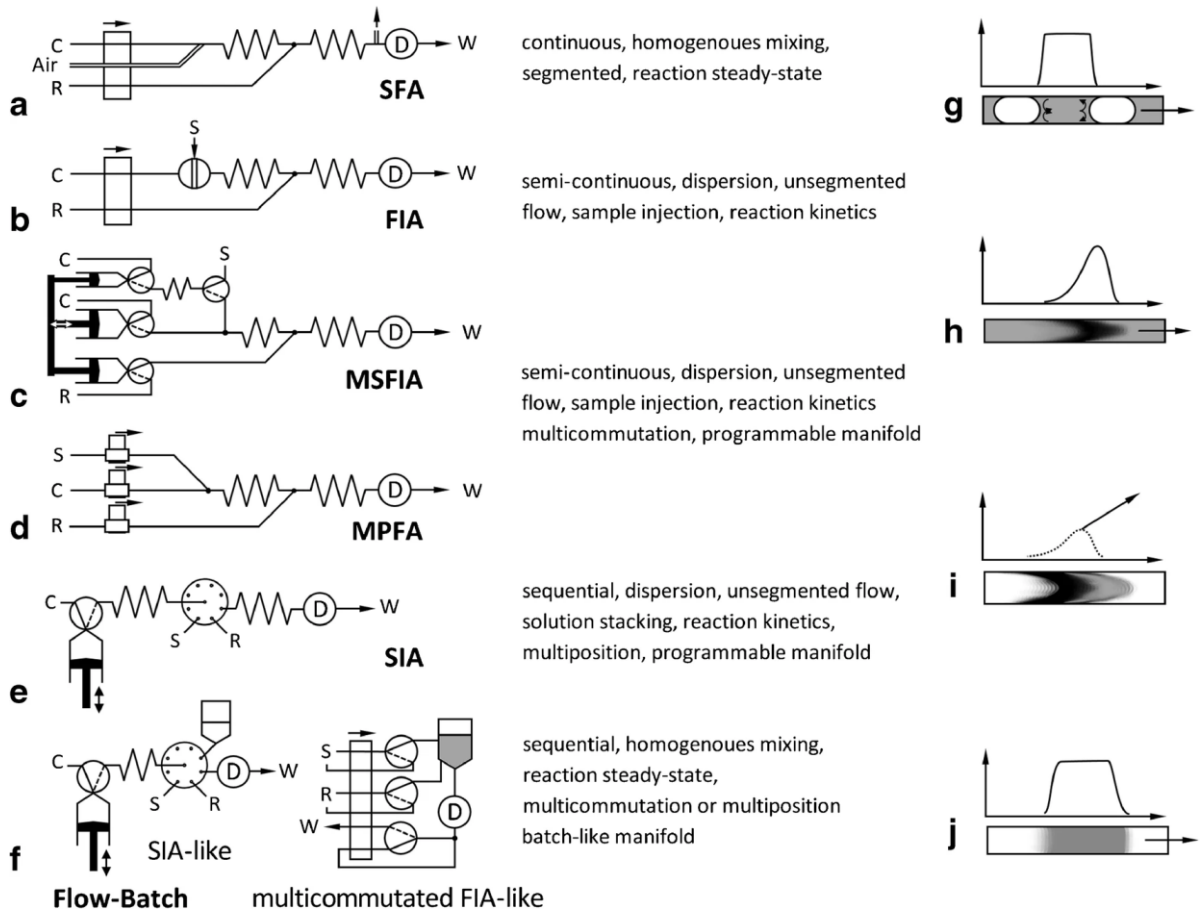

Figure 1: Different configurations, basic features, and mixing patterns with the characteristic signals of the flow techniques. a) SFA: Segmented Flow Analysis, b) FIA: Flow Injection Analysis, c) MSFIA: Multisyringe Flow Injection Analysis, d) MPFA: multipumping flow analysis, e) SIA: Sequential Injection Analysis, f) Flow-batch approach. Taken with permission from [14].

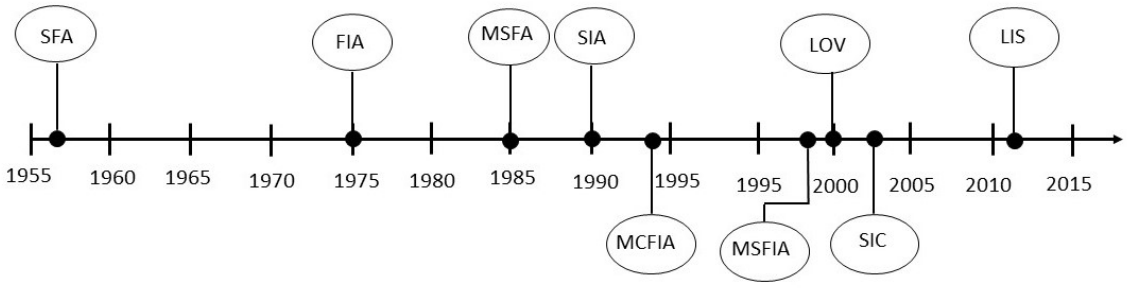

Figure 2: Historical milestones of the flow techniques. SFA: Segmented Flow Analysis, FIA: Flow Injection Analysis, MSFA: Mono Segmented Flow Injection Analysis, SIA: Sequential Injection Analysis, MCFIA: Multicommutated Flow Injection Analysis, MSFIA Multisyrige Flow Injection Analysis, LOV: Lab-On-Valve, SIC: Sequential Injection Chromatography, LIS: Lab-In-Syringe.

3.1.3.3. Sequential Injection Analysis

A typical SIA manifold as shown in Figure 3 comprises of a bi-directional syringe pump, a selection valve, and a detector, all connected by flexible tubing. The selection valve features 6-10 lateral ports and one central port. The detection cell is usually placed on a lateral port of the selection valve, other ports are used for samples and reagents aspiration. The selection valve and the detection cell might be connected by the additional reaction coil for effective mixing. The tube connecting the syringe pump and the central port of the selection valve is called holding coil and allows aspiration of solutions before the next step of pushing them towards the detector without the risk that they enter the void of the syringe pump and eventually cause carry-over problems. The principle of the technique is a sequential aspiration of the sample and reagents into the holding coil and mixing of the solution zones that is enhanced further upon flow reversal [24]. The emerging product is propelled toward the detection cell by the carrier stream as it is displayed in Figure 4.

The SIA technique has several advantages over FIA. For one, there is a high simplicity in using only a single-channel manifold system. The instrumental robustness is higher mainly due to the replacement of the peristaltic pump by the computer-controlled bi-directional syringe pump enabling aspiration of the precise volumes, fast reaction time, and reduced flow pulsation [35]. In addition, the aspirated volumes required for the analysis and the reaction time are controlled by programmable flow-rates, which can be changed in the control program. Therefore, the consumption of reagents and samples is significantly reduced compared to FIA [26]. A higher versatility is further given by the incorporation of the selection valve, the centre (“heart”) of the instrumental setup. Various external devices and detectors can be positioned on the lateral ports to implement a particular application. The tubing is typically made from an inert material such as polytetrafluorethylene (PTFE) or polypropylene. [36].

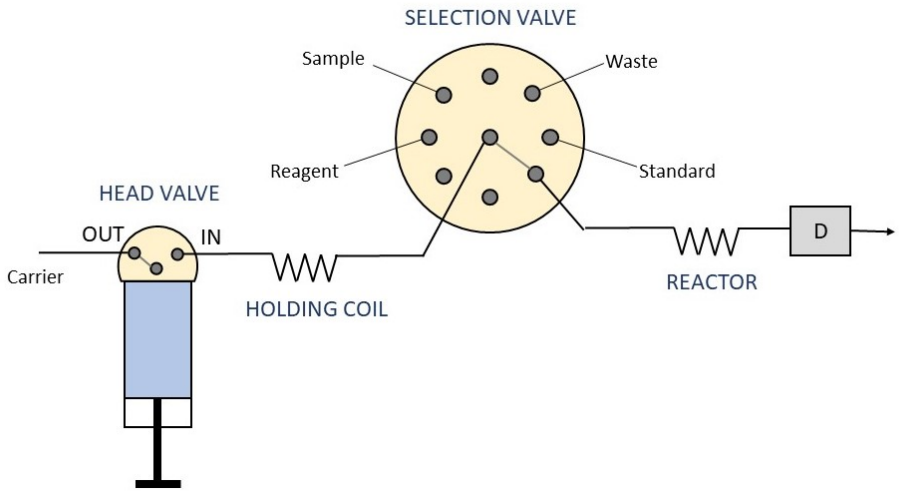

Figure 3: Configuration for Sequential Injection Analysis. D: Detector.

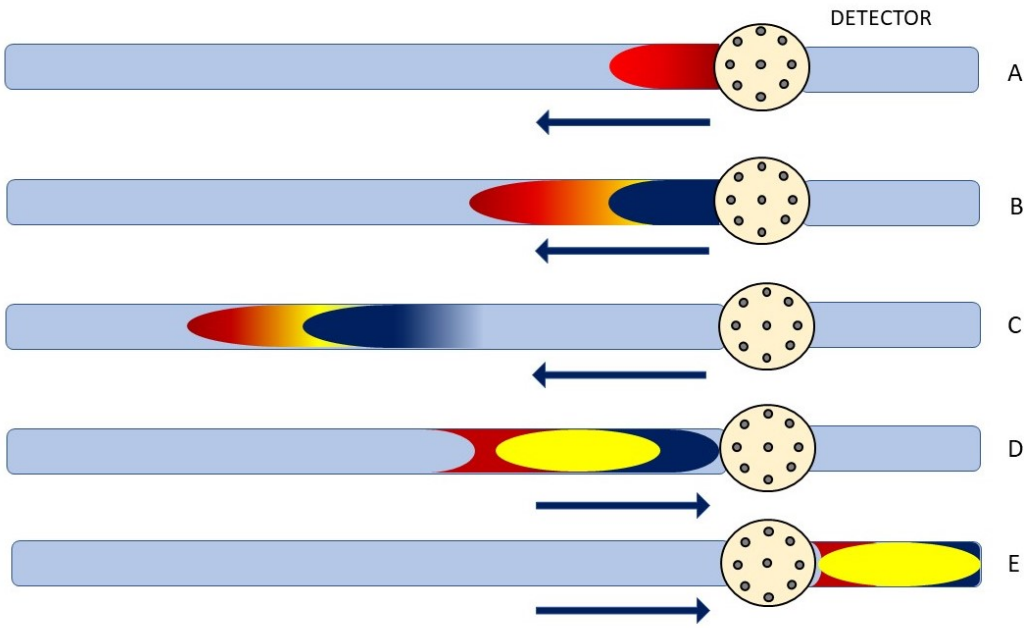

Figure 4: Flow pattern in Sequential Injection Analysis. A: Aspiration of the reagent zone, B: Aspiration of the sample zone, C: Dispersion of the zones, D: Flow reversal, and mixing of both zones, E: Detection of the reaction product.

Besides simple chemical reactions or dilution of the sample by the placement of additional devices (e.g. mixing chamber, membranes, derivatization columns) to the selection valve port, SIA enables to perform more complex procedures such as different extraction methods, or enzymatic reactions and immunoassays [35]. From the SIA technique other FT have been derived, e.g. Lab-on-valve (LOV) and LIS (described in chapter 3.1.3.4 and 3.1.3.5) extending its possible applications.

The feasibility to automate a wide range of analytical processes is projected in many different analytical fields including pharmaceutical [37,38] and environmental analysis [39,40], food control [41], or industrial process control [42]. In the pharmaceutical analysis, SIA is used mostly for quality control of drug formulations, SIA is also suitable for monitoring of drug dissolution/release [43].

3.1.3.4. Lab-On-Valve

Lab on-valve is another “evolution state” of the FT derived from SIA by placing a single monolithic platform on the top of the multiposition selection valve instead of the genuine stator. The channel system or “LOV manifold” shows inner diameters typically ranged between 0.5 and 2.0 mm [44]. This allows miniaturization of the analytical procedure based on pressure-driven liquid handling at low microliter levels. The manifold is typically made of polymethacrylate or more chemically resistant transparent polymers such as hard polyvinylchloride or polyetherimide. The insertion of optical fibres into valve channels enables in-line spectrophotometric or fluorescence measurements. Besides this, the treated sample, e.g. after analyte derivatization, can be collected into vials and measured at-line. Finally, the device can also be on-line hyphenated to a separation technique. It must be said that such options exist also for other FT, above all SIA. The LOV technique can be used the same way as SIA for monitoring of coloured reactions or coupled on-line with the separation techniques. In addition, a suspension of the

sorbent particles or "beads" can be aspirated into the LOV manifold and there used to create a renewable packed microcolumn inside a flow channel for µSPE or, if correspondingly modified beads are used, for enzymatic reactions and immunoassays (described in more detail in section 3.2.3.1) [45]. The typical configuration of Lab-on-Valve for this so-denoted Bead-Injection Analysis approach is shown in Figure 5.

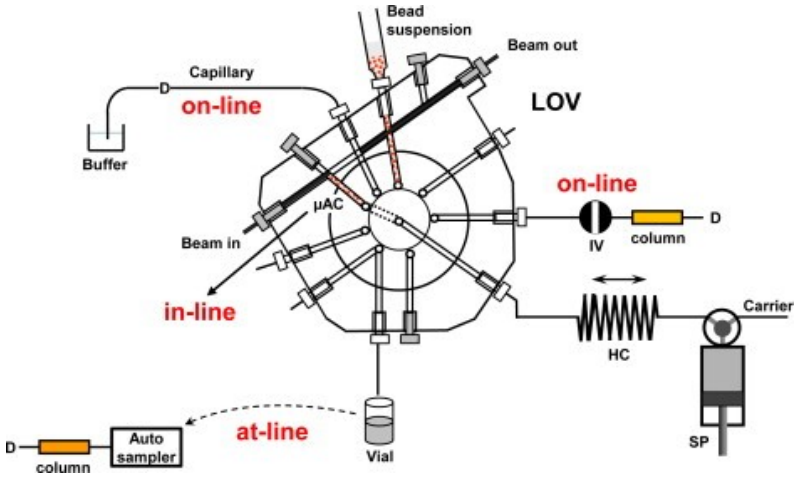

Figure 5: Configuration of the Lab-On-Valve format with indicated possibilities of detection. Taken with permission from [44].

3.1.3.5. Flow-Batch Analysis

Flow-Batch Analysis (FBA) can be described as a combination of batch-wise and flow techniques automation. Since FT have a limitation in terms of homogeneous mixing of a wide range of volumes or viscosity, connection of the manifold to a mixing chamber can be a solution, which allows to perform in-batch processes controlled by FT.

Using an open mixing chamber [46] it is possible to sequentially aspirate the solutions via a flow system or to add the sample directly into the chamber by pipetting and proceed with further modifications via the flow system. The possible use of a stirring bar inside the chamber is advantageous in terms of homogenization of the mixture and cleaning, a step that is very important to reduce carry-over between consecutive samples. However, the stirring rate must be slow in order not to spill the liquid out, the open environment of the chamber might be a source of contamination of the sample or, if toxic reagents or solvents are used, evaporation can harm the operator and present another source of error. On the other hand, sensing devices can be easily placed into an open chamber or standard addition by pipetting can be carried out.

Many different approaches of FBA has been proposed since the introduction in 1988 [47]. FBA can be performed in nearly all other FT including multicommutated FIA, MSFIA, or using individual solenoid pumps in combination with a mixing chamber. The early configurations consisted of a flow system comprising solenoid valves for individual flow line control all driven by a peristaltic pump connected to the mixing chamber [48]. Later approaches used a mixing chamber placed on the lateral port of the selection valve being a part of an SIA instrument [49,50]. The advantage of this approach is the possibility to aspirate millilitres volume of the sample, homogeneous mixing of as many solutions as required, with variable volumes, and step-by-step addition with pausing in-between as needed and

performing reactions in stop-flow mode to increase the method sensitivity [23]. The flow-batch approach can be used for many different applications such as automated titrations, standard additions, or for sample preparation, in particular dilution, as well as the easy integration of electrodes as sensing elements into a flow system [48]. Many of these steps are not possible to perform easily in tube-and-dispersion-based FT while they are very interesting for sample preparation procedures. For example, dispersive liquid-liquid microextraction (DLLME) has been automated using a mixing chamber connected to a modified SIA system denoted as dual-valve SIA (DV-SIA) [49], this configuration and others will be described in about automation of LLE chapter 3.2.5.

FBA is a simple and versatile automation approach and outcomes are quite easy to comprehend as it lacks the concept of sample dispersion but also, certain advantages such as the self-cleaning action of the carrier, typical for most FT, are missing.

3.1.3.6. Lab-In-Syringe

Lab-In-Syringe (LIS) is a flow-batch approach that uses the void of the syringe pump of an SIA system as a size-adaptable chamber for the various procedural steps listed above instead of the additional mixing chamber, which comes with some interesting advantages. It can also be considered as a hybrid of SIA and FBA. A recent tutorial on the technique is highlighted [25].

LIS technique was firstly applied for DLLME using a dispersive solvent. The dispersion was created by rapid injection of the sample into the mixture of the disperser and extraction solvent since the inlet diameter is wider than in normal syringe [4]. Later, Horstkotte and co-workers added a stirring bar into the syringe driven by an external magnetic field [51]. This improvement solved the problem with emulsion created by dispersive solvent and phase separation was induced simply by the stop of the stirring and gravity. In addition, by stirring rapid homogenization is achieved and facilitates cleaning of the syringe, which can help the method's repeatability, and shorten the analysis time by simplifying procedural steps [25]. On the other hand, the stirring bar inside the syringe creates a large dead volume which disables complete emptying of the syringe. This problem can be overcome by turning the syringe upside-down and aspiration of a small volume of air [23].

The stirring device generally consists of the direct current motor, strong magnets and the stirring bar. The different possibilities of the configuration of the stirring device are shown in Figure 6. In the first works, the stirring was enabled by integrated two oppositely magnetized iron rods that were placed onto the syringe barrel creating the external magnetic field driven by a relay-controlled motor (Figure 6A). Later, a stir bar driver was used consisting of the plastic ring with integrated magnets. This device can be easily used for both configurations of the syringe (Figure 6B and C). To simplify the stirring device the neodymium magnets were placed on the top of the direct current motor (Figure 6D), in addition, the visibility of the processes inside the syringe was improved.

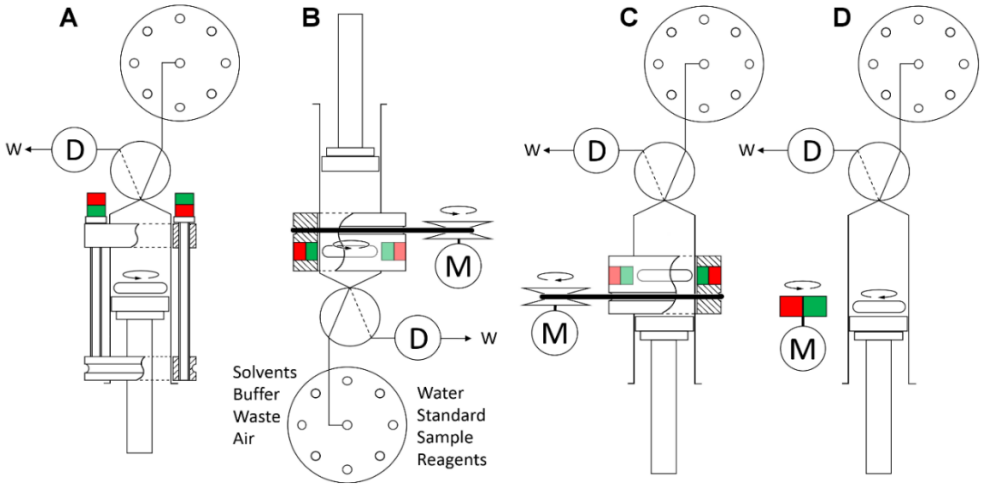

Figure 6: Stirring devices using in Lab-In-Syringe technique. The device is made of: A: integrated two oppositely magnetized iron rods driven by the motor, B: Plastic ring with integrated magnet for upside-down orientation of the syringe, C: Plastic ring with integrated magnet for normal orientation of the syringe, D: Magnets placed on the motor. Adapted with permission from [25].

The cleaning step is faster and more efficient than in FBA because the syringe does not have to be filled completely (required filling volume is around 20% of the syringe volume) and the syringe piston wipes the syringe walls, which contributes to the cleaning efficiency. As Figure 7 implies, four steps are required to clean the chamber in FBA compared to just two steps required using LIS [25].

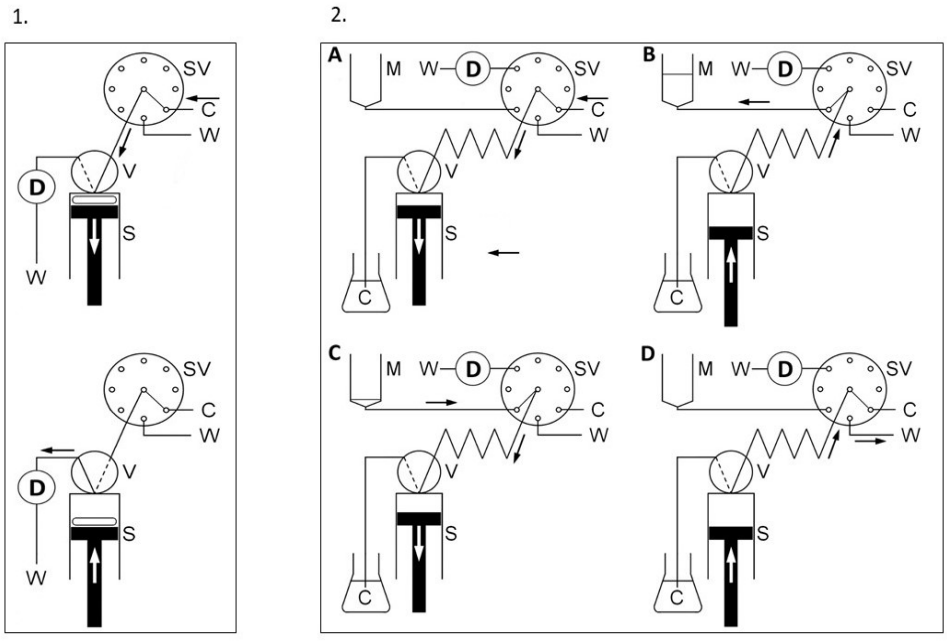

Figure 7: Comparison of cleaning procedures of the LIS technique (1.) and flow-batch approach (2.).

In contrast to FBA, LIS uses an entirely closed void which is advantageous for the use of organic solvents or other toxic reagents. Moreover, higher stirring rates can be applied. Yet using a closed environment risk of overpressure has to be taken into account when gases are generated during the reaction [23,51].

Detection can be carried out either in a flow-through detection cell placed on the lateral port of the selection valve or, since the syringe is transparent, inside it. For this, optical fibers can be positioned in a 180° angle with the syringe between them using a purpose-made adaptor [7]. Homogeneous mixing inside the syringe makes the Schlieren effect negligible for both detection modes (spectrophotometric and fluorimetric) and signals are rather of rectangular than peak shape [25].

Besides spectrophotometric detection LIS have been coupled with different advanced detection techniques such as GFAAS [52], ICP-AES [8,53], GC with FID and MS [54,55] or HPLC with UV and MS detection [4,9,56] enabling determination of mixtures of elements and compounds with high selectivity and sensitivity.

Change of the pressure inside the syringe can be achieved when the syringe head valve is turned to a closed port and either lifting or lower the piston (positive or negative pressure, respectively). This can be used e.g. to enhance evaporation of a volatile analyte at reduced pressure or forcing a gaseous compound to dissolve at increased pressure. By drilling a through-hole or flow channel through the piston of the syringe we can create a second inlet to the syringe void, which can be used for continuous pumping of the sample through the syringe acting as a flow-through reactor as explored in experimental work 4 of this dissertation [57] or the channel can be used for the transport of the gaseous analytes from the headspace of the syringe for the GC separation [54] or drop creation [58].

Compared to classical FT where manifold cleaning is automatic and quasi-simultaneously done during the analysis by the carrier flow, the LIS technique requires an additional cleaning step after every measurement due to the involved dead volume. This significantly prolongs the time of the analysis by ca. 1 min. Another disadvantage is given for applications that require gradient formation where SIA is more feasible than LIS. Finally, cooling or heating of solutions can be performed faster and more efficiently inside a tubing manifold due to the larger surface [25].

The main features of the LIS technique are robustness, compactness, low cost of the instrumentation, and high operation versatility, in particular for performing liquid phase extractions. LIS is suitable foremost for sample preparation procedures benefiting from treating larger volumes (millilitres) of the sample so as typically used in the environmental analysis [23]. Up to this date, many different applications of LIS mostly directed to sample preparation, have been proposed, most of all automation of DLLME. Here, compared to manual performance, LIS can be considered as a green technique due to the low consumption of solvents [59]. Specific applications for the automation of sample preparation will be discussed in chapter 3.2.5.4.

3.1.4. Approaches for hyphenation of flow techniques with detectors

3.1.4.1. General modes of hyphenation

Flow techniques can be coupled with various detection and separation techniques ranging from spectrophotometry to sophisticated ICP-AES or ICP-MS as well as from simple separations carried out on monolithic columns coupled to a SIA system (known as Sequential Injection Chromatography - SIC) to advanced separation techniques as HPLC, GC, or CE. This chapter is mostly focused on techniques used in the included publications.

Generally, four different modes are distinguished concerning the coupling of techniques that derive from operation modes of monitoring and process analysis [34,60]:

1. Off-line: The sample, previously treated in the flow system, is transferred manually from the flow system to the detection instrumentation that can be situated remotely and the time delay between the first and the second process can be significant. A typical example is the collection of extracts obtained from a flow-automated extraction procedure and their transfer to an HPLC, possibly equipped with an autosampler, in one batch after finishing all extractions.
2. At-line: The extract is transferred from one instrument to another but on the place of operation, e.g. by a robotic arm collecting the extract and placing the vial into the autosampler of the second system.
3. On-line: Hyphenation of the two systems through a flow interface such as an injection valve that switches immediately after loading. This mode was applied mostly in the presented experimental works of this dissertation. The differentiation between at-line and on-line is not always clear and easier understood considering process monitoring. Here, at-line monitoring assumes a significant time difference between sampling and signal that makes feedback control to the process impossible. At-line can also be understood as a snap-shot of the sample composition while on-line delivers continuous data.
4. In-line: Sample preparation and detection are integrated into one system, e.g. measuring the extract inside the flow system used for sample preparation, possible during the preparation. In-line is most often related to electrode-based detection techniques where the signal is obtained quasi-instantaneously. In process monitoring, in-line would refer to the continuous measurement of the sample composition inside the process, which is direct in time, location, continuous, and fully representative.

3.1.4.2. Hyphenation of flow techniques with spectrometry and fluorimetry

The typical detection techniques used in flow analysers are optical detection principles including UV/VIS spectrophotometry, fluorimetry, and, to a lesser extent, chemiluminescence emission. For all these techniques, the sample is often measured in flow-through detection cells that are mostly commercially available. Optical fibres are often used to connect the detection flow cell to a light source and the detector.

The most often used detection technique is spectrophotometry that is based on the absorption of light by the sample and contained analytes or produced derivatives. An important limitation of this technique can be the so-denoted “Schlieren effect” for those FT where a carrier is used. This is because the mixing

of solutions is incomplete, and a concentration gradient is created leading to an inhomogeneous refraction index. This causes partial reflection and refraction of the light beam when the sample passes through the detection cell, which is observed as a baseline disturbance. The Schlieren effect increases the signal-to-noise ratio and therefore affects the LOD of the method. It can be minimized by using a carrier solution similar in composition or refraction index to the sample, homogenous mixing/static mixers, or by real-time subtraction of the signal on an analyte insensitive wavelength, the so denoted reference wavelength [24]. An elegant alternative is the so-called stop-flow measurement mode where only the change in signal of a colorimetric reaction is registered with the sample-reagent mixture stopped inside the detection cell. The stopped flow mode is therefore also ideally suited to register reaction kinetics. In unsegmented flow, the detection can be deteriorated also by the presence of the air bubbles. Bubbles can act as a lens as well as a mirror causing the light to be refracted or reflected, which results in a sudden increase of the sample absorbance.

In the first analysers based on air segmentation, U-shaped glass detection cells were used. These cells required special holders to be integrated into the spectrophotometer and specific for each instrument and showed significant loss of light. Thus, they were replaced by Z-shaped flow cells with lower cost and uncomplicated connection to the system using the same tubing fittings as for the flow manifold. To avoid the accumulation of air bubbles in the cell, the liquid should flow from the bottom of the cell and should be regularly flushed with the appropriate cleaning solution. The windows for the optical paths are made from transparent material (quartz, glass, sapphire) whereas the other parts of the cell should be made from non-transparent, ideally black, easily wettable material [24].

Spectrophotometry is the least sensitive detection technique of the ones typically employed in FT but using longer path lengths of the detection cells such as liquid waveguide capillary cells being based on total reflection of the passing light, the sensitivity can be increased more than 100 times.

To increase sensitivity and selectivity of the measurement solid-phase spectrometry can be applied. In this case, the beam attenuation is measured after interaction with the analyte adsorbed on the solid particle. Besides the absorption of the beam, the radiation scattering by the solid particle occurs. This can cause high blank value and decrease repeatability. To overcome this problem, the solid phase should be made of transparent material, and by using a reference wavelength to compensate for the high blank.

Fluorimetry is a very sensitive and far more selective detection technique than spectrophotometry, therefore quite often used in FT. It is based on the excitation of molecules with conjugated double bonds, which emit radiation of a longer wavelength when returning to the ground energy state. The geometry of the flow cell in terms of illumination is characterized by a viewing angle of 90°. Fluorimetric detection is of interest in SIA for the detection of active substances in pharmaceutical formulations as filling materials very often do interfere little while the active compound can be fluorescent [61]. FT are also and very favourably used to automate chemical assays based on chemiluminescence emission due to their unique reproducibility in terms of mixing and timing which enables measurement only milliseconds after adding the reagent in a reproducible way, e.g. by a confluence, that is required to make this simple and highly sensitive detection technique reliable and robust [62]. Typical analytes act either as catalyst or inhibitor of redox-reaction and selectivity is achieved by the specific chemiluminescence reagent and careful optimization of the reaction conditions.

3.1.4.3. Chemometric approaches in spectral analysis

Flow techniques are in principal non-separative so that in general selectivity is achieved via e.g. analyte-specific reagents, enzymes, selective detection techniques, or matrix elimination. However, simple mixtures of various analytes can be selectively resolved by a difference in reaction kinetics or, using spectra-yielding techniques (UV-Vis spectrometry and fluorimetry), by chemometric approaches. For this, multichannel or fast scanning spectrometers must be used to obtain sufficient information for spectra discrimination [63].

One of the most frequently used approaches is a multivariate spectral analysis that assumes that the spectrum of a sample containing more than one analyte can be represented as a linear combination of the spectra of each analyte plus an unknown part that cannot be explained and that can be considered as sample specific background caused by matrix components. In the absence of any other absorbing substance or equal matrix for standard and sample, *n* analytes can be quantified by the knowledge of *n* absorbances values measured at *n* individual wavelengths, ideally representing high differences in molar absorptivity between all analytes. In reality, the error becomes smaller the more data are taken into account. This approach was used for the determination of three isomers of nitrophenols in one experimental work forming part of this dissertation (Publication 4). Here, three isomers of mono-nitrophenols were quantified simulating the unspecific background signal by a polynomial term [57].

A similar approach was proposed by Manera et al. [64] who used a multivariate least-squares regression model and a multiple standard calibration approach for the determination of a mixture of nitrophenol isomers. A training set of 15 different mixtures with randomly selected concentrations for each analyte was required for calibration. From the data of the training set, the prediction set was created for the determination of the components from an unknown mixture [65]. Nevertheless, this model did not count with a contribution to unknown interferences in the sample. Instead, the interference contribution to the spectra was minimized simply by the selected reduced wavelength range of the spectra.

3.1.4.4. Hyphenation of flow techniques with ICP-AES

Inductively coupled plasma-atomic emission spectrometry (ICP-AES) is an optical detection technique belonging to the group of atomic spectrometric methodologies. ICP-AES detection is based on the nebulization of the sample and atomization of its components coming along with the thermal breakdown of any organic compounds contained. Most elements can be measured by this technique by excitation of the atoms by a plasma torch that is generated by inductive heating at radio frequencies. The excited atoms emit light while returning to the ground state at wavelengths that are specific for each element. The plasma is generated by ionization of a gas (typically argon) reaching temperatures of several thousand degrees Celsius. The instrumentation consists of a nebulizer, spray chamber, the plasma torch, and a spectrophotometer. Qualitative information about the analyte is obtained from the specific emitted wavelength and the intensity of the radiation corresponds to the analyte concentration [66]. The typical configuration of ICP-AES is shown in Figure 8 and described in more detail below.

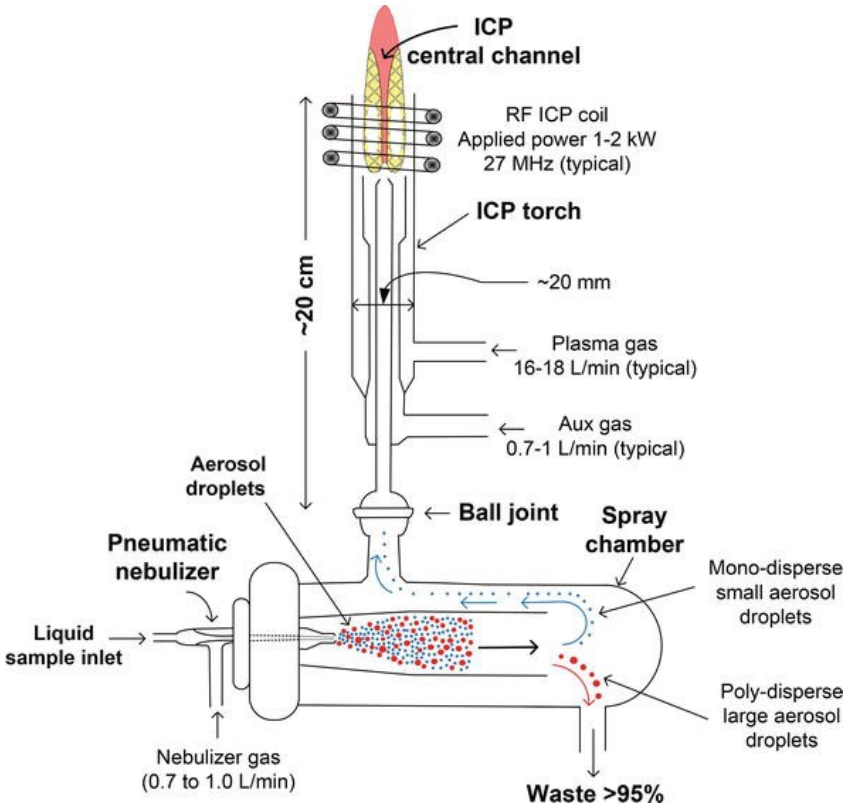

Figure 8: Schematic picture of ICP-AES instrumentation showing concentric pneumatic nebulizer, spray chamber, and plasma torch. Taken from [67] (open access).

Instrumentation:

The nebulizer is used to create fine droplets of the liquid sample. It is one of the most important parts of the ICP instrument because the sensitivity of the instrument depends on the reproducible generation of the aerosol that can reach the plasma discharge. A variety of pneumatic nebulizers (e.g. concentric or cross-flow type) and ultrasonic nebulizers were designed for this purpose varying in material, spray characteristics and dimensions.

Pneumatic nebulization uses viscous drag forces that arise from a gas flow passing over the sample surface and disintegrate the liquid into fine droplets [68]. The sample is aspirated by the peristaltic pump into a capillary of the nebulizer by the flow of 0.5-4 mL/min and spraying gas flow is ranging from 1 to 2 L/min [69]. Generally, the highest efficiency of droplet introduction into the plasma torch even at optimal conditions reaches only a few percents [68].

Ultrasonic nebulizers use a piezoelectric transducer and oscillating membrane to create the aerosol of droplets with a smaller diameter and with narrow particle size distribution and much higher efficiency than pneumatic nebulizer (efficiency up to 30%). However, this nebulizer has a higher memory effect then pneumatic nebulizer and is not suitable for samples with high salt content as it is prone to the deposition of salt and suspended particles [68].

Before the aerosol reaches the plasma torch, the finest drops must be separated from the large one, which can cause plasma instability. This process happens inside the spray chamber, which is connected to the nebulizer. The drops must be small enough to achieve efficient desolvation and analyte vaporization and atomization as a requirement of atom excitation. Depending on the spray chamber

design, all droplets above a certain volume, are removed and discarded to waste while only smaller droplets, ca 1-5% of the sample, enter the plasma torch [66]. A large volume of the chamber requires also a long time to fill the chamber with the aerosol, which consequently requires prolonged time for signal stabilization and cleaning, i.e. return to the baseline. Among the different designs, cyclone spray chambers show a reduced volume and improved time performance [68].

The plasma torch consists of three concentric quartz tubes. By the central tube, the nebulized sample is carried into the plasma torch, the intermediate tube is used for auxiliary plasma gas flow and the outer plasma argon gas flow is used as a cooler. A Tesla coil produces "seed electrons", which initiate the ionization of argon. The free electrons are accelerated by the electric and magnetic field created by a copper coil connected to the radiofrequency generator. The transition of the energy from the coil to the electrons is called inductive coupling. The chain reaction of the high-energy electron with the argon atoms changing the gas into the plasma consists of argon atoms, ions, and electrons creating the ICP discharge of very high temperature [66,68].

The radiation, which is emitted by the excited electrons of the sample is polychromatic, i.e. showing element-specific spectral lines. The radiation is collected by focusing optics (e.g. mirrors) and refracted by the optical grating or prism. The detector is in older instruments a photomultiplier which can be combined either with a monochromator with a single exit slit and detector or polychromator with multiple exit slits and detectors for each slit. Modern instruments use the polychromator in combination with semiconductor photodetectors such as charge-coupled devices [70].

Typically, the emission is read from the side of plasma (Radial plasma Figure 9A) with the advantage of instant venting of gases and heat. Nevertheless, modern instruments allow also axial view (Figure 9B) to the plasma torch providing longer path length thus improving the sensitivity. The axial view might be more prone to matrix effects since the path is going also through the atomization zone and cooler tail of the plasma [70].

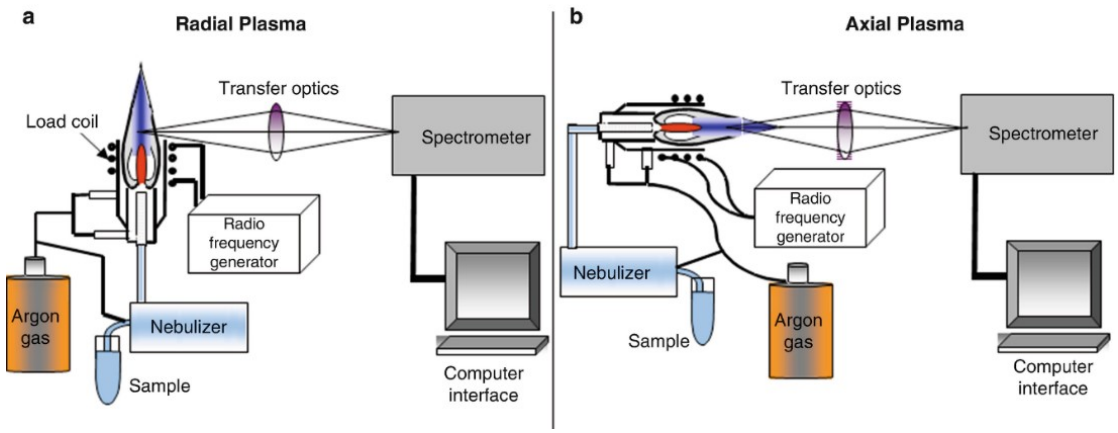

Figure 9: Configuration of the ICP-AES for A) Radial measurement B) Axial measurement. Taken with permission from [71].

Interferences

The matrix can affect the process of nebulization, desolvation, atomization, or ionization. The presence of salt or organic compounds can change the viscosity of the solution and thus they can change the quantity of nebulized sample. The desolvation process can alter by the difference in volatility of the

analyte and matrix. Therefore, suitable sample preparation prior to the analysis can overcome these problems [70]. As one choice, liquid-liquid extraction can be applied where FT can be beneficially coupled on-line to the ICP-AES, e.g. via an injection valve, to automate the sample pretreatment procedure. Nevertheless, it must be considered that organic solvents can lower the temperature of the plasma and that generally, larger solvent amounts in the injected sample are not compatible with ICP measurements. For instance, chlorinated solvent decrease electron density, and carbon deposition on the sampling cones can occur. Therefore, back-extraction into an aqueous matrix is generally required as it was done for the experimental work related to publication 1 [8]. Alternatively, appropriate instrumentation adaptation for the solvent introduction using a heated spray chamber is possible that has been used in the experimental work related to publication 2 [53].

Hyphenation with flow techniques

FT and ICP-AES are advantageously coupled for trace elemental analysis when certain sample preparation, such as analyte preconcentration or clean-up from the matrix, is required. Since FIA and ICP-AES both operate in continuous carrier flow they can be coupled easily. The coupling of noncontinuous FT such as SIA or LIS with ICP is also possible and is presented in two publications in this thesis. Often, a low-pressure injection valve acts as interphase of both instruments. The sample can be then injected into the instrument by the filling of the injection loop with the extract and valve switching upon which the extract is carried to the nebulizer [8]. Another possibility is to use an air-segmented flow injection methodology where the air is used as a carrier to create a small sample plug. This approach was used in one experimental work of this thesis using a sample plug of only 5-15 µL. By this methodology, background interferences are minimized because the sample volume is very low and the oxygen content of the air segments improved carbon combustion and decreases its deposition [53].

Comparison with other techniques for elemental analysis

One of the most advantageous features of this technique is the ability of simultaneous analysis of a wide range of elements with very high selectivity. Among the few limitations is the detection of Argon itself, which is used as plasma gas and elements presented in the solvents of the sample such as H, N, O, and C. Also, halogens are problematic due to their high excitation energies. The wide linear dynamic range (four to nine orders of magnitude) is much higher compared to atomic absorption techniques ranging from only one to two orders of magnitude. Whereas AAS operation is usually sequential, i.e. one element at the time, by ICP-AES it is possible to analyse simultaneously more elements [66]. On the other hand, an advantage of Flame AAS (FAAS) is the ease of operation and low cost of the instrumentation. GFAAS has a lower detection limit but 2-3 min are required for the analysis of each element whilst ICP-AES analyses the whole sample containing several elements at the same time. ICP can be also coupled with MS detection providing even lower detection limits and an extended range of possible elements for analysis. Table 1 shows a comparison of the selected parameters of ICP-AES, ICP-MS, FAAS, and GFAAS.

Table 1: Comparison of detection techniques used for elemental analysis. Adapted from [70].

Parameter	ICP-AES	ICP-MS	FAAS	GFAAS
LOD	ppb	ppt	ppb	ppt
Linear range (Orders of magnitude)	10^5	10^5	10^3	10^2
Sample throughput (elements/time/sample)	5-30/1 min	All/2-6 min	1/20 s	1/5 min
Number of detectable elements	>75	>75	>68	>50
Precision (short term)	0.2 - 3%	1 - 3%	0.1 - 1%	1 - 5%
Sample volume	High	Low	Very high	Very low
Cost	High	Very high	Low	Medium

3.1.4.5. Hyphenation of flow techniques with liquid chromatography

Liquid chromatography (LC) is a widely used and well-known analytical technique therefore the basic principles are not explained in this chapter. Coupling of FT with liquid chromatography can be carried out either prior to separation for automation of required sample preparation procedures or post-separation for derivatization of the analytes to facilitate their detection [34].

The simplest configuration combining FT with analyte separation is known as sequential injection chromatography, which means the integration of the separation column directly into the flow analyser. A typical SIC system consists of an SIA system with the separation column placed on the lateral port of the selection valve and the flow-through detection cell connected, e.g. with optical fibres to the lamp and UV/VIS detector, downstream. SIC allows moderately fast separations of simple mixtures. Working with a low-pressure system implies some limitations in the possible use of separation columns, being normally short monolithic or core-shell columns (25-50 mm of length). Another challenge is the maximum volume of the syringe pump for the mobile phase, which might not be sufficient to elute all the analytes, yet the syringe can be easily refilled. Compared to the HPLC, SIC robustness is generally lower than for HPLC due to the limited pressure stability of the employed pumps (e.g. syringe type) and the back-pressure that requires the use of lower flow rates than typically applied to monolithic columns in HPLC. Nevertheless, SIC provides separations with lower consumption of the solvents thus the costs per analysis are reduced. Portability and easy liquid manipulation enabling on-line derivatization are also advantageous [72].

Possible uses of FT for pre-chromatographic sample preparation can be liquid phase extractions, analyte trapping techniques (SPE), or membrane-based extractions to transfer the analytes into a suitable solvent, preconcentrate, and clean from the matrix interferents.

The interphase for the on-line coupling of FT and LC is typically the high-pressure injection valve of the HPLC connected to the flow instrumentation. Sample processed in the FT (e.g. extract after LLE)

can be introduced into the sample loop of the HPLC valve and injected on the chromatographic column after switching of the valve. Simple sample preparation can be also done by placing a short solid-phase extraction (SPE) column ahead of the injection valve, then the trapped analyte is eluted by FT and loaded in the injection loop of HPLC [73]. Another possibility is to place the SPE column onto the injection valve, where the analyte is loaded by FT but eluted with the mobile phase of the HPLC [74]. By flow system, post-column reactions such as the introduction of the fluorescent label prior to fluorimetry detection can be carried out. Coupling of FT with HPLC combines the advantage of both techniques enabling faster analysis, lower waste generation, high reproducibility, sensitivity, selectivity, and safety of the operator [34,60].

Since 1980 more than 800 articles have been published related to sequential injection chromatography and hyphenation of FT with LC with steady numbers from the nineties till present. The number of articles for each year is shown in Figure 10; the data were taken and processed from the database available in the flow injection tutorial web page [75].

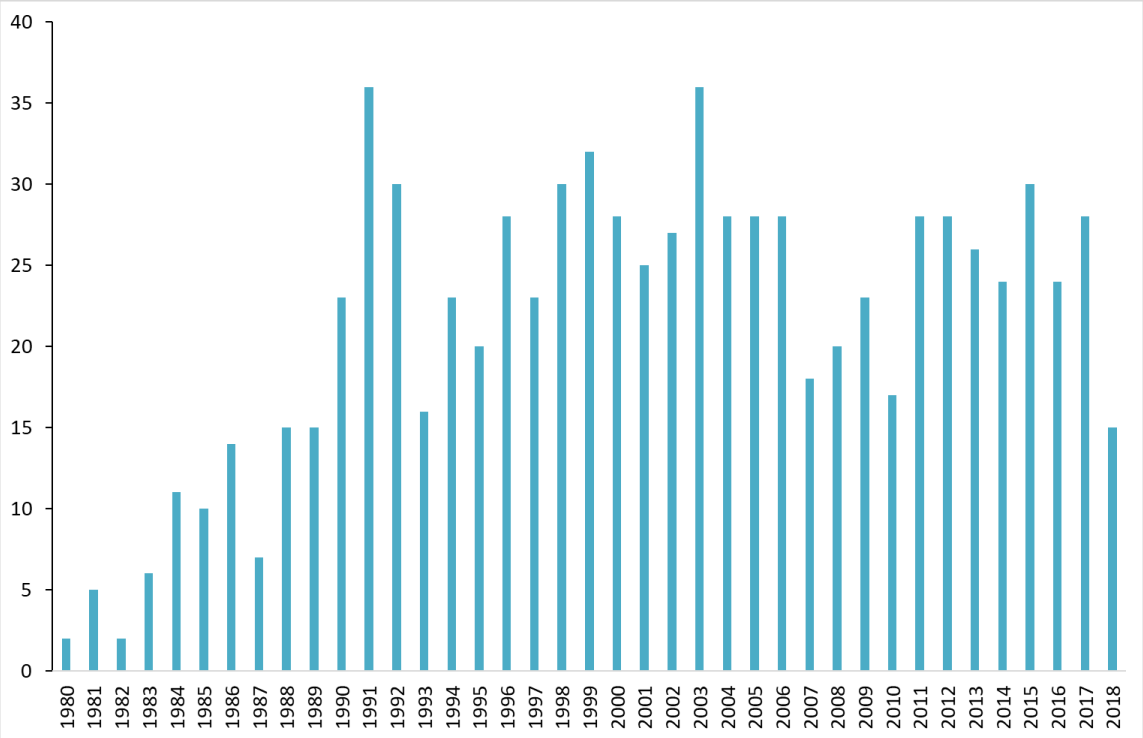

Figure 10: Number of articles for each year related to flow techniques coupled with liquid chromatography.

3.2. Sample preparation and possibilities of automation

3.2.1. Importance of preparation of the sample prior analysis

Sample preparation is an important step of the entire analytical process because most of the samples cannot be injected directly into advanced detection instruments. There is no universal sample preparation procedure and for each task, a suitable method has to be selected and optimized individually for the specific sample matrix, concentration level and type of analyte, and the used analytical detection technique [15]. The main tasks for the sample preparation are clean-up, i.e. elimination or minimization of the possible interferences from the sample matrix, selective isolation, preconcentration of the analyte if the concentration levels are lower than the LOD of the intended detection technique, and transfer of the analyte into a medium that is compatible with the used detection technique [76]. Sample preparation involves different kinds of operations ranging from simple ones such as weighing, solution mixing, dilution, or filtration to the more complex analyte extraction or derivatization procedures, which usually requires more steps [77]. However, these multistep processes are still the most problematic ones in terms of the time (typically 80% of the total analysis time), precision, cost, and environmental impact [78].

Miniaturization of the extraction procedures is a way how to make sample preparation more cost-effective and environmentally friendly, mainly by lower consumption of solvents. Moreover, higher preconcentration factors can be sometimes achieved, e.g. using a single drop of the extraction solvent as the acceptor phase inside the aqueous sample. The number of steps should be kept as low as possible to make the procedure faster and to improve repeatability. Besides, green sample preparation should avoid toxic solvents.

Procedural miniaturization and, in particular, automation of the miniaturized sample procedures is a hot topic in the development of modern fast and green techniques with high reproducibility, which overcome several hindrances of manual sample handling such possibly lower reproducibility, or contact with toxic reagents and solvents [15].

The simplest approach for the sample preparation of highly concentrated samples is known as “dilute and shoot”, i.e. the sample is diluted which reduces interferences but is applicable only if the analyte is well above the LOQ concentration level. Another popular sample preparation, e.g. concerning the analysis of blood plasma, is protein precipitation, which is based on the denaturation of the proteins by miscible organic solvents such as acetonitrile, strong acids, or other agents. The approach works for wide range polarity of the analytes, it is cost-effective without long-lasting optimization and might be automated using 96-well plates [78]. Other extraction techniques will be discussed in the following chapters with the emphasis of those used in the publications included in the thesis.

3.2.2. Solid-phase extraction

3.2.2.1. General principles and modes of solid-phase extraction

SPE is a widely used sample preparation technique applied mostly to analyte preconcentration and clean-up. It is based on the selective adsorption of the analyte on a sorbent (or resin) from a liquid, generally aqueous sample. The classical arrangement of SPE consists of cartridges connected to a vacuum manifold via the plastic stopcock. The cartridges are filled with a resin in the range of 30 mg to 10 g and trapped between polypropylene frits. By applying vacuum, a pressure difference is generated enhancing the passage of the loaded solutions through the sorbent. The typical procedure is shown in Figure 11: 1.) conditioning the resin with an appropriate solvent (e.g. methanol) or buffer to swell the resin and increase the accessible surface area and wash out possible interferences, 2.) loading the sample, often buffered, 3.) cleaning the resin with a washing solution 4.) elution of the analyte and collecting the eluate into a vial. Two approaches are used: Either the analyte is retained and eluted which allows also its preconcentration if the loaded sample volume exceeds the eluent volume, or the resin is selective for the interferences of the matrix for sample clean-up, i.e. retention of the interferents and collecting the sample during loading omitting the following steps [77,79].

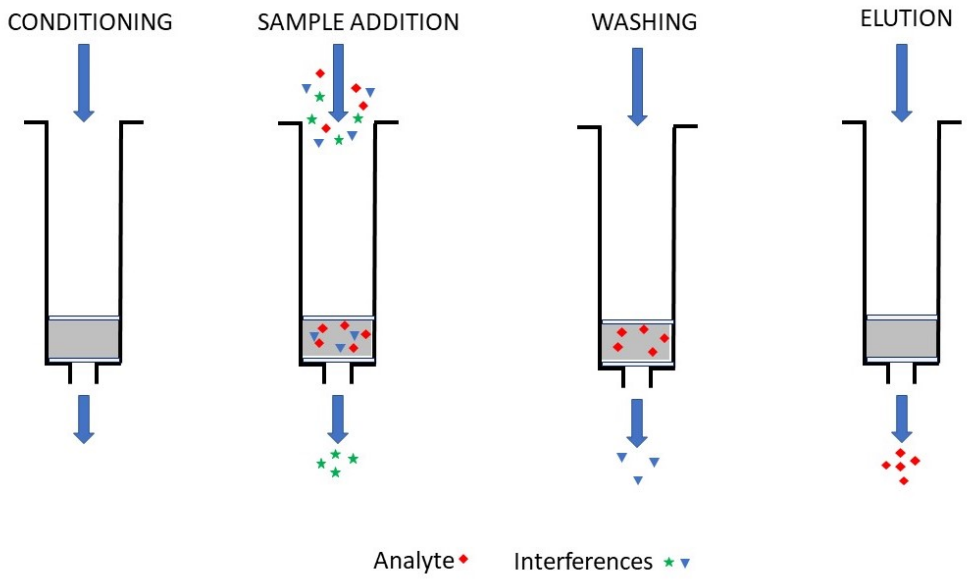

Figure 11: The typical procedure of SPE consisting of sorbent conditioning, sample loading, sorbent washing, and analyte elution.

The mechanism of retention of analytes is described as a partition between the two phases, the solid resin, and the liquid sample, where the analyte affinity must be stronger for the sorbent during loading. The types of interactions include nonpolar, polar, or ionic exchange and depend on the used type of sorbent and given functionalities. The sorbent must be always selected according to the physico-chemical properties of the analyte to achieve high selectivity, affinity, and capacity. Typically used sorbents are based on bonded silica (C8 or C18), ion-exchange, or mixed-modes materials. Besides, sorbents based on particle polymer, monoliths can be also applied for SPE. Novel materials such as molecularly imprinted polymers (MIP), restricted access material (RAM), or graphitized carbon were

also proven as suitable for SPE. Besides cartridges, SPE can be carried out in other formats including discs, membranes, polymer fibres or coatings, well-plates, sorbent-filled pipette tips, or small monolithic columns [79,80].

SPE cartridges are also used for analyte preconcentration from large volumes of sample (e.g. for water analysis) For this, the so-denoted breakthrough volume must be evaluated. A breakthrough occurs when the analyte is eluted from the column with the sample being loaded either because the retention is insufficient, or the loading capacity of the sorbent is exceeded. The breakthrough volume is then the maximum volume of the sample possible to use still avoiding the breakthrough effect [81].

The main advantages of SPE are high analyte recoveries and the achievable preconcentration factor, lower consumption of organic solvents compared to LLE (usually miscible and non-chlorinated ones), usability for the wide range of analytes due to the wide range of selectable sorbents, and the possibility for miniaturization and automation. On the other hand, SPE is still a multistep and tedious procedure with possible low batch-to-batch reproducibility. Moreover, single-use plastic cartridges compared to miniaturization methods still reveal high sample and solvent consumption which makes the technique rather uneconomical with disputable environmental impact [78].

3.2.2.2. Miniaturized approaches in solid-phase extraction

Modern miniaturized SPE approaches reduce drastically the consumption of the sample and solvents, some approaches are even solvent-free. Also, the amount of sorbent is decreased and, in some cases, can be used repeatedly. Moreover, the number of steps is usually reduced and on-line hyphenation with detectors is in the most cases possible (Automation of SPE will be described in chapter 3.2.3)

Microextraction by packed sorbent (MEPS) is a miniaturized SPE technique introduced in 2004 [82]. Only 1-2 mg of a sorbent is packed either inside the void of a microsyringe of 100-250 µL volume as usable on many autosamplers or placed between the syringe and connected needle as a small cartridge. The packed material can be coated for more selective extraction. The possible volume of the sample varies from 50 µL to 1000 µL. SPE methods can be transferred to the MEPS format by downscaling of the volumes. The volume of eluent is greatly reduced compared to typical SPE cartridges which enables the direct injection of the eluate to LC or GC using the so-prepared syringe in their autosamplers without further modification of the instrument. Therefore, automation is very easy and does not require any special equipment.

The MEPS approach is suitable for the extraction of a wide range of analytes even from complex matrices, thus suitable for biological samples yet cartridges are costly but can be generally reused for 10-100 samples [78].

Solid-phase microextraction (SPME), developed in 1989 [83], is an extraction method based on adsorption and desorption of the analyte on a fibre or wall of a fused-silica capillary (in-tube SPME) coated with a selective sorbent of the high active surface. The desorption can be either thermal or by a solvent [84]. The fibre is usually integrated into the needle of a microsyringe and is removable. Also, the fibre is typically made of fused-silica coated with an organic polymer (e.g. polydimethylsiloxane, PMDS) or metallic wire and is immersed either directly into the sample or used in head-space mode

[78]. Direct immersion can cause damage to the fibre by the components of matrix especially by proteins in biological samples (biofouling). Therefore, the headspace approach is preferred in combination with GC and the entire procedure is solvent-free. In this case, the fibre is inserted into the injection port of the GC where thermal desorption of the analyte takes place as shown in Figure 12. The important requirement is the high volatility of the analytes for efficient desorption. Despite the low absolute recoveries (below 5%), the sensitivity is sufficient in combination with GC because the analyte is desorbed and injected into the system quantitatively. Commercial fibres are also very costly but can be used for a long time if used for head-space extraction. In-tube SPME is favourably used for on-line sample preparation prior HPLC separation where the coated capillary is used as an injection loop. Sample is repeatedly passed through the capillary and the analytes are desorpted by the mobile phase [15,85].

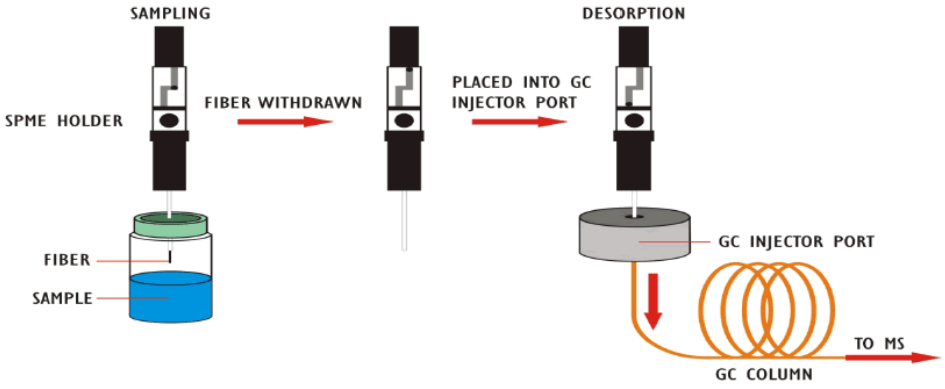

Figure 12: Solid-phase microextraction procedure with on-line desorption in GC-MS. Taken from [86] (open access).

Stir-bar sorptive extraction (SBSE) is based on the same effect as SPME, where the stir bar is coated with the sorbent that is often a layer of PMDS. The stir bar is placed into the sample or into the headspace of the sample according to the used mode of operation. The extraction kinetics depends on a thickness of the coating (typically 0.3-1 mm), size of the bar, and stirring velocity. Extraction times in SBSE are very long, typically up to several hours. For SPME and SBSE techniques, the analytes should be hydrophobic with logP values of at least 2.5 [15,87]. However, novel sorbents for polar analytes have been proposed such as coatings of polyurethane or based on RAM or MIP. In addition, derivatization of more polar analytes can be done during the extraction step [78]. The main disadvantages are the manual removal and drying of the stir bar after extraction and the requirement for special thermal desorption interphase for GC analysis.

3.2.3. Automation of solid-phase extraction

SPE is a popular sample preparation technique since a wide range of sorbents are commercially available, which increases the applicability and acceptance of this technique. However, the number of steps required is time-consuming. Therefore, attempts toward automation in order to reduce the time of the analysis and to increase the robustness of this technique have been made. The following chapters discuss different modes of automation of SPE using sorbent particles, in particular the LOV format and on-line SPE/column switching in HPLC with and without the aid of coupled FT analysers.

3.2.3.1. Automation by Lab-On-Valve Bead Injection Analysis

Automation of SPE by FT can be carried out simply using a short SPE cartridge or column packed with sorbent particles or an extractive disk in the flow system, e.g. placement at the lateral port of the selection valve in an SI-analyser. However, after reusing such a column over a longer time it might get blocked by the particles from the matrix, or at least, the back-pressure increases due to the accumulation of matrix components, which could result in leakages. Moreover, repeated usage can cause carry-over effects by strongly retained components including analytes and irreversible changes of the sorbent surface by the matrix with decreasing the lifetime of a column [88].

A way how to overcome the listed challenges is the use of renewed packing of a microcolumn from a suspension of the sorbent particles, in this approach often called beads. The related technique is therefore known as Bead Injection (BI) that is typically performed in a Lab-On-Valve system (BI-LOV). It is based on in-situ packing of a microcolumn by aspiration of the bead suspension, its transfer into one of the LOV manifold channels, and their trapping by an integrated frit or a stopper. That can be a movable rod, tubing, or an optical fibre with an inner diameter smaller than the diameter of the beads. Thus, the liquid can flow through the microcolumn without the sorbent loss. After each analysis, the beads are discarded to the waste by reversed flow and replaced by a new column packing. The most critical step is to assure adequate trapping without losing beads nor exceeding the pressure limits of the syringe pump. A smaller issue is to keep the beads in a homogeneous suspension, which can be done by continuous stirring or circulation of the suspension by the peristaltic pump [45,88].

For successful handling, the beads in the flow system, spherical beads of uniform size, large diameters (> 100 μm), and good wettability are preferred. Although, molecularly imprinted polymers with irregular shape and a wider range of sizes have been presented as feasible [89]. Commercially available polymeric resins such as those based on polystyrene-divinylbenzene or, in particular, soft particles of agarose are possible to be used directly. Resins can be further modified with specific ligands and antibodies to enhance the sorption of the analytes, or incorporation of chromogenic reagents can be carried out to enable selective detection of the analytes [88]. Magnetic beads, originally developed for immunoassays [90], can be also applied for μSPE [91].

Detection is possible either during the elution of the analytes from the sorbent with the detection flow cell downstream of the microcolumn or directly on the surface of the beads by optosensing. In this case, no elution of the analytes is required thus high preconcentration factors can be achieved. For elemental

analysis, the beads can be also transported to a graphite furnace for the pyrolysis followed by atomic absorption spectrometry (AAS) [92].

The BI-LOV technique was applied for μSPE for the determination of trace concentration of analytes. For example, this technique was used for the preconcentration of the iron(II) from the seawater using cellulose-based chelating sorbent with a post-column colour reaction [93]. Recently, the LOV format was coupled to the HPLC was applied for SPE of oestrogens in wastewaters using highly selective MIP as sorbent [94].

3.2.3.2. Automation of solid-phase extraction by column switching technique

On-line SPE coupled with the HPLC enables preconcentration of a large volume of sample used e.g. for trace-level analysis of environmental samples. Besides, the sample is cleaned from the sample matrix before the separation of analytes on the analytical column.

The switching column technique is based on the loading of the sample on a short trap column or a capillary placed in the injection valve of HPLC when the valve is in the loading position (Fig. 13 A). The column is then cleaned by a weak solvent (e.g. water) to remove the polar matrix without elution of analyte of interests. Afterwards, the valve is switched to the injection position (Fig 13 B) and the analytes are eluted with the mobile phase of the HPLC onto the analytical column for their separation. When the valve is switched to the loading position, the extraction column can be cleaned with solvent concurrently with the separation step [95]. This switching time must be optimized to ensure enough cleaning time to remove matrix components without losing analytes. Operation of SPE column loading and elution can be in counter or co-current mode. The counter-current or back-flush mode is usually preferred since it minimizes peak broadening but all particles that have been settled onto the SPE cartridge will be flushed onto the HPLC columns, so sample filtration is imperative before use. When the column is flushed by the eluent in the sample direction as the HPLC mobile phase, the extraction column acts as a filter, thus the analytical column is protected but particle would accumulate in the extraction column [96].

The described configuration was applied for the analysis of emerging pollutants such as bisphenols or antibiotics in environmental matrices using a wide range of sorbents including MIP [97] and RAM [98]. Also, the technique is suitable after certain treatment for biological matrices such as urine [99], plasma [100], or saliva [101].

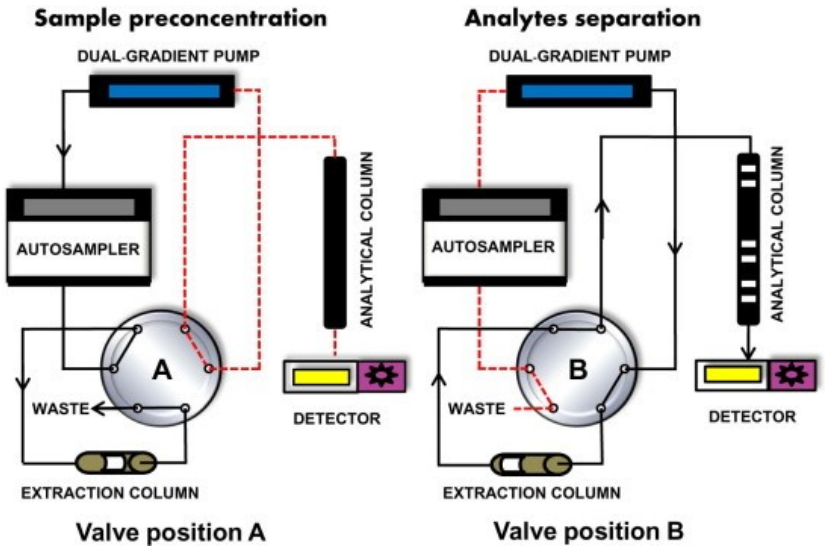

Figure 13: Configuration for switching valve system of the HPLC. A: Loading position for analyte preconcentration, B: Inject position for analyte separation. Taken with permission from [95].

The column switching technique is performed using two HPLC pumps, one for loading of the sample onto the column and elution to a second column for analyte separation. Nevertheless, the on-line SPE HPLC can be also coupled with FT with the extraction column placed in the injection valve as interphase. The flow manifold increases the versatility of the system enabling preliminary sample treatment (e.g. addition of reagent, dilution, or LLE). The synchronization of the HPLC software and the FT automated sample pretreatment requires to trigger one instrument with the second where the secondary instrument has to wait for the primary to initiate the next analysis cycle. Adjustment of the timing of both operations surely requires some experience but optimized, the analyses can run unattended. For parallel loading and separation, using gradient elution, the switching time to injection position for the interfacing injection valve must be optimized to elute all analytes onto the second column for analyte separation. Moreover, the separation gradient\ composition of the mobile phase must be carefully adjusted and usually, it is a compromise between the mobile phase strong enough for elution of all analytes from the extraction column and effective separation of the analytes on the separation column. If the eluent is not capable to elute all the analytes, carry-over effects of the most retained analytes appear. As it was mentioned above, the column might get blocked with time and cause increased back-pressure and leakage. Therefore, regular cleaning of the column is required. The frequency depends mostly on the sample matrix.

3.2.4. Liquid phase extraction

3.2.4.1. General principles and modes of liquid-phase extraction

Liquid-phase extraction is a sample preparation technique where the analyte is transferred from liquid, gas, or solid sample into a liquid acceptor phase, the extraction solvent. During this process, the target analytes are cleaned from most of the matrix and might also be preconcentrated depending on the employed volumes of sample and extraction solvent. For efficient performance, the extraction solvent must be immiscible with the sample, typically being aqueous and the analyte has to be more soluble in the solvent than in the sample. The mass transfer is then based on diffusion or, if the mixture is agitated, on efficient convection. The ratio of the analyte A in the two phases in the equilibrium state is given by the distribution constant K_D defined as:

$$K_D(A) = {}^{c_{A,org.}}/_{c_{A,aq.}}$$

where $c_{A,org.}$ is the concentration of the analyte in the extract and $c_{A,aq}$ is the concentration of the analyte in the sample. For the characterization of compounds, generally, the decadic logarithm of K_D, i.e. $\log K_D$ or shortly logD is used.

The fraction of the analyte extracted E depends on the K_D and the volume ratio between the extractant V_E and sample V_S and is described as:

$$E = {}^{K_D \cdot \frac{V_E}{V_S}}\Big/_{(1 + K_D \cdot \frac{V_E}{V_S})}$$

The extraction should be optimized to achieve the highest K_D at a reasonable phase ratio. For $V_E > V_S$, the extraction might be efficient, but the analyte is diluted, and the solvent consumption will be unacceptably high. Therefore, a volumetric ratio of $\frac{V_E}{V_S} < 1$ is typically used for the analyte with high distribution constant. For the analytes with lower K_D it is preferred to apply multiple extractions with the fresh solvent [102].

The extraction is based on different chemistries. Besides the distribution of the single molecules, analytes can be transferred to the organic solvent as ion-pairs (to compensate for a permanent charge of the analyte) or as chelates (metal analysis). To achieve a high distribution constant for the analyte, parameters such as the extraction solvent, the sample pH in case of analytes with acid-basic properties, or the type and concertation of the chelating or ion-pairing reagent are optimized. In respect of the extraction solvent, the ability to form hydrogen bonds, the presence of aromatic functionality, or the dipole moment are of interest. The extraction kinetics depend further on the agitation rate, temperature, and viscosity of the phases [103].

Classical LLE has been favourably used for decades for its simple performance and required apparatus consisting only of separation funnels, flasks, or beakers. Usually, several millilitres up to litre volumes of the sample are agitated with the extraction solvent and phase separation is reached simply by gravity or aided by centrifugation.

For large volumes of the sample, continuous LLE can be used. Generally, there are two possibilities: either the solvent is continuously propelled through the sample or the sample is continuously flowing through or around a constant small volume of the solvent yielding high preconcentration factor as it was presented in publication 4 [57].

For the first option, glass-apparatus can be used that enables continuous cycling of the solvent which passes the sample as a liquid, is then outside the sample vessel evaporated to yield the extracted analyte, hereafter condensed and re-used for extraction [104]. This procedure is useful for the analytes with a low distribution coefficient and this configuration is also mostly used in the industry.

Classical approaches of LLE are not environmentally friendly nor economical due to large volumes of organic solvents required and waste produced. Moreover, they involve tedious sample manipulation and reach limited enrichment factors. Novel miniaturized and automated procedures have been developed whose main advantages are reduction of the cost per analysis, increased time efficiency, higher preconcentration factors, and lower impact on the environment by using far less or less harmful miscible extraction solvent.

3.2.4.2. Liquid phase microextraction approaches

Static versus dynamic mode

Miniaturized methods based on LLE principles are replacing conventional LLE for sample preparation in various analytical tasks. They have become popular for their low consumption of extraction solvent (some of the approaches are free of organic solvent), high achievable preconcentration factors, low instrumentation requirements, and low cost.

The extraction can be carried out either in static or dynamic modes according to the hydrodynamic features. In static mode, the solvent is immersed into a fixed volume of the sample. The analytes are diffusing into the solvent but the extraction rate decreases with the time as the extraction equilibrium is approached and the analyte concentration in the sample and thus the concentration gradient decreases.

On the other hand, in dynamic mode, sample, solvent, or both phases are continuously exchanged. The extraction efficiency is lower compared to static mode because of the shorter contact time but favoured by the fluid flow. On the other hand, the extraction rate does not decrease or at least not as rapidly. This is because there is no significant reduction of the analyte concentration in the sample or no saturation of the extraction solvent since at least one phase is continuously exchanged [105].

Single drop microextraction (SDME)

SDME was the pioneer of the miniaturized extraction methods developed in the mid-90s by Liu and Dasgupta [106] applied to the extraction of gases into a drop of the aqueous reagent in a flow system. Later, the same authors proposed a drop of solvent immersed in a drop of aqueous sample (drop-in-drop) [107]. In the same year, the manual performance of sample preparation using a single drop was proposed [108].

The drop can be either directly immersed into the sample (DI-SDME) or can be exposed to the volatile analytes in the head-space above the sample (HS-SDME). The applications of both configurations are summarized in recent reviews [109,110].

The SDME technique is based on the extraction (generally non-exhaustive) into a small drop of acceptor phase hanging from the support, which is most often the needle tip of a microsyringe. The drop size ranges typically from 0.2 to 5 μL but can be as large as 30 μL yet with increasing problems in stability [111]. In head-space mode, the acceptor should be of low volatility while in the mode of direct immersion, the used extraction solvent must reveal very little solubility in the sample. High viscosity and surface tension are ideal to create a stable drop in both cases. In addition, the used solvent should be compatible with the intended analytical instrumentation yet due to the small volume, this is of lower importance.

DI-SDME (Figure 14A) is limited to the use of only low stirring rates in order to avoid drop instability. Moreover, particles present in samples can cause problems such as accumulation on the drop surface. To yield a very high preconcentration factor the drop can be exposed to the continuously flowing sample. This technique is known as continuous flow microextraction and was developed in 2000 by Liu and Lee [112]. DI-SDME can also be performed as liquid-liquid-liquid microextraction (LLLME) [113], i.e. using a three-phase system where the ionizable analytes are extracted in its non-ionic form into an organic layer on the top of the sample and while simultaneously back-extracted into an aqueous drop immersed in the organic phase in which the analytes are ionized. The organic solvent acts as a liquid membrane and the concentration gradient is maintained. The drop can also float on the surface of the sample without any support and can be collected by solidification of the floating organic drop, where the sample after extraction is placed into the ice bath and the organic extract rapidly solidifies and can be removed with a spatula, melted at the room temperature, and analysed [114]. Vice versa, also solidification of the sample has been reported [115] allowing the decantation of the extractant so that a wider range of solvents can be used.

HS-SDME (Figure 14B) is advantageously used for volatile and semi-volatile analytes such as alcohols [116], or benzene, toluene, ethylbenzene, and xylenes (BTEX) [117] in highly complex matrices. Moreover, the drop does not have to be an organic solvent but also be an aqueous solution. The sample can be vigorously stirred to enhance mass transfer without affecting the drop stability. The extraction rate is determined by the transfer from the sample to the headspace being the slowest step. The mass transfer can be enhanced by the stirring, applying ultrasound energy or microwave radiation, or by a decrease of pressure [109].

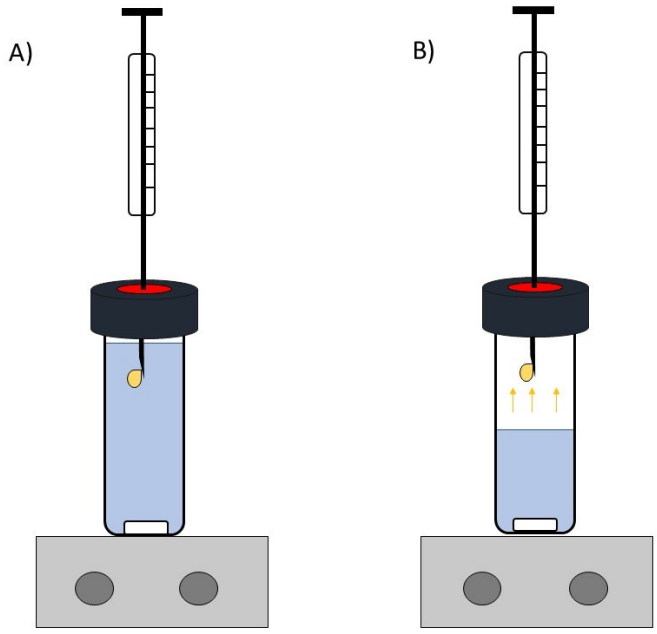

Figure 14: Configurations of single drop microextraction A) Direct immersion SDME B) Headspace SDME.

Hollow fibre liquid-liquid microextraction (HF-LLME)

HF-LLME using mechanical support for the extraction solvent was introduced in 1999 by Pedersen-Bjergaard and Rasmussen. In the two-phase system, pores and lumen of the hollow fibre are filled with the organic solvent. In the three-phase system, the lumen is usually filled with an aqueous acceptor phase of certain pH into which the analyte can be back-extracted in its ionized form [105]. The chemical principle is the same as for the three-phase SDME and based on a pH and permanent concentration gradient of the non-ionic analyte form.

The hollow fibre is usually made of polypropylene with a length of 1.5-10 cm. It is soaked in the organic solvent and the excess of the solvent is washed away so that in the pores remains about 15 to 20 µL. The organic solvent should be immiscible with water, have selective extraction properties, and should have a sufficiently high viscosity for stable immobilization in the pores. For these reasons, 1-octanol or dihexyl ether are often selected [118]. The fibre can be sealed from one side and supported from the other side by a syringe needle, which also handles the liquid inside the lumen [119]. Another option is to use a fibre sealed on both ends that are immersed in the sample containing a stirring bar. This technique is known as solvent bar microextraction [120].

The hollow fibre acts as a protection and microfilter of the extract inside the lumen, thus this technique can be used also for highly complex and dirty samples [121]. Moreover, high enrichment factors are generally yielded, and the extracts and compatibility with the detection techniques depend on the selected mode. In the two-phase system, the organic extract can be directly injected into the GC and in the three-phase system, the analytical instrument is most often HPLC or CE [118]. Nevertheless, there are some drawbacks related to the manual handling of the hollow fibre and syringe, which can be a source of extract contamination [121]. The typical configuration is shown in Figure 15.

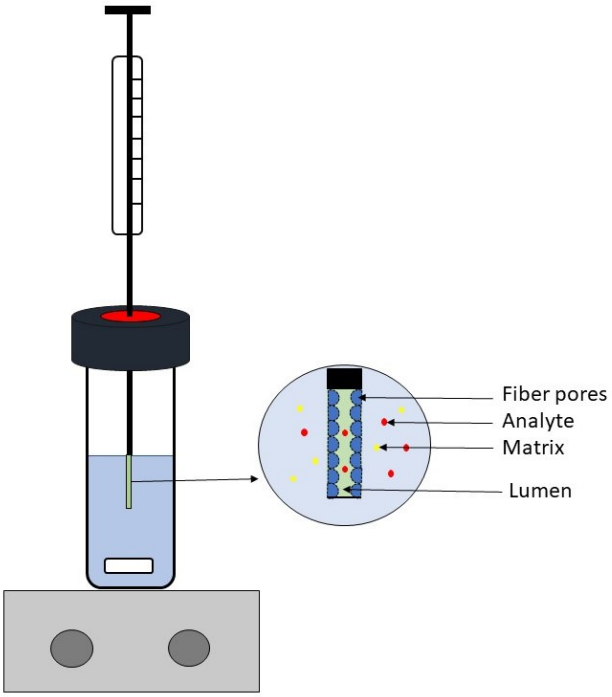

Figure 15: Configuration of hollow fibre liquid-liquid microextraction.

Dispersive liquid-liquid microextraction (DLLME)

DLLME is nowadays widely used for sample preparation since this technique is fast, cheap, simple, yields high enrichment factors, and produces low volumes of organic waste. The procedure is shown in Figure 16.

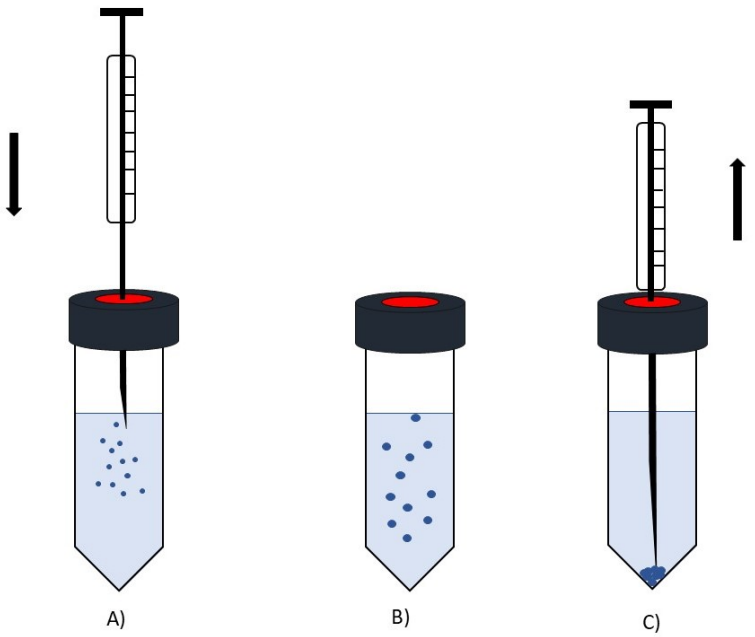

Figure 16: Typical procedure of dispersive liquid-liquid microextraction. A) Injection of the extraction and disperser solvent B) Dispersion of the extraction solvent and rapid extraction of the analyte C) Sedimentation of the extraction solvent by centrifugation and collection of the extract by the syringe.

The original DLLME method developed in 2006 by Rezaee [122] and co-workers was based on a two-phase system. An extraction solvent with a higher density than water mixed with the water-soluble disperser solvent is rapidly injected into the aqueous sample resulting in the creation of a cloudy solution. This is caused by the immediate dissolution of the disperser solvent in the sample leaving behind very small droplets of the immiscible extraction solvent. The immensely large surface area between the phases enables a very fast mass transfer. After the extraction, the cloudy state is broken by centrifugation, spontaneous sedimentation, and droplet coalescence, or by solvent-assisted demulsification. The bottom phase, i.e. the extraction solvent containing the analytes, is collected by a microsyringe and injected into the detection instrumentation. This configuration has several requirements on the extraction solvent such as its higher density than water, very low solubility in the aqueous phase, the ability to extract analyte of interest, and the compatibility with the analytical instrumentation. Only a few organic solvents meet these requirements and are often from the group of halogenated hydrocarbons, which are toxic solvents and excellent solvents for diverse plastics. The disperser solvent has to be miscible with both phases; therefore, MeOH, acetonitrile (ACN), or acetone are often utilized.

DLLME can sometimes lead to the formation of stable emulsions, in particular, in the presence of surface-active substances and when the disperser solvent is used. In this case, centrifugation is imperative to assure separation of the phases but this step prolongs the procedure and complicates its automation [123,124].

Alternative approaches have been also introduced. Solvents lighter than water replaced halogenated hydrocarbons. The disperser solvent was omitted using different stirring force such as ultrasound, or vortexing to achieve solvent dispersion instead, which allows spontaneous phase separation without required centrifugation [125].

For easy recovery and collection of floating solvents, special purpose-made extraction flasks have been developed, e.g. featuring a narrow neck or a stopper with a capillary to confine the solvent droplets [126]. Another way of the collection of the solvent is by solidification of the floating organic drop.

3.2.4.3. Homogeneous liquid-liquid extraction and QuEChERS

Homogeneous liquid-liquid extraction uses two miscible phases, usually an aqueous sample and a polar organic solvent which initially form a homogenous phase, i.e. showing an infinitely large interface. Therefore, the extraction kinetics at phase separation is very fast, and long extraction time is not required. The phase separation can be induced by various approaches depending also on the solvent used, such as by the addition of kosmotropic compounds, i.e. via salting- or sugaring-out [127,128], via a change in temperature [129], by a change of the pH value using a “switchable solvent” that shows acid-base properties [130], or by the addition of a small amount of ternary solvent [131]. Typically used solvents are ACN, ethyl acetate, acetone, or isopropanol. The technique is appreciated for the extraction of moderately polar analytes and for its low cost, time efficiency, and the possibility of using less toxic solvents that are compatible with often used analytical techniques such as liquid chromatography [132].

Salting-out, i.e. the increase of the ionic strength, is likely the most frequently used approach to induce phase separation. The salting-out phenomenon can be explained by a formation of multiple-layered hydration shells around the added ions by electrostatic attraction of water, which means that a lower volume of water is available for dissolving the organic solvent thus causing its oversaturation and displacement from the homogenous solution. The salting-out effect of the ions increases with the high ion charge density and small ion size. The lyotropic series describes the ability of anions (citrate, CO_3^{2-} > SO_4^{2-} > Cl^- > NO_3^- …) and cations (Mg^{2+} > Ca^{2+} > Li^+ > Na^+ > NH_4^+…) to precipitate hydrophobic substances in polar solvents. According to this, the most often used salts are magnesium sulphate, ammonium sulphate, and sodium chloride also due to the high solubility, low cost, and unlikelihood of precipitating other matrix components of these substances [133]. Salting-out extraction (SALLE) is advantageously used for moderately polar analytes such as sulphonamides [134], tetracycline [135], or artificial sweeteners [136] yielding high extraction efficiencies. The method can be used even for complex matrices because the solvent to sample ratio must be small to achieve phase separation so that the relative loss of solvent by absorption to the sample matrix, e.g. particles, and the risk for emulsion formation are negligible. Using larger volumes of the extraction solvent makes also the collection of the extract simpler than for miniaturized approaches. However, the preconcentration factor is small and a secondary preconcentration of the analytes can be required and imperative for trace analysis [132].

QuEChERS (quick, easy, cheap, effective, rugged, and safe) is a multistep sample preparation introduced in 2003 [137]. The procedure covers SALLE in combination with dispersive SPE (dSPE) as a secondary extract clean-up. This procedure is mostly applied for the determination of multiclass residues from complex food samples (juices, eggs, wines, rice). Before the extraction, proper homogenization of the sample is required usually by dry ice. Then 10 g of the sample is added into ACN in the ratio 1:1 (w/vol). ACN is suitable for most of the applications for moderately polar analytes because is selective and compatible with chromatographic instrumentation. For the more hydrophobic analytes, acetone or ethyl acetate can be used, nevertheless, lipids or waxes can be co-extracted in this case. Type and amount of salt influence the water content in the organic layer. The salt of choice is usually anhydrous $MgSO_4$ in combination with ACN giving high recoveries for moderately polar analytes. Nevertheless, in the organic part residues of water may remain, which can cause co-extraction of the polar compounds from the matrix. Sodium chloride can be added to regulate the polarity of the extract and increase selectivity. dSPE is conducted to remove unwanted co-extracted compounds from the matrix. Compared to traditional SPE there is no need for vacuum manifold and no conditioning and elution step since it is used for the adsorption of matrix compounds [138]. The procedure was applied for the determination of pesticides from fruit and vegetables by GC or LC with mass spectrometry detection [139,140], veterinary drugs from meat or eggs [141,142], or mycotoxins from wine and beer [143,144].

3.2.5. Automation of liquid-liquid extraction

This chapter is focused mainly on the automation of liquid-phase microextraction techniques covered in this dissertation being SDME, DLLME, and SALLE. More information about the automation of other extraction techniques can be found in comprehensive reviews about the automation of static and dynamic non-dispersive [20,145] and dispersive [23] LLE.

3.2.5.1. Automation of liquid-liquid extraction by autosamplers

Autosamplers enable automating LLE procedures mostly in vials but it is also possible to carry out the procedure directly in the syringe void similarly to the LIS approach. By an autosampler, it is possible to aspirate precise volumes of the sample, reagents, and solvent and dispense them into a vial for mixing or derivatization. An extract can be collected and directly injected into a hyphenated analytical instrument. Some autosamplers have already integrated positions, e.g. for vortexing or magnetic stirring, and manage to automate even complex microextraction procedures. In-parallel extractions by passive diffusion are possible but position for vortexing, heating, or stirring is usually just one, therefore these kinds of sample processing are usually sequential [20,23].

For instance, autosampler can be used for the automation of HS- and DI-SDME, as well as the automation of HF-LLME [146]. In the dynamic mode, the HS-SDME drop was repeatedly exposed in the headspace and retracted into the syringe. For DI-SDME the solvent was inside the syringe in the thin layer and the sample was repeatedly aspirated and dispensed. All the steps along with agitation, drop formation, exposure, and retraction, and delivery for the analysis were automated.

An autosampler was also used for the automation of DLLME coupled to GC-MS for the determination of phthalate esters. The use of a low-density extraction solvent as well as solvent-based demulsification, ease the automation of the procedure. The procedure was fully automated, however, using only 100 µL syringe, the injections of all the solvents had to be repeated several times, which significantly prolonged the procedure. Moreover, the addition of a large volume of ACN for demulsification alters in principle the solubility of the analytes in the sample which consequently decreases the extraction efficiency [147]. The problem with a long time of the procedure was solved using a two-rail autosampler with a one 2.5 mL syringe for extraction procedure and a second 10 µL syringe for injection of the extract into GC-MS [148].

Ionic liquid-based DLLME was also automated by an autosampler system with an additional SPE step. The procedure was used for the extraction of benzoylurea from water based on HLLE with first ionic liquid and induction of the phase separation by the addition of second ionic liquid. The emulsion was then loaded on the SPE column to retain extract droplets and the analytes were eluted by ACN and injected into HPLC-UV. The whole procedure was automated enabling pretreatment of 4 samples simultaneously and phase separation by centrifugation was omitted using SPE [149].

Autosamplers are combined with chromatographic instruments usually already by the producer. Therefore, both parts are often controlled by the same software, which makes the system user friendly, robust, and reliable. Therefore, they are the first choice for commercial laboratories. Nevertheless, DLLME automation by autosamplers is not frequently investigated due to the problems with

compatibility of the used solvent with HPLC or commercial unavailability of vials enabling the reliable collection of the extract. Therefore, the development in this field is mostly focused on modifications of the autosampler systems such as the addition of modules for vortexing or centrifugation to enable the automation of the entire procedure [23].

3.2.5.2. Automation of liquid-liquid extraction using classical flow techniques

LLE automated by FT has several advantages compared to batch extraction such as elimination of contamination from outside in the closed system, high sampling rate, and self-cleaning feature of the system avoiding carry-over [23]. The earliest approaches of flow automation of LLE were published in 1978 by Karlberg [150] who used a phase segmenter, an extraction coil, and a phase separator (Figure 17) to create a solvent-segmented sample stream comparable to continuous air-segmented flow analysis (see section 3.1.3.2). The typical procedure starts by the injection of the sample into the carrier stream (or continuous aspiration of the sample), which can be mixed with a reagent on the way to the phase segmenter, e.g. to adapt the extraction conditions. The stream of sample merges with the organic phase by confluence and continues via the extraction coil, in which the mass transfer from the sample into the segments of solvent takes place. To achieve a steady flow rate of the organic solvent and avoid the contact of the organic solvent with the peristaltic pump tubing, a displacement bottle can be used. The aqueous phase is removed in the phase separator while the extract continues to the detector. The phase separator is the most important component since it must ensure complete separation of the organic phase from the aqueous sample. Phase separation is generally based on the difference in densities or selective permeability of the solvent through a hydrophobic membrane [151].

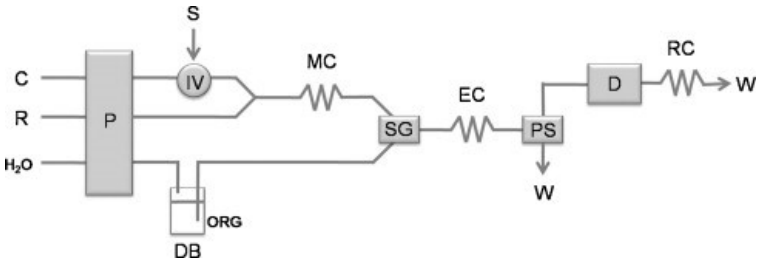

Figure 17: Configuration of the flow-based manifold for liquid-liquid extraction. C: carrier, R: reagent, P: propulsion unit, S: sample, IV: injection valve: MC: mixing coil, DB: displacement bottle, ORG: organic solvent, SG: segmenter, EC: Extraction coil, PS: phase separator, D: detector, RC: restriction coil, W: waste. Taken with permission from [151].

A similar manifold as shown in Figure 17 was used for elemental analysis by GFAAS but integrating back-extraction of the metal analytes to an aqueous phase. After the first phase separation, the organic phase was transferred to another segmenter and mixed with the stripping agent. After passing the second phase separator the aqueous extract was injected to the detector [152].

Automated back-extraction in a FI\SIA system was presented also by Wang et and Hansen in 2002. The system consisted of two extraction coils. In the first, the metal analytes and reagents were continuously pumped by a peristaltic pump and were merged with the extraction solvent aspirated by a syringe pump. A dual-conical gravitational phase separator was used to get the pure organic extract, which was merged in the second extraction coil with a back-extraction solution containing nitric acid

and stripping agent. After the secondary phase separation, the extract was injected into the GFAAS [153] and ICP-MS [154].

To achieve low dispersion of the sample in the carrier stream, monosegmented flow system can be used. In the two-phase system for the determination of cadmium, the plug of the sample was trapped between the two air bubbles followed by a plug of organic solvent. The mass transfer between the phases was based on the creation of a thin water film on the wall of the glass tube. This configuration allows efficient extraction, high sample throughput, and low carry-over effect without the requirement of phase separation [155].

The wetting film can be also created with the organic solvent on the wall of hydrophobic PTFE tubes of extraction coil, which forms a pseudo-stationary phase. The film is stabilized by the aspiration of air, and the excess of the organic solvent is removed by aspiration of air or water. Afterwards, the sample is aspirated into the extraction coil and the analytes of interest are extracted into the thin organic film. Finally, the analyte is back-extracted into a suitable acceptor and carried towards the detector or directly flushed out as the organic film that is dissolved by a secondary solvent. The extraction film is renewed for each analysis cycle. This configuration does not require a phase segmenter and allows quantification of both phases in one cycle and the consumption of the organic solvent is low. The most challenging part is the reproducible formation of the film (thickness, intactness, etc.) and the stability of the wetting film during the procedure [151]. The wetting film approach was used for instance for the extraction of nitrophenols from wastewater [156].

Flow-based DLLME in the flow analysis was introduced in 2009 by Anthemidis and Ioannou for elemental analysis of Cu and Pb by FAAS. The mixture of extraction and disperser solvent (xylene and methanol) and chelating reagents were aspirated into the holding coil and mixed with the sample by the aid of an auxiliary peristaltic pump forming a cloudy solution. The complexed metals were rapidly extracted into the solvent droplets that were retained on a microcolumn filled with PTFE-turnings. Hereafter, the solvent was eluted by methyl isobutyl ketone and injected into the detector [157].

3.2.5.3. Automation of liquid-liquid extraction by flow-batch approaches

For LLE automated by Flow-Batch techniques, an extraction chamber is utilized that is operated by the flow system. Compared to LLE carried out in a tubing manifold of the flow system, phase separation in-batch is achieved simply by gravity. The large dead volume of the chamber requires proper cleaning and limits the versatility of operation, e.g. only slow stirring speed is feasible are among the disadvantages described already in section 3.1.3.6.

DI-SDME was automated in an SIA system using a stirred extraction chamber in which the solvent drop was supported by the sampling probe of the autosampler of a GFAAS instrument for the enrichment and posterior analysis of chromium. The SIA system controlled the aspiration of sample and reagents to the extraction chamber while the formation of the drop and the injection to the detector was carried out by the pump of the autosampler [158]. DI-SDME was also carried out inside a flow-through extraction chamber for trace metal analysis. The drop was attached to a glass capillary, the sample was continuously flowing around the microdroplet, and cadmium was extracted as a complex with diethyldithiophosphate [159].

Automation of DLLME was done by SIA-based flow batch analysis and the first attempts were made by our research group in collaboration with the University of P.J. Šafárik in Košice presenting the technique called Dual-Valve SIA (DV-SIA). The system consisted of two SIA instruments connected both to an extraction chamber. The first system (SIA1) handled all the required solutions including the extraction solvent towards the chamber while SIA2 was exclusively used for handling the extract. By this, problems due to passing a biphasic solution through the detection cell (Schlieren effect) were overcome [49]. Air-assisted DLLME was carried out in two subsequent works where SIA1 was used only for the aqueous solutions and SIA2 only for the organic phase. Solvent dispersion was achieved by controlled aeration in the extraction chamber. The system was used for the determination of traces of copper in pharmaceuticals [160] as well as thiocyanate ions in saliva as ion-pair complex [161]. Finally, the system, shown in Figure 18, was used for DLLME based on using a disperser solvent and high flow rate injection of the solvent mixture to achieve dispersion [162].

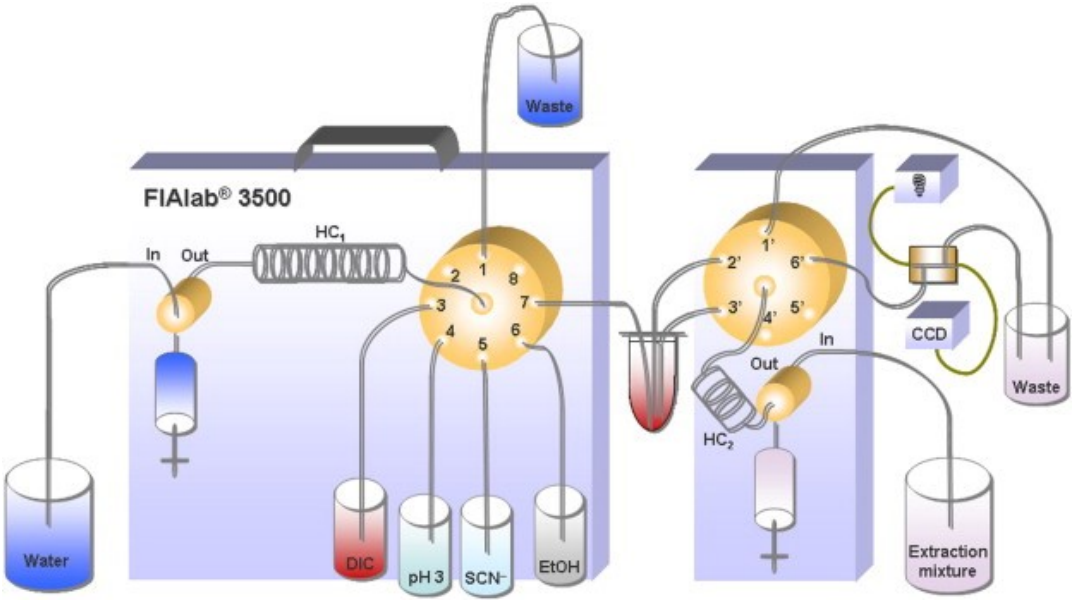

Figure 18: DV-SIA used for flow-batch dispersive liquid-liquid microextraction for the determination of thiocyanate ions. HC: holding coil. Taken with permission from [162].

Recently, the flow-batch automation of HLLE has been reported by Bulatov's group. A mixing chamber was placed on a lateral port of the selection valve of an SIA system. Phase separation from an acetonitrile-water mixture was induced either by the addition of concentrated glucose solution (sugaring-out) [163] or highly concentrated sodium hydroxide solution (7 mol/L) [164] or the addition of NaCl (salting-out) using octylamine as a novel extractant for HLLE [165]. Air bubbling was used to promote extraction. The respective analyser systems were coupled to HPLC-UV or combined with in-line spectrophotometric detection for the determination of tetracycline in urine [165], procainamide in urine [166], or diclofenac in saliva [164], respectively. The system used for alkaline-induced phase separation and the in-line optical probe is shown in Figure 19.

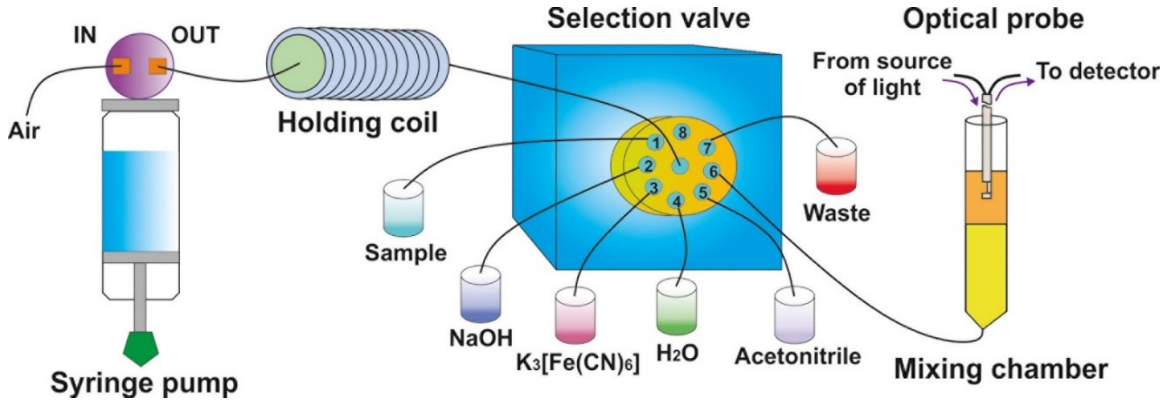

Figure 19: Flow-batch automated salting-out extraction with alkaline-induced phase separation for the determination of diclofenac from human saliva. Taken with permission from [164].

3.2.5.4. Automation of liquid-liquid extraction by Lab-In-Syringe

The LIS technique is similar to flow-batch automation, yet LIS has demonstrated to be more versatile and shows some important advantages as it was discussed in section 3.1.3.6. The differences include a more efficient and faster cleaning and size adaptable constantly closed vessel that helps to avoid carry-over and environmental contamination of the sample during treatment.

DLLME has been automated by LIS most frequently. The first publication introducing LIS technique was focused on automation of DLLME using solvent lighter than water and the disperser solvent. Because the syringe inlet has a wider diameter than disposable plastic syringes the procedure begun with the aspiration of extraction and disperser solvents unlike the manual procedures and the dispersion was achieved by aspiration of a sample using a high flow rate [4]. In the subsequent work, spectrophotometric detection was carried out directly in the syringe void after phase separation of the organic extract lighter than water. Optical fibres were placed oppositely on the upper part of the syringe for the detection of Rhodamine B dye [7]. The following publications were focused on in-syringe derivatization and complexation for determination of copper as a complex with bathocuproine [167] and aluminium in seawater with lumogallion using fluorometric detection [168].

The stirring-assisted LIS was used for the determination of aluminium omitting disperser solvent [51] or for the determination of chromium using reaction with diphenylcarbazide [160]. The upside-down orientation of the syringe enables complete emptying. This configuration was for the first time applied for the determination of cationic surfactants using solvent denser than water (chloroform). The extract was kept in a short holding coil while the aqueous phase was discarded to the waste. The extract was then re-aspirated and cleaned as it is shown in Figure 20 [161].

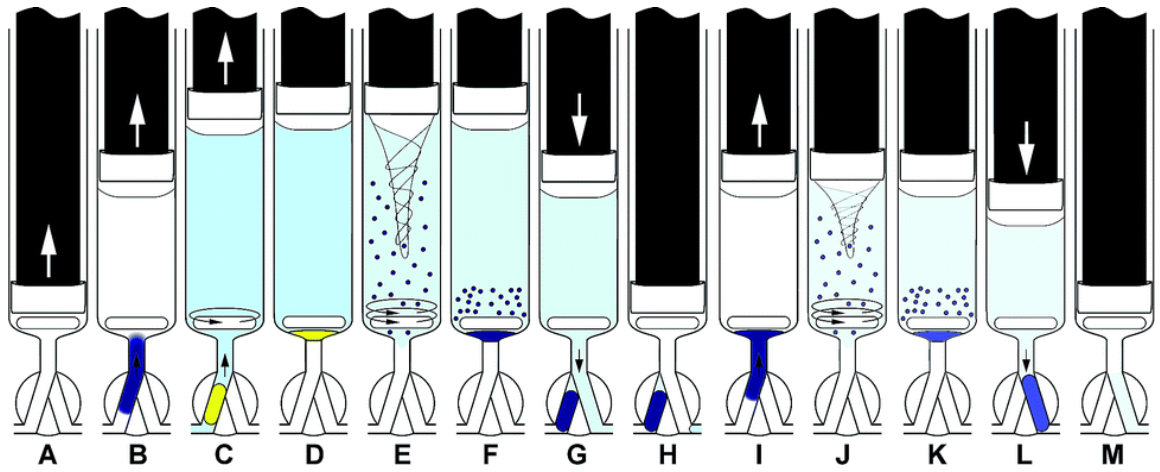

Figure 20: Lab-In-Syringe automated procedure for dispersive liquid-liquid microextraction. A-B) Aspiration of the sample and buffer, C-D) Mixing and aspiration of the solvent, E) DLLME, F) Solvent sedimentation, G-H) Keeping extract in the holding coil and discharging the sample to the waste, I-J) Re-aspiration of the extract and cleaning with water, K-M) Extract sedimentation and injection to the detector. Taken with permission from [169].

Feasibility of LIS-automated dispersive back-extraction into the aqueous phase was demonstrated for the determination of heavy metals in different matrices. This work is included in the dissertation thesis (Publication 1) [8]. A secondary inlet (enabled via the drilled-through syringe piston) in the syringe piston was used to carry out continuous DLLME for the determination of nitrophenols by multivariate spectral analysis. The sample was semi-continuously flowing through the syringe containing a fixed volume of the extraction solvent. The publication is included in this dissertation (Publication 4) [57].

HS-SDME was automated inside of the syringe pump by Šrámková et al. [5] for the determination of ethanol in wine (Figure 21). Negative pressure was applied for the enhancement of analyte volatilization. The drop was an aqueous reagent of potassium dichromate and sulphuric acid. The detection was based on the reduction of chromium(VI) to chromium(III) by ethanol. The unreacted chromium (VI) was detected spectrophotometrically. A similar system was later used for HS-SDME of the mercury by an amalgamation process inside an aqueous drop containing Pd nanoparticles as a trapping agent with subsequent detection by GFAAS [52]. Šrámková et al. also proposed LIS-automated HS-SDME using the syringe in an upside-down position with a secondary inlet used for drop formation. The system was used for the determination of ammonia in environmental water [58]. The same upside-down configuration with a secondary inlet was also used for the automation of headspace extraction of benzene, toluene, ethylbenzene, and xylenes from water samples. The system was coupled to GC with a flame ionization detector (FID). The use of any extraction solvent was omitted and analytes in the gaseous phase were directly injected into the analytical instrument [54].

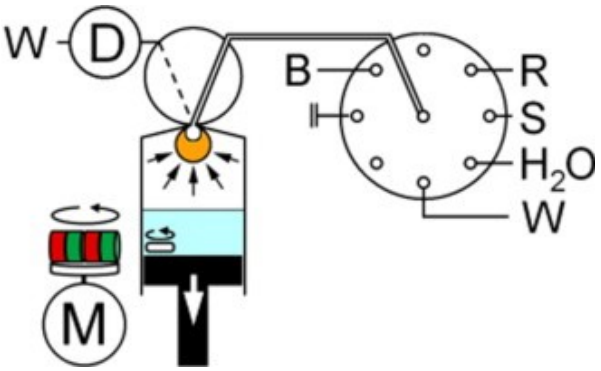

Figure 21: Lab-In-Syringe automated headspace single drop microextraction. Taken with permission from [5].

DI-SDME of lead from drinking water was also proposed by our group and the work is included in this dissertation (Publication 3). In short, two configurations were proposed. The first one used the syringe in an upright position and a drop of a toluene-hexanol mixture was floating inside the sample supported by an insert in the syringe inlet. In the second configuration, the orientation of the syringe was upside-down and a small stirring cross was placed into the drop of chloroform that increased the surface of the extraction phase and extraction speed without leading to solvent dispersion [6].

Besides the flow-batch automation of HLLE, LIS automation of sugaring-out extraction by the LIS technique was presented by Timofeeva et al. [9] for the determination of pesticides in fruit and berry juices. ACN was used as an extraction solvent and a saturated glucose solution acted as the phase separation agent. The system was coupled with HPLC-MS/MS detection. LIS automation of SALLE in combination with secondary clean-up and preconcentration by on-line SPE for determination of sulphonamides from urine is included in this dissertation (Publication 5).

This dissertation contributes to this topic by new applications of DLLME and SALLE and as a new mode of LIS-automated treatment by DI-SDME. The selected methods using the LIS technique are summarized in Table 2.

Table 2: Selected methods using LIS technique listed according the used extraction technique and time.

Analyte	Matrix	Extraction method	Detection	Syringe position	Ref.
Benzo(a)pyrene	Tap water	DLLME-disperser solvent	HPLC-UV	Upright	[4]
Rhodamine B	Water samples, soft drinks	DLLME-disperser solvent	UV-Vis-on drop sensing	Upright	[7]
Copper	Environmental water	DLLME-rapid aspiration of the sample	UV-Vis-long path detection	Upright	[167]
Aluminium	Coastal seawater	DLLME-disperser solvent	Fluorescence	Upright	[168]
Chromium (VI)	Environmental water	DLLME-stirring	UV-VIs	Upright	[170]
Cationic surfactants	Drinking water	DLLME- stirring	UV-Vis	Upside-down	[169]
Cadmium, Copper, Lead	digestive fluids, seawater, soil leachate	DLLME- stirring	ICP-AES	Upside-down	[8]
Nitrophenols	Surface water	Continuous DLLME-stirring	UV-Vis	Upside-down	[57]
Pesticides	Fruit and Berry juice	Sugaring-out LLE	HPLC-MS/MS	Upright	[9]
Ethanol	Wine	HS-SDME	UV-Vis	Upright	[5]
Mercury	Environmental water	HS-SDME	GFAAS	Upright	[159]
Ammonium	Environmental water	HS-SDME	UV-Vis -on drop sensing	Upside-down-secondary inlet	[58]
Benzene, toluene, ethylbenzene, xylenes	Surface water	HS extraction	GC-FID	Upside-down-secondary inlet	[54]
Lead	Tap water	DI-SDME	UV-VIs	Upright and upside-down	[6]

3.3. Bioaccessibility studies

3.3.1. Terminology

For monitoring of potentially hazardous effects of newly emerging contaminants including both inorganic and organic compounds in environmental, food, and biological samples/matrices, reliable methods that can mimic natural processes are highly desirable because the evaluation of the contaminant bioaccessibility and bioavailability are required as a part of the risk assessment.

The term bioavailability is defined by ISO norm no. 17402:2008 [171]. It comprises a dynamic process of three successive steps as follow:

1. the bioaccessibility, which is the maximum fraction of contaminants leached from a solid to a liquid medium
2. Environmental bioavailability, which is the fraction of the bioaccessible pollutant that can permeate through the membrane of a living cell
3. Toxical bioavailability, which covers the internal processes in the organism such as pollutant distribution, metabolism, and accumulation

From bioaccessibility studies, we can obtain useful information concerning contaminants present in the environment. If the contaminant has a high bioaccessibility there is also a higher risk that it becomes bioavailable to organisms and can accumulate in the food chain. The main reason why the bioaccessibility is evaluated more often than the bioavailability is that there is no need for in-vivo experiments. Consequently, this parameter is much easier and economic to study [172].

3.3.2. Batchwise versus dynamic methods

In batch experiments, sequential extraction procedures are performed that are based on the extraction of the analyte of interest from a given solid sample (usually soil or food) into a certain volume of a leaching agent. Generally, the experiments are starting with the weakest possible eluent (water, diluted salt solutions) following subsequently by stronger ones (e.g., the addition of oxidizing or reducing chemicals in the metal determination or mixture of water and polar organic solvent for organic pollutants). The extract is analysed after a long extraction time to ensure that the equilibrium is reached. Such static leaching tests are used very often and give us basic information about the contamination of the sample. Nevertheless, this mode has several disadvantages such as:

1. There is no information about the leaching kinetics.
2. The procedures are very long (up to 2 days).
3. The procedures are laborious as they involve many steps including manual handling.
4. The results might be an underestimation of the actual bioaccessibility since contaminants may re-absorb on other solids during the long extraction time.
5. During the static extraction desorbed compounds from the surface of solids are not removed which is however considered in bioaccessibility theory: soil permeation with precipitation water and

absorption of components by organisms will lead to a concentration gradient that is in static experiments not obtained.

6. The pH of the leaching agent might change in the case of analytes extraction from solid samples of buffering capacity, e.g. soil.

To overcome these limitations flow-through dynamic extraction was proposed to mimic genuine leaching conditions in nature. In this mode, a fresh portion of the leaching agent is continuously pumped through a container or column that holds the solid sample. By this, the actual bioaccessibility of the leaching into the environment is emulated more realistically. The kinetic profiles are usually monitored as a function of the exposure time obtaining more realistic information about the extractability of pollutants. Compared to batch-wise procedures, the solid-to-liquid ratio is a less crucial factor since the extraction is eventually exhaustive [172,173].

3.3.3. Automation of the bioaccessibility studies by flow techniques

Flow techniques are suitable for the automation of process monitoring and dynamic procedures such as bioaccessibility studies described above. Both FIA and SIA techniques have been utilized with different types of containers for solid samples. A lot of bioaccessibility studies were carried out in FI-TRACE group and the used configurations and components were summarized in two reviews [172,173].

Flow-through sample containers:

A stirred flow chamber is a container accommodating the solid sample in an amount of 0.25 - 5 g [173], which is constantly agitated by the magnetic stirrer. The volume of the chamber can be up to 10 mL [172]. The chamber enables the use of a large amount of sample without a significant increase in back-pressure. Yet, the dead volume is high causing carry over between different extractants [172]. This type of chamber was used for the leaching study of cadmium and copper from cultivated soil [174] or iron fraction for corrosion products from natural gas pipelines [175].

Very often, a cylindrical column has been used as a sample container for leaching studies because of its commercial availability. Cartridges for SPE can be also used for sample packing after removal of the upper part and the sorbent or fluoropolymer tubes containing the sample using quartz-wool plugs as frits were employed. The amount of sample that can be packed is lower than 5-200 mg and by this significantly below what can be used in the stirred flow chambers. Therefore, this type of column is not suitable for highly heterogeneous samples from which the representative sample is difficult to obtain. On the other hand, the dead volume is much lower. This type of column was for instance applied for the study of the leaching of various metal species from crushed rocks [176], for the determination of bioaccessible arsenic in seafood [177], or for the packing of microplastics [74].

A biconical microcolumn, i.e. with an inner shape similar to a diabolo (double conical shape at both ends), was proposed as an alternative to the cylindrical column. This column showed improved hydrodynamic properties which promoted the contact of the sample and extractant by allowing the circulation of the solid particles. Up to 300 mg of the sample can be packed without facing problems of frit clogging and with only minimal dead volume [172]. This type of column was used for the evaluation of the effect of the natural weathering of incineration ashes of municipal waste [178].

As a general challenge, the sample cannot be packed tightly in either cylindrical or biconical microcolumns as this will lead to high back-pressure and deteriorate the effective mixing of the sample with the extractant and cause low reproducibility [172]. Thus, the large-bore column was designed for the accommodation of large bulk of samples up to 1 g with the possible extraction flow rate up to 10 mL/min [179].

Propelling devices

Peristaltic pumps are most often used for dynamic leaching studies. They are suitable for continuous pumping of the extractant through the sample container and enable straightforward on-line connection to continuously operated detectors such as ICP-AES, or ICP-MS [180]. The problem arises when the pumping tubes are wearing out over time and are damaged by reactive extractants such as strong acids or oxidizing solutions. This can result in an unstable and gradually decreasing flow-rate that affects the method reproducibility.

Computer-controlled syringe pumps provide improved accuracy in flow rate and used volumes. A syringe pump is usually part of any sequential injection system where the sample container can be placed on a lateral port of the selection valve. Compared to FIA different modes of solution handling (uni-directional, bi-directional, stopped-flow, etc.) can be done without any changes in the configuration. The stopped-flow mode can be applied to increase the contact time of the leaching agent with the sample. Another possibility of increasing the contact time is the repeated passage of the same leaching solution via a closed-loop setup. To avoid unwanted compaction of the sample in an extraction column, the flow of the leaching agent can be reversed in fixed intervals [172,181].

SIA system or just a single syringe pump was used for dynamic leaching of heavy metals from soil [182] as well as antioxidants from food samples using the surrogate gastric and intestinal juices as the leaching agent [181]. A leaching study of the emerging contaminants from mussels to the gastrointestinal fluids was studied using a LOV format with integrated SPE cartridge for analyte preconcentration followed by LC-MS/MS detection [183].

In this dissertation leaching of phthalates from microplastics into the seawater was recently studied using a flow-through platform coupled on-line with HPLC-DAD (Publication 6, Figure 22) [74].

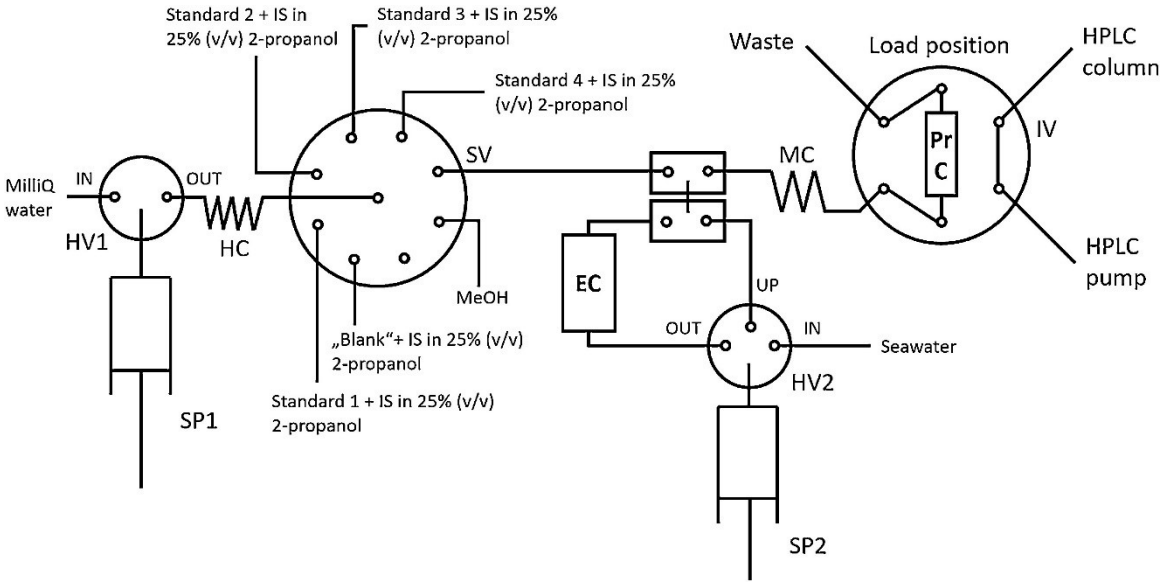

Figure 22: On-line system for the study of the leaching of plastic additives from microplastics to the seawater. EC: extraction column, HC: holding coil, HV: head valve, MC: mixing coil (40 cm, 0.8 mm id), PrC: C18 monolithic preconcentration column (10 × 4.6 mm); SV: selection valve; IV: HPLC injection valve; SP: syringe pump. Taken with permission from [74].

4. RESULTS AND DISCUSSION

4.1. List of publication included in the thesis

1. B. Horstkotte, **K. Fikarová**, D.J. Cocovi-Solberg, H. Sklenářová, P. Solich, M. Miró, Online coupling of fully automatic in-syringe dispersive liquid-liquid microextraction with oxidative back-extraction to inductively coupled plasma spectrometry for sample clean-up in elemental analysis: A proof of concept, Talanta 173 (2017) 79-87.

   $IF_{2017} = 4.244$

   Times cited: 10, JCR category: analytical chemistry, Quartile in category: Q1

   Candidate's contribution: Carry out the experiments during 5 months stay at the University of Balearic Islands, optimization of the extraction method, data processing, revision of the manuscript

2. R. Sánchez, B. Horstkotte, **K. Fikarová**, H. Sklenářová, S. Maestre, M. Miró, J.L. Todolí, Fully Automatic In-Syringe Magnetic Stirring-Assisted Dispersive Liquid-Liquid Microextraction Hyphenated to High-Temperature Torch Integrated Sample Introduction System-Inductively Coupled Plasma Spectrometer with Direct Injection of the Organic Phase, Analytical Chemistry 89 (2017) 3787-3794.

   $IF_{2017} = 6.042$

   Times cited: 13, JCR category: analytical chemistry, Quartile in category: Q1

   Candidate's contribution : My optimization results and system configuration from previous work with some changes were used for similar work with the on-line coupling of LIS with ICP-AES with direct measurement of the organic phase. Manuscript comments and revisions.

3. I.H. Šrámková, B. Horstkotte, **K. Fikarová**, H. Sklenářová, P. Solich, Direct-immersion single-drop microextraction and in-drop stirring microextraction for the determination of nanomolar concentrations of lead using automated Lab-In-Syringe technique, Talanta 184 (2018) 162-172.

   $F_{2018} = 4.916$

   Times cited: 14, JCR category: analytical chemistry, Quartile in category: Q1

   Candidate's contribution: System set-up and first optimization study. Manuscript comments and revisions.

4. **K. Fikarová**, B. Horstkotte, H. Sklenářová, F. Švec, P. Solich, Automated continuous-flow in-syringe dispersive liquid-liquid microextraction of mono-nitrophenols from large sample volumes using a novel approach to multivariate spectral analysis, Talanta 202 (2019) 11-20.

   $IF_{2019} = 5.339$

   Times cited: 3, JCR category: analytical chemistry, Quartile in category: Q1

   Candidate's contribution: Carry out the experiments, optimization of the method, measurement of the surface water samples, data evaluation, writing and revisions of the manuscript

5. **K. Fikarová**, B. Horstkotte, D. Machián, H. Sklenářová, P. Solich, Lab-In-Syringe for automated double-stage sample preparation by coupling salting out liquid-liquid extraction with online solid-

phase extraction and liquid chromatographic separation for sulfonamide antibiotics from urine, accepted in Talanta 15.7.2020. https://doi.org/10.1016/j.talanta.2020.121427.

IF_{2019}= 5.339

Times cited: 0, JCR category: analytical chemistry, Quartile in category: Q1

Candidate's contribution: Carry out the experiments, optimization of the method, measurement of the urine samples, data evaluation, writing and revisions of the manuscript

6. **K. Fikarová**, D.J. Cocovi-Solberg, M. Rosende, B. Horstkotte, H. Sklenářová, M. Miró, A flow-based platform hyphenated to on-line liquid chromatography for automatic leaching tests of chemical additives from microplastics into seawater, Journal of Chromatography A 1602 (2019) 160-167.

   IF_{2019} = 4.049

   Times cited: 1, JCR category: analytical chemistry, Quartile in category: Q1

   Candidate's contribution: Carry out the experiments during 7 months stay at the University of Balearic Islands, optimization of the method, leaching study from the CRM microplastics, data processing, writing, and revisions of the manuscript.

4.2. Comment on publication 1

Online coupling of fully automatic in-syringe dispersive liquid-liquid microextraction with oxidative back-extraction to inductively coupled plasma spectrometry for sample clean-up in elemental analysis: A proof of concept

This experimental work was carried out in cooperation with the University of the Balearic Islands at the FI-TRACE group of Prof. Dr. M. Miró in 2015 during a four months Erasmus+ stay.

DLLME is a very rapid extraction technique typically achieving high preconcentration factors. In particular, the latter characteristic is highly important in transition and heavy metal analysis since the concentration levels of the analytes are in the range from ppb to ppt. An additional advantage is that DLLME extracts do not usually show many remains of the sample matrix. On the other hand, the procedure is applicable only to liquid samples. A secondary clean-up by analyte back-extraction into an aqueous phase is advantageous for highly complex matrices as well as to transfer the analytes into a solvent that is compatible with the specific detection instrumentation. Dispersive back-extraction automated by the FT was accomplished only a few times as it is more typical for dual-stage clean-up to use solvent immobilized on the hydrophobic membrane from which the analyte is eluted by appropriate stripping agent.

The aim was to develop an automated and rapid sample preconcentration and clean-up method suitable for the elemental analysis in trace levels. The detection technique of choice in the elemental analysis is usually ICP-AES or ICP-MS. However, DLLME aims analyte extraction into a water-immiscible solvent while ICP instruments are typically not compatible with high contents of organic solvents in the sample as it can deteriorate the process of nebulization and excessively cool down the plasma. One of the possible approaches how to overcome this problem in an automated manner is to back-extract the analytes into an aqueous phase. To the best of our knowledge, the back-extraction of metals was automated by LIS technique for the first time in this work.

The automation of DLLME by LIS technique in combination with back-extraction for on-line coupling to ICP-AES was studied for the first time and applied to the determination of Cd, Cu, and Pb cations in troublesome matrices (coastal seawater, surrogate digestive fluids, soil leachates) that cannot be analysed directly with this technique. For the automation of the back-extraction procedure, the up-side down orientation was more advantageous allowing a complete emptying of the syringe and the possibility to keep the organic extract of lower density than water inside the syringe after the accomplished extraction followed by the back-extraction step. In this configuration, the motor was attached to the side of the syringe, i.e. not moving with the syringe piston, since the stirring bar was in a fixed position, in the inlet of the syringe, as it is shown in Figure 23.

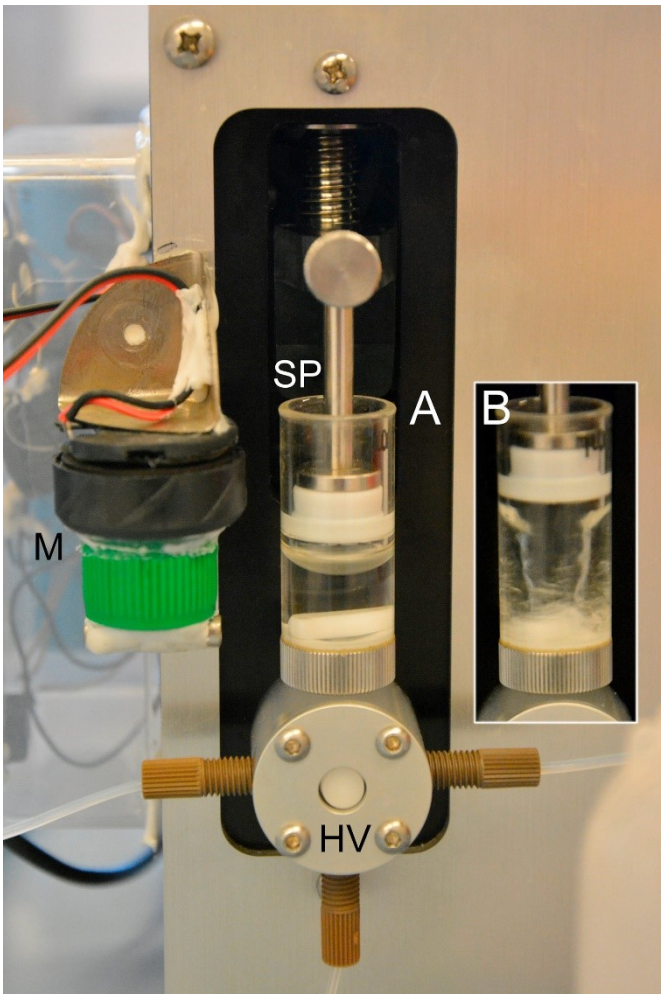

Figure 23: Configuration of the syringe with a fixed motor. A) after phase separation, B) during the stirring. HV: head valve, M: motor, SP: syringe pump. Taken with permission from [8].

The selected analytes were extracted into a hydrophobic solvent as metal complexes. Therefore, two commonly used chelating reagents were tested: ammonium pyrrolidine dithiocarbamate (APDC) and diethyl dithiophosphate (DDTP). APDC was preferred because of the lower stability constant of the metal chelates than for DDTP, which was considered advantageous for the aimed back-extraction.

Toluene was selected as an extraction solvent for its low solubility in water (0.047 g/100 mL), high extraction efficiency for the metal complexes, and lower density than water which was suitable for the selected upside-down orientation of the syringe. As an alternative, methyl isobutyl ketone was tested but the high solubility in the aqueous phase of 1.91 g/100 mL made the solvent inapplicable for ICP measurements.

The syringe piston and inlet part as well as the stirring bar were made from PTFE. Therefore, toluene stuck to these parts and it was another challenge to promote the floatation of the solvent during phase separation. This problem was solved at the beginning by quick activation and deactivation of the motor. As a more efficient solution, the aspiration of a small volume of isopropanol was found and the procedure was modified.

Three different approaches were tested for the back-extraction of the analytes to the water phase. At first 2.85 mol/L nitric acid was tested as a back-extraction agent in batch experiments. Nevertheless, by

this simple approach, we achieved only low recoveries (24-72%). Therefore, the use of a stripping agent was tested to enhance the back-extraction which showed higher chelate stability constant than the ones of analytes to replace them from the formed chelates kinetics. Palladium was found in the literature as a suitable candidate and was added to the nitric acid (0.42 mol/L and 1.5 mol/L). Three different back-extraction times were tested for this approach (60 s, 120 s, 180 s). While for Cd and Pb the back-extraction yield was almost quantitative already in the lowest back-extraction-time, for Cu the mass recovery was more pronounced with longer back-extraction time, yet, achieving a maximum 76% recovery for 180 s of back-extraction.

To achieve the highest preconcentration factor possible, back-extractant volume was reduced from 3.5 mL to 1 mL. However, it was found that at the correspondingly higher concentration, Pd^{2+} precipitate as a complex with APDC. Therefore, this approach was also not considered as suitable for in-syringe back-extraction.

Consequently, the third novel approach involving oxidative back-extraction was tested. Dithiocarbamates can be oxidized, which results in a decrease of their chelating ability and breaking of the complexes. By the addition of KIO_3 in the concentration of 20 mmol/L to the 1 mol/L HNO_3 using 100 s back-extraction time, we achieved the quantitative back-extraction of all the analytes.

The used ICP-AES was equipped with a concentric nebulizer for high dissolved solids content and cyclonic spray chamber to avoid possible problems of matrix carry-over that was then not observed in sample analysis. The sensitivity of the method could be further improved using a more efficient nebulizer, which was unfortunately not available for this work. The measurements were done in axial torch mode. The interphase for the on-line coupling was the injection valve with the injection loop of 200 µL.

This work demonstrated the capabilities of using the LIS technique for sample pretreatment in elemental analysis. The on-line coupling of the technique to ICP-AES enabled fully automated sample clean-up, preconcentration, and determination of the selected metals from complex matrices including seawater, surrogate gastric juice, and soil leachates. The method sensitivity could be further improved by a decrease in the volume of the back-extractant, increase the volume of the injection loop, or the use of a more efficient nebulizer of ICP instrument. In the following work (Publication 2), the sensitivity was improved by the direct injection of the extract yet with the need of using a modified ICP instrument for this purpose. Figure 24 shows graphical abstract for the publication.

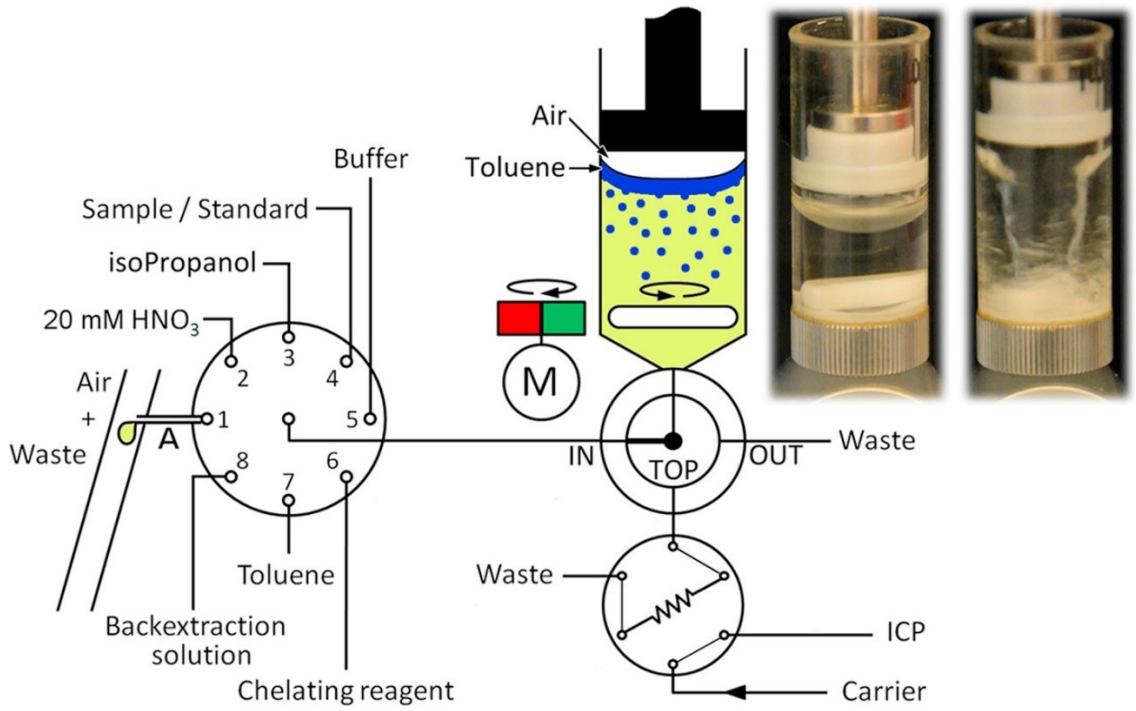

Figure 24: Configuration of LIS system for DLLME followed by back-extraction coupled on-line to ICP-AES. Graphical abstract for Publication 1 [8].

4.3. Comment on publication 2

Automatic in-syringe magnetic stirring-assisted dispersive liquid-liquid microextraction hyphenated to high-temperature torch integrated sample introduction system-Inductively Coupled Plasma Spectrometer with direct injection of the organic phase

This experimental work follows up on the previous experiments and was done in collaboration with the University of the Balearic Islands and, in particular, with the University of Alicante with the group of Prof. Dr. Jose-Luis Todolí. This group has developed a special heated nebulization system for the direct injection of complex samples into ICP-AES that removed most of the typical matrix effects. This nebulizer system also enabled the direct injection of organic solvents. The contribution of the candidate was in the development of the automated DLLME method, which was further modified for the intended purpose.

As it was mentioned in section 4.2, the injection of the organic solvent into the ICP-AES can deteriorate the measurement and even cause the shutdown of the plasma torch. Therefore, it is not common to inject organic solvents into ICP directly and only a limited number of papers exist on this topic. A significant number was published by the collaborating research group at the University of Alicante. In this work, LIS automated DLLME was coupled on-line to ICP-AES with direct injection of the extract for the first time.

Cd, Cu, Pb, and Ag were selected as model analytes. The extraction procedure was taken over from the previous work with few changes: i) xylene was selected as an extraction solvent as the collaborating group has used this solvent in previous works, ii) DDTP was used as complexing reagent aiming for the formation of highly stable complexes, iii) the syringe was in the upright position allowing an easy collection of the floating droplets as the extraction solvent was lighter than water. On the other hand, this orientation of the syringe can lead to a more pronounced carry-over contamination due to the dead volume caused by the stirring bar located inside the syringe. Therefore, the cleaning procedure was also re-optimized.

To enable direct injection of the organic extract, a high-efficiency concentric micronebulizer combined with a heated spray chamber denoted in literature as a “high-temperature torch integrated sample introduction system” was applied. The solvent was limited by the use of an injection valve to only 5-15 µL and transported by an airflow. The oxygen in the air bubbles increased the carbon combustion, which minimized the carbon deposit.

In comparison to DLLME with the back-extraction step, by DLLME without posterior back-extraction, higher enrichment factors were yielded thus the lower limit of detection was reached. On the other hand, the method sensitivity using back-extraction can be still improved using higher efficiency nebulizer, a lower volume of back-extraction solution, and injection of the larger volume of extract. Besides, a secondary clean-up of the extract is achieved by the back-extraction, which is advantageous for very complex matrices, and no special equipment of ICP instrument is required. Figure 25 was used as a graphical abstract for the publication showing the used configuration and ICP torch.

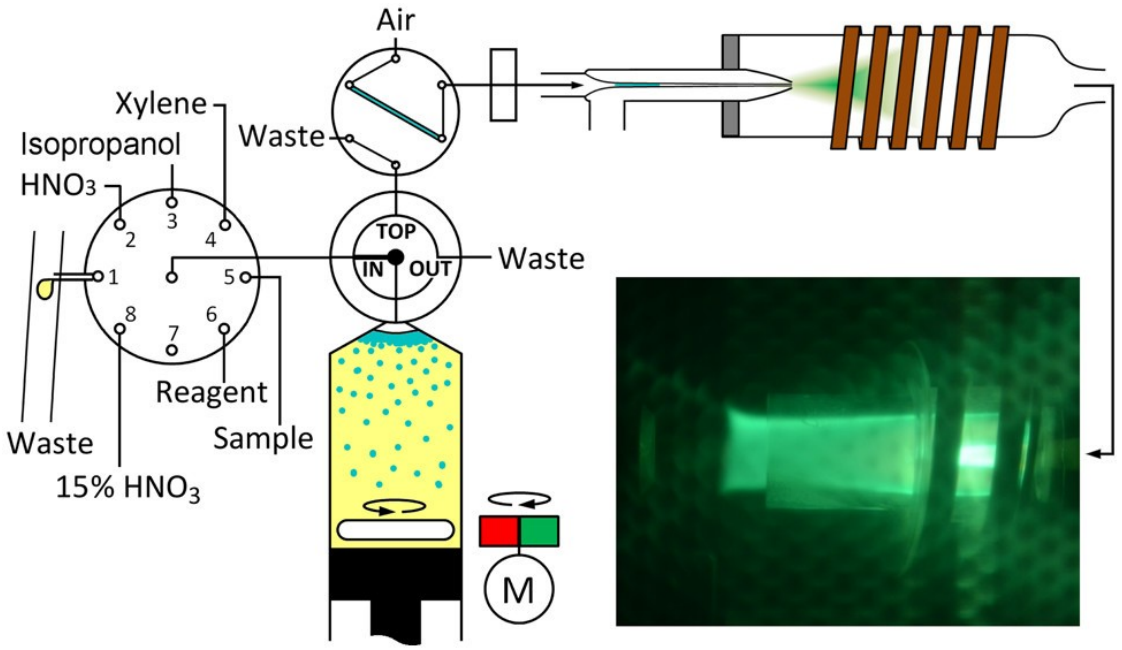

Figure 25: Configuration of the system for LIS automated DLLME with direct injection of the organic extract. Graphical abstract for publication 2 [53].

4.4. Comment on publication 3

Direct-immersion single-drop microextraction and in-drop stirring microextraction for the determination of nanomolar concentrations of lead using automated Lab-In-Syringe technique

In this experimental work, the automation of direct-immersion single-drop microextraction by the Lab-In-Syringe was studied using two operational modes. Both modes were optimized, and the resulting methods were critically compared for the analysis of lead in drinking water using the reaction with dithizone as a sensitive chromogenic complexing reagent.

DI-SDME is a popular miniaturized extraction technique with very low consumption of the organic solvent with possible high enrichment factors. The critical factor is the drop stability, which can be impaired by the fast stirring as well as by manual handling. Therefore, automation of this technique can improve significantly the repeatability of the technique.

A general objective was to develop an automated method that enables analyte extraction by a solvent without the need of solvent dispersion that i) would require fast stirring during extraction, ii) therefore stirring control to enable phase separation, iii) risk of emulsion formation, and iv) need to relatively large solvent volumes. Initial work was therefore done without any stirring control but only placing a motor on the piston of the syringe pump.

The first proposed method (method A) used the syringe pump in an upright position with a solvent lighter than water for drop formation. Of particular importance was the stabilization of the drop, which is mostly influenced by the selection of the organic solvent, drop volume, and stirring rate. These parameters were optimized, and the syringe inlet was modified by inserting a piece of PTFE tubing, which decreased the dead volume and improved the reproducibility of the drop formation and stabilization. In addition, a small volume of air was aspirated after the extraction solvent, resulting in the formation of a “shell” around the bubble. This trick improved the drop stability, prevent the sticking of the drop to the PTFE surface of the syringe inlet, and increased the contact area.

Toluene was selected as an extraction solvent with high extraction efficiency for the selected complex of lead with dithizone. However, the low viscosity of toluene led to the formation of an unstable drop. Therefore, the addition of the more viscous solvent (hexanol) was tested to stabilize the drop. The ratio of toluene and hexanol was studied while a mixture of toluene and hexanol 80:20 (% v/v) was finally selected as a compromise between extraction efficiency and repeatability. The volume of the drop was studied in the range of 35-65 µL. With the increase of the solvent volume, the signal decreased due to the dilution factor and lower surface-to-volume ratio. Nevertheless, the repeatability increased with the higher volume, therefore a volume of 60 µL was finally selected.

Since the DI-SDME has typically slow extraction kinetics due to the low interfacial surface, longer extraction times than for DLLME were required. The tested range was between 20-640 s with a steadily increasing signal and the selected time was 300 s as a compromise between the sensitivity and time of the analysis.

The second method (method B) used the syringe pump in the upside-down orientation and chloroform was selected as an extraction solvent with a higher viscosity than water with a high extraction efficiency for the analyte. In this configuration, a cross-shape stirring bar allowing in-drop stirring without creation an unwanted dispersion of the solvent was used. Due to the high hydrophobicity of the PTFE surface of the syringe inlet and the stirring bar, the drop covered the siring bar and remained in the centre of the syringe inlet. The slow stirring rate was used (570 rpm) to avoid unwanted solvent dispersion.

Since dithizone has been found more soluble in chloroform than in the toluene-hexanol mixture, the reagent was used already combined with the extraction solvent. Some of the optimization parameters were adopted from the previous method. Nevertheless, drop volume and extraction time were re-optimized for this method. The extraction time was studied in the range of 30-300 s and it was found that quantitative extraction was achieved using time exceeding 180 s, therefore extraction time of 150 s was selected as sufficient. This time is very short in comparison not only to the previous method but also to DI-SDME in general. The extraction volume was tested in the range of 45-210 µL, and as in method A, 60 µL was adopted as a compromise between method sensitivity and repeatability.

To improve the selectivity of the method, three commonly used masking agents for lead determination were tested to inhibit signal of other metal cations and anions possibly being present in tap water e.g. Ca^{2+}, Mg^{2+}, and Cl^{-} and NO_3^{-}, which did not interfere significantly. Significant interferences were found for Fe^{3+}, Cd^{2+}, Zn^{2+}, and Cu^{2+}. A mixture of potassium sodium tartrate and potassium cyanide were required to mask all the mentioned interferents, except for Cd^{2+}, whose interference persisted but its presence in drinking water is highly unlikely.

While the extraction efficiency of method A was only 33%, the extraction of Pb-dithizone complexes using method B was quantitative. The reason was probably in higher solubility of the complex in chloroform than in mixture of toluene and hexanol, the higher surface area of the drop using in-drop stirring and lower adsorption of the complexes on the PTFE surfaces when using upside-down orientation. Sample throughput for method A was due to the longer extraction time 10 h^{-1} while for method B was improved to 12 h^{-1}.

In method B, the LOD was about three-times lower than in method A and reached the legal limits set by the EU authorities of 10 µg/L. Therefore, Method B was proven as more suitable for the determination of lead in tap water.

Automation of DI-SDME by LIS was achieved using simple and portable instrumentation without the requirement for remote stirring control in method A. Compared to LIS-automated DLLME solvent consumption was reduced. A high stirring rate, which required additional modifications of the driving device was not needed. Consequently, there was no problem with the long phase separation or emulsification. Longer extraction time typical for SDME was significantly improved by in-drop stirring in method B. LIS technique versatility was confirmed by automation of DI-SDME in two different configurations, which can be both used for sample preparation. Figure 26 shows graphical abstract for the publication.

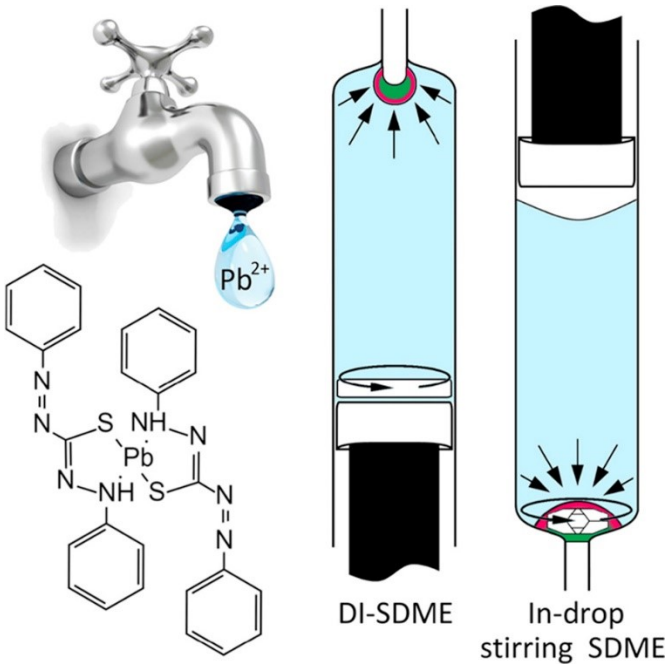

Figure 26: Configuration of DI-SDME using solvent lighter than water solvent denser than water. Graphical abstract for Publication 3 [6].

4.5. Comment on publication 4

Automated continuous-flow in-syringe dispersive liquid-liquid microextraction of mono-nitrophenols from large sample volumes using a novel approach to multivariate spectral analysis

In this work, we introduced for the first time the automation of continuous DLLME with posterior back-extraction and spectrophotometric detection of nitrophenols. The experimental work aimed to develop an automated extraction method emulating the fluidized bed SPE concept, which is a process mostly known from industry based on fluidization of the solid particles by liquid, which is flowing through the particles, stirring, or vibration. [184]. The aim was to show that this concept can be also used for LLE using LIS technique. Fluidization of the solvent has the same advantages as the fluidization of the beads such as high surface to volume ratio, fast mass transfer, and effective mixing. In addition, using the dispersed solvent instead of the particles, there is no risk of system blockage.

The system consisted of two connected syringe pumps, where the first pump featured a drilled-through piston that enabled a flow channel to the syringe void. By this, the syringe void acted as a size-adaptable flow-through extraction chamber containing a floating extraction solvent. The fluidization/dispersion of the solvent droplets was accomplished synergically by the stirring in the level of the organic solvent and the flow rate of the sample.

Four modes of operation were considered for the flow-through syringe pump: The syringe could be used either in upright or upside-down orientation with the extraction solvent either lighter or denser than water. Using the syringe in the upright position (Figure 27A, B) has a disadvantage in the impossibility to empty the syringe completely due to the dead volume given by the stirring bar placed inside the syringe. On the other hand, in upside-down orientation, by aspiration of a small volume of air, it is possible to dispense all the liquid out. Using a solvent denser than water (Figure 27C), discarding of the sample, and keeping the extract in for further back-extraction are more complicated. In addition, chloroform as the most common solvent denser than water had a low extraction capacity for nitrophenols, which were selected as model analytes. Therefore, the final selected configuration (Figure 27D) was upside-down with a floating solvent.

The second syringe pump was used to propel the sample through syringe 1 in semi-continuous flow. Alternatively, any other pump such as peristaltic could have been used.

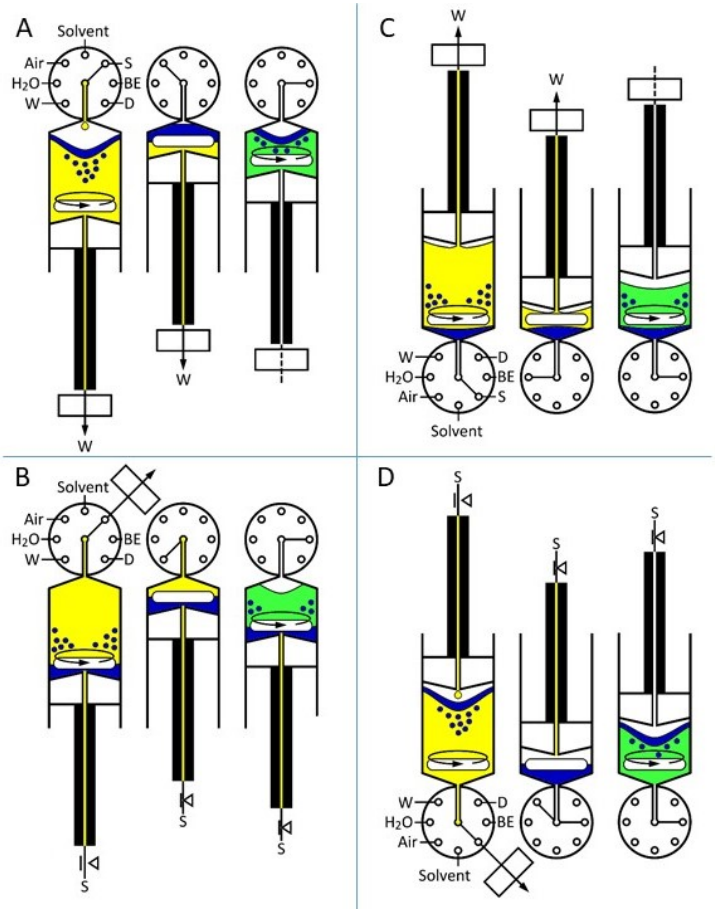

Figure 27: Possible configurations for continuous DLLME. A) Upright position with solvent lighter than water, B) Upright position with solvent denser than water, C) Upside-down position with solvent denser than water, D) upside-down position with solvent lighter than water.

For the efficient dispersion and minimization of the solvent loss, the stirring bar was lifted inside the syringe to be leveled with the water-solvent interphase, which is detailed in the material section of the article. The most critical parameters to achieve a minimal loss of the organic solvent were stirring rate and flow rate of the sample. The stirring rate should be fast to enable efficient dispersion of the solvent to the fine droplets, but the flotation velocity of very small droplets decreases. Therefore, there is a higher risk to lose the solvent by the flow of the sample. The tested stirring rates were 700-1130 rpm and flow rates 10-40 µL/s. We observed that a stirring rate of more than 1100 rpm enables the most efficient dispersion and the effect of the flow rate in the selected range was insignificant, however, at a flow rate 40 µL/s the accumulation of the solvent in the second syringe was observed. Therefore, we selected 30 µL/s flow rate of the sample at 1150 rpm stirring rate.

Nitrophenols are environmental pollutants of high concern that can be extracted into an organic solvent in acidic medium and can be back-extracted in alkaline conditions to an aqueous solution where they have a characteristic yellow colour with absorbance maxima around 400 nm in the visible range. To distinguish between different isomers of mono-nitrophenol (ortho-, para-, meta-nitrophenol) multivariate spectral analysis can be advantageously applied to omit their separation by chromatographic techniques.

In this work, we applied a new approach to multivariate spectral analysis. In short, the absorbance of the back-extract was a linear combination of the spectra of the standard of each nitrophenol isomer of

certain concentration and the fourth-order polynomial, which described baseline alteration due to remains of humine substances in the back-extract and measurement errors. The sum of the squared deviations between the measured and simulated values in the measured wavelength range of 270-470 nm were minimized using a nonlinear solving function in MS-Excel. Using the reference wavelength at 490 nm were all the spectra were close to zero, simplification of the background signal polynomial term was possible.

The volume of the sample was studied up to 24 mL where the linear increase of the signal was confirmed achieving an effective preconcentration factor around 25. The volume of the sample could be further increased but the time of the analysis would be significantly prolonged. Figure 28 shows graphical abstract of the publication.

The results of this work were presented at 21st ICFIA 2017 (Saint Petersburg) in a poster form and was awarded the Best Poster prize.

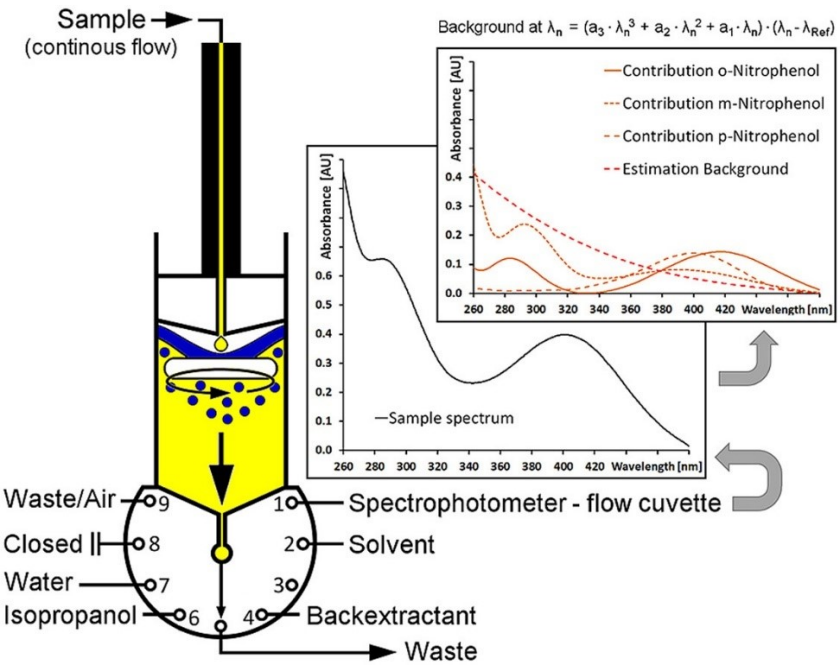

Figure 28: Lab-In-Syringe automated continuous dispersive liquid-liquid microextraction. Graphical abstract for Publication 4 [57].

4.6. Comment on publication 5

Lab-In-Syringe for automated double-stage sample preparation by coupling salting out liquid-liquid extraction with online solid-phase extraction and liquid chromatographic separation for sulfonamide antibiotics from urine

This experimental work was focused on the development of an automated dual-stage procedure for sample clean-up and analyte preconcentration with subsequent on-line-coupled HPLC separation. Sulphonamides were selected as model analytes of moderate polarity that are extractable into a polar organic solvent. The system consisted of an autosampler for the aspiration of all reagent solutions and the sample, an automatic syringe pump equipped with 2.5 mL glass syringe, which acted as an extraction chamber for salting out homogenous liquid-liquid extraction, and an HPLC system with an injection valve that acted as interface and that integrated a 1 cm home-made SPE column filled with anion exchange resin.

At first, salt for the phase separation and extraction solvent miscible with water were selected. Contrary to manual procedures where the salt can be added as a solid directly to the sample in order to obtain a saturated solution, for in-flow procedure nearly saturated solution (80%) was prepared. The disadvantage of this approach is the high viscosity of the solution and further dilution of the salt solution with sample and reagents. Therefore, the used flow rate must be very slow, and sufficient volume had to be aspirated to induce phase separation. The selected salt in preliminary experiments was a mixture of 2 mol/L $MgSO_4$ and 1 mol/L NaCl based on unpublished optimization results the ability of different salts to induce phase separation and achieve high purity of both aqueous and organic phases was evaluated.

As a possible extraction solvent acetone, 1-propanol, and ACN were tested. ACN was selected as a suitable candidate based again on the evaluation of phase separation and purity of the extract containing a minimal amount of water.

For the on-line SPE based on anion-exchanger, a home-made 10 mm column was prepared. To achieve uniform packing of the particles, the resin was suspended in 50% MeOH and loaded into the column using a vacuum. Three different polymeric resins were tested based on strong anion exchanger and weak anion exchanger and quantitative recovery was obtained for Strata™-X-A 33 µm Polymeric Strong Anion exchanger from Phenomenex.

An autosampler was used for the aspiration of all the solutions and sample. The advantage over the selection valve used usually in SIA is enough positions for all the samples, standards, and reagents so that there is no need for changing solutions. On the other hand, the dead volume of the connecting tube must be taken into account together with sufficient cleaning of the tube, also from outside, to prevent carry-over effect and contamination of the solutions.

In SALLE the preconcentration factor is typically very low. Therefore, additional sample preparation for analyte preconcentration is desirable. In the case of highly complex samples, secondary clean-up can improve matrix elimination. In this work, we combined for the first time LIS automated SALLE with on-line SPE and HPLC separation in one closed system. Figure 28 illustrates the clean-up of the extract

from the urine matrix comparing direct injection of the urine, solely on-line SPE, and SALLE with on-line SPE using the internal standard for posterior scaling. A significant reduction was achieved using a combination of SALLE and SPE compared to a single SPE procedure or direct injection of diluted urine.

The LIS automated procedure was synchronized with HPLC so that when the procedure had finished the valve with the SPE column switched to the inject position, HPLC was triggered and the separation method started. After approximately 2 minutes the valve turned back to the load position and the next run of sample preparation begun while the analytes were separated. Both separation and sample preparation took almost the same time, therefore there was no delay and both systems run in-parallel, which increased the sample throughput.

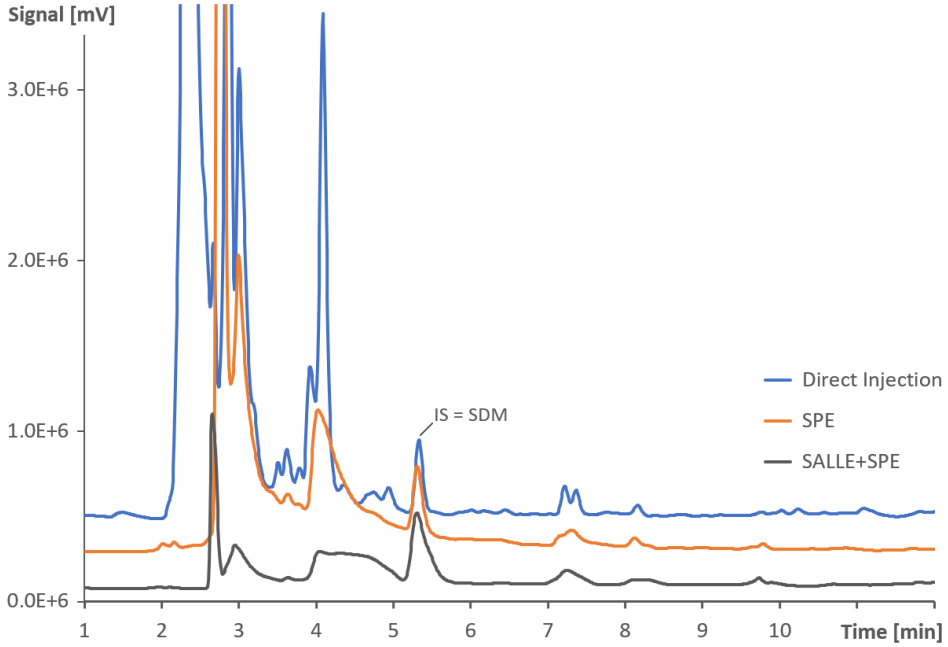

Figure 29: Comparison of urine sample spiked with the internal standard after direct injection to the HPLC, solid-phase extraction, and Salting-out extraction and combination with SPE. Taken from [185].

4.7. Comment on publication 6

A flow-based platform hyphenated to on-line liquid chromatography for automatic leaching tests of chemical additives from microplastics into seawater

This experimental work was carried out during a 7 months internship at the University of the Balearic Islands in the FI-Trace group of Prof. M. Miró Lladó and was supported by the Erasmus+ program of the European Union, and the project STARSS (Reg. No. CZ.02.1.01/0.0/0.0/15_003/0000465) co-funded by ERDF.

In the frame of this work, for the first time, the dynamic leaching of plastic additives from microplastic materials into seawater was studied. For the on-line leaching study, certified reference microplastic materials (polyethylene of medium density (PE) and poly(vinyl chloride) (PVC)) were tested with incurred seven phthalates and bisphenol A (contained only in PE).

A fully automated system consisting of a sequential injection analyser instrumentation equipped with 0.5 mL syringe (SP1), auxiliary stand-alone syringe pump (SP2), and HPLC instrument with a diode array detector was set up. Artificial seawater was pumped by SP2 through a stainless-steel column that contained ca. 50 mg of microplastic particles. The leached analytes were trapped in a 10 mm C18 monolithic column, which was placed in the sampling loop of the injection valve of used HPLC. The analytes were then eluted and separated by the mobile phase that was based on ACN:water gradient (40%-90% (v/v) ACN).

From the initial experiments, we observed that out of the 9 species only 4 most polar phthalates namely dimethyl-phthalate (DMP), diethyl-phthalate (DEP), benzyl-butyl-phthalate (BBP), and di-n-butyl-phthalate (DnBP), as well as bisphenol A, were leached in detectable levels. Therefore, the method was optimized for these analytes.

The calibration of the system was carried out in the on-line system by the matrix matching approach. This means that each calibration solution was on-line diluted with seawater in a ratio of 1:1. The calibration solutions could not be prepared in an aqueous environment due to the observed loss of the analytes by adsorption on the used glassware and the inner surfaces of the tubing of the flow system manifold. Therefore, we had to consider adding an organic modifier such as MeOH, or 2-propanol to overcome this problem. Nevertheless, the addition of more than 40% MeOH or 30% 2-propanol caused precipitation of seawater. Thus, the maximum tested contents were 30% MeOH and 25% 2-propanol.

At first, peak areas of standards prepared in different content of MeOH and 2-propanol and a mixture of both solvents were compared with the standards prepared in 100% MeOH, to find the lowest concentration of the organic solvent in which the analytes are not adsorbed on the surfaces. The concentration of 25% 2-propanol and a mixture of 30% MeOH and 10% 2-propanol were found sufficient for analyte stabilization in the solution.

Nevertheless, the addition of the organic modifier into the standard solution causes a breakthrough of the most polar analytes, especially DMP. Therefore, the type and the content of the organic modifier as well as the length of the preconcentration monolithic column had to be selected to achieve minimum

stacking of the analyte on the glassware and concurrently lowest breakthrough of DMP. A satisfactory compromise was obtained by the addition of 25% 2-propanol and using a 10 mm C18 monolithic column. This way, the recovery of DMP was only 40% but the determination sensitivity was sufficient for on-line analysis of the leaching process.

Two different internal standards (IS) were added to the calibration solution for quality control of the loading of the column. The use of two IS was necessary because of the difference in analyte polarity of DMP, DEP, and BPA which was a group of very polar analytes (IS-ethyl paraben) and BBP and DnBP which were less polar analytes (IS-benzyl benzoate). The IS in the 25% 2-propanol was not only in the calibration solutions but was also on-line merged with the seawater that has passed through the microplastic column to detect potential clogging of the preconcentration column by seawater matrix.

The obtained data from the on-line leaching study were fitted to a first-order kinetic model to evaluate the extraction rates of the analytes and enable to calculate the bioaccessible pool of each analyte. The most polar analytes DMP, DEP, and bisphenol A were leached rapidly from both tested materials. After 30 fractions of 1 mL each, the bioaccessible pool (> 79% of the initial concentration) was leached corresponding to an extraction time of 120 min. The following equation of exponential decrease of the general form was found to be a suitable mathematical model to describe the leaching behaviour of all target analytes:

$$Y(t) = A \cdot exp^{(-k \cdot t)}$$

where A is the maximum dynamic bioaccessibility of species per weight unit of microplastics (µg/g) at the initial time and k (min^{-1}) is a specific constant describing the leaching kinetics.

From the kinetic constant k, we can conclude that DMP and BPA leached twice as fast as DEP in the case of PE, and DMP leached thrice as fast as DEP from PVC. The difference was assumed to be due to higher hydrophobicity of the PVC material. As a careful estimation, the potential environmental risk can be more likely higher for DMP and BPA that will leach faster during plastic decomposition and weathering degradation to microplastics.

Apart from the publication of this experimental work in the scientific journal (Journal of Chromatography A), the results were presented by the author at Flow Analysis XIV 2018 (Bangkok, Thailand) in oral form. This presentation was awarded the 2nd prize for oral presentation of young researchers.

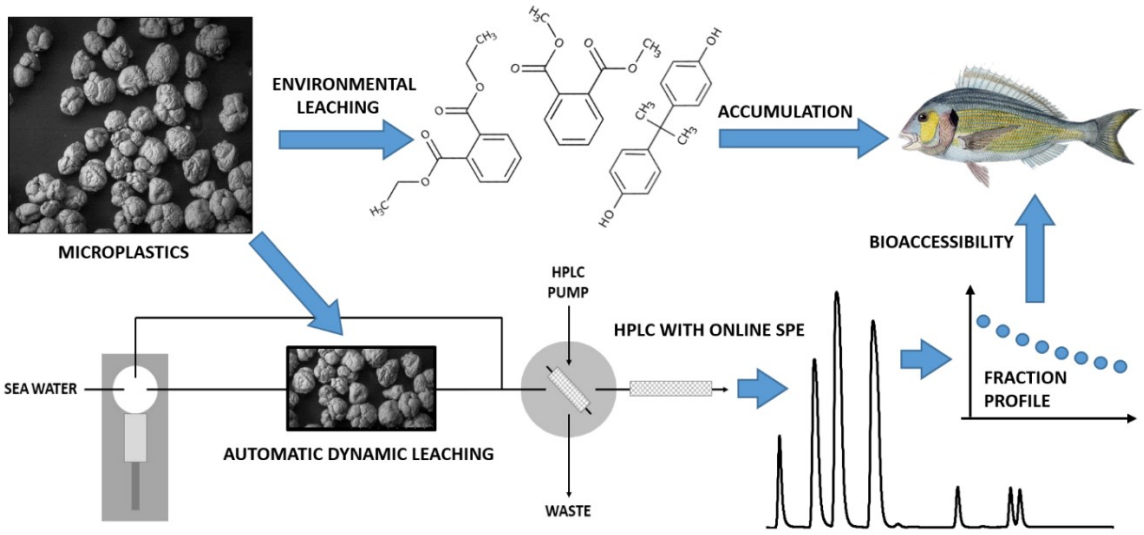

Figure 30: Simplified configuration for the on-line leaching study of the phthalates and bisphenol A from microplastics into the surrogate seawater. Graphical abstract for publication 6 [74].

5. CONCLUSION

This thesis, based on experimental work, is dealing with the automation of the sample preparation and bioaccessibility studies using FT. The Lab-In-Syringe technique was proven to be a suitable tool for the automation of some of the used (micro)extraction techniques, i.e. single drop microextraction, dispersive liquid-liquid microextraction, or homogeneous liquid phase extraction. Moreover, on-line hyphenation with various detection techniques was proven feasible.

The proposed automated procedures required minimal manual manipulation (e.g. changing of the solutions), were rapid, allowed to yield high preconcentration factors, repeatability, and a high degree of sample clean-up with minimal waste generation.

Apart from sample preparation, FT can be used with the high benefit for various kinetic studies including leaching studies of analytes from different solid matrices such as soil or food. One work included in this dissertation was focused on the automation of dynamic leaching of the plastic additives from microplastics into seawater as a recently emerged environmental problem and a “hot topic” in the field of environmental analysis.

All the proposed methods were validated and applied to real samples to prove the suitability for the measurement of the respective analytes of interest. The presented works are published in scientific journals and are listed in the Web of Knowledge in the first quartile (Q1). Publication 1, 2, and 5 were carried out in cooperation with the prof. Miró’s group (FI-TRACE) at the University of the Balearic Islands in Spain and Publication 2 also with the University of Alicante in Spain.

To summarize, each work included in this dissertation contributes to the development of FT by novel configurations of LIS (i.e. secondary inlet to the syringe for continuous DLLME, different configurations for DI-SDME), hyphenation of LIS to ICP-AES for trace metal analysis, the combination of LIS automated HLLE with on-line SPE in one system or novel application for the on-line dynamic leaching study of microplastics. The broad spectrum of applications presented in the thesis is demonstrating the high versatility of the FT in the field of sample preparation and bioaccessibility studies.

6. LIST OF OTHER OUTPUTS OF THE CANDIDATE

6.1. List of oral presentations at national and international conferences

1. K. Fikarová, B. Horstkotte, M. Miró, H. Sklenářová, Study Of "Lab-In-Syringe" Analysis for the automation of microextraction procedures, 7th Postgradual and 5th Postdoctoral conference, 7th-8th February 2017, FaF UK Hradec Králové, p. 68.
2. K. Fikarová, B. Horstkotte, H. Sklenářová, Automated continuous in-syringe dispersive liquid-liquid extraction and back-extraction for the determination of nitrophenols in surface water, 8th Postgradual and 6th Postdoctoral conference, 24th-25th January 2018, FaF UK Hradec Králové, p. 49.
3. K. Fikarová, M. Rosende, D. J. Cocovi Solberg, B. Horstkotte, H. Sklenářová, M. Miró, Automatic investigation of the bioaccessibility of phthalates and bisphenol A from microplastics in seawater under flow-through dynamic extraction conditions using an on-line switching valve HPLC system, 2nd STARSS conference, 25th-27th November 2018, Hradec Králové.
4. K. Fikarová, M. Rosende, D. J. Cocovi Solberg, B. Horstkotte, H. Sklenářová, M. Miró, Study of the bioaccessibility of phthalates and bisphenol A from microplastics in seawater using an on-line switching valve HPLC system, 14th International Conference on Flow Analysis, 2nd-7th December 2018, Bangkok, Thailand, p. 94. 2nd prize for student presentation.
5. K. Fikarová, M. Rosende, D. J. Cocovi Solberg, B. Horstkotte, H. Sklenářová, M. Miró, Study of the bioaccessibility of phthalates and bisphenol A from microplastics in seawater using an on-line switching valve HPLC system, 9th Postgradual and 7th Postdoctoral conference, 23th -24th January 2019, FaF UK Hradec Králové, p. 55.
6. K. Fikarová, F. Maya, B. Horstkotte, H. Sklenářová, F. Švec, F, Biomimetic crystallization of metal organic framework for the preparation of hybrid monolith in capillary format for the selective extraction of drugs, 10th Postgradual and 8th Postdoctoral conference, 22nd-23rd January 2019, FaF UK Hradec Králové, p. 21.

6.2. List of posters presented at conferences

1. K. Fikarová, B. Horstkotte, M. Miró, H. Sklenářová, Automated sample dispersive liquid-liquid extraction/backextraction coupled on-line to ICP-AES, Czech chromatography school-HPLC 2017, 12th-15th March 2017, Rožnov pod Radhoštěm, Czech Republic.
2. K. Fikarová, B. Horstkotte, D.J. Cocoví, M. Miró, H. Sklenářová, P. Solich, Automated sample dispersive liquil-liquid extraction/back-extraction coupled on-line to ICP-AES, 45th international symposium on high performance liquid phase separations and related techniques, 18th-22nd June 2017, Prague, Czech Republic, p.225.
3. K. Fikarová, B. Horstkotte, H. Sklenářová, P. Solich, Automated continuous in-syringe dispersive liquid-liquid extraction and back-extraction for the determination of nitrophenols in environmental samples, 21st International Conference on Flow Injection Analysis and Related Techniques, 2nd-9th September 2017, St. Petersburg, Russia, p. 104. Best poster award.
4. K. Fikarová, M. Rosende, D. J. Cocovi Solberg, B. Horstkotte, H. Sklenářová, M. Miró, Automatic bioaccessibility analysis of phthalates and bisphenol A from microplastics in seawater under flow-through dynamic extraction conditions using an on-line switching valve HPLC system, Czech chromatography school-HPLC 2019, 12th-15th May 2019, Zaječí, Czech Republic.
5. K. Fikarová, B. Horstkotte, H. Sklenářová, P. Solich, Automated salting-out liquid-liquid extraction in Lab-In-Syringe coupled to online SPE and HPLC for the determination of sulfonamides in urine 48th International Symposium on High-Performance Liquid Phase Separations and Related Techniques, 16th-20th June 2019, Milan, Italy.

6.3. List of lectures and posters with co-authorship

ORAL PRESENTATIONS:

1. Continuous dispersive liquid-liquid extraction of mono-nitrophenols from water samples exploiting the Lab-In-Syringe technique and a novel approach to multivariante spectrum analysis including back-extract background simulation, B. Horstkotte, **K. Fikarová**, P. Solich, H. Sklenářová, Flow Analysis XIV, Bangkok, Thailand.

POSTERS:

2. Direct immersion single-drop extraction and in-drop stirring extraction for lead determination in drinking water using Lab-In-Syringe technique, I. Šrámková, B. Horstkotte, **K. Fikarová**, H. Sklenářová, P. Solich, 19th International Symposium on Advances in Extraction Technologies (EXTECH 2017), Santiago de Compostela, Spain

3. Lab-In-Syringe automation of flow-through dispersive liquid-liquid microextraction integrating dispersive backextraction and simplified multivariante spectrum analysis with background modelling for the determination of nitrophenols in environmental waters, B. Horstkotte, **K. Fikarová, H.** Sklenářová, F. Švec, P. Solich, 20th EuroAnalysis. Istanbul, Turkey, Poster award

4. Lab-In-Syringe: A versatile technique for automation of liquid phase microextraction approaches, B. Horstkotte, **K. Fikarová**, I. H. Šrámková, H. Sklenářová, P. Solich, 20th EuroAnalysis. Istanbul, Turkey

6.4. Grant projects and fellowships

1. STARSS project, CZ.02.1.01/0.0/0.0/15_003/0000465, 2017-2019: *Establishment of Specialized Team for Advanced Research on Separation Science*. Ph.D. student co-worker of the project, project guarantor prof. RNDr. Petr Solich, CSc. (Member of the team since 1.3. 2017)
2. ERASMUS+ 2018, financial support for the Internship at the University of the Balearic Islands
3. Frantisek Svec Fellowship 2019, financial support from the CASSS community for the internship at the University of Tasmania
4. Fond Mobility of Charles University, financial support for the internship at the University of Tasmania

6.5. Foreign internships

1. University of the Balearic Islands 09/2015-12/2015(4 months, Erasmus + program), an internship at prof. Manuel Miró's group (FI-TRACE)

   Topic: Lab-In-Syringe automation of DLLME for the determination of heavy metals by ICP-AES.
2. University of the Balearic Islands 03/2018-07/2018 and 09/2018-11/2018 (7 months, Erasmus + program), an internship at prof. Miró's group

   Topic: Study of the bioaccessibility of phthalates and bisphenol A from microplastics in seawater using an on-line switching valve HPLC system
3. University of Tasmania 08/2019-12/2019 (4 months, CASSS Fellowship, Fond mobility), an internship at ACROSS group under the supervision of Dr. Fernando Maya Alejandro.

   Topic: Biomimetic crystallization of metal-organic framework for the preparation of hybrid monolith in capillary format for the extraction of pharmaceuticals

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8. SUPPLEMENT

Supplement 1: Online coupling of fully automatic in-syringe dispersive liquid-liquid microextraction with oxidative back-extraction to inductively coupled plasma spectrometry for sample clean-up in elemental analysis: A proof of concept – manuscript

Supplement 2: Fully Automatic in-syringe magnetic stirring-assisted dispersive liquid−liquid microextraction hyphenated to high-temperature torch integrated sample introduction system-Inductively Coupled Plasma Spectrometer with direct injection of the organic phase – manuscript

Supplement 3: Direct-immersion single-drop microextraction and in-drop stirring microextraction for the determination of nanomolar concentrations of lead using automated Lab-In-Syringe technique – manuscript

Supplement 4: Automated continuous-flow in-syringe dispersive liquid-liquid microextraction of mono nitrophenols from large sample volumes using a novel approach to multivariate spectral analysis – manuscript

Supplement 5: Lab-In-Syringe for automated double-stage sample preparation by coupling salting out liquid-liquid extraction with online solid-phase extraction and liquid chromatographic separation for sulfonamide antibiotics from urine – manuscript (just accepted)

Supplement 6: A flow-based platform hyphenated to on-line liquid chromatography for automatic leaching tests of chemical additives from microplastics into seawater – manuscript

Supplement 7: Certificate for Best poster award (ICFIA 2017, Saint Petersburg)

Supplement 8: Certificate for 2nd place for oral presentation of young researchers (Flow Analysis XIV 2018, Bangkok)