Inkjet Printable Molecularly Imprinted Polymers for Determination of Penicillin V

Master’s Thesis

to obtain the academic degree of
Diplom-Ingenieur

in the Master’s Program
Chemistry and Chemical Technology
"The proof of the pudding is in the eating."

Old English proverb
Acknowledgements

The presented work was carried out as a part of the project 'AquaNOSE' (864893), funded by the Austrian Bundesministerium für Verkehr, Innovation und Technologie via the Forschungsförderungsgesellschaft. The major part of experimental work was performed at the research facilities of Profactor GmbH, Im Stadtgut A2, 4407 Steyr-Gleink, Austria. Thank you to the FFG and Profactor for offering me that opportunity.

First and formost, I would like to thank Dr. Leo Schranzhofer for supervising my work at Profactor. Thank you with for all the support, trust and useful advice! I further would like to thank the whole Profactor team for providing me all kinds of aid during the experimental work.

I would also like to thank Univ.-Prof. Dr. Oliver Brüggemann for the competent and uncomplicated supervision on the part of Johannes Kepler University and for his helpful advice on polymer chemistry issues.

Another word of thanks goes to Univ.-Prof. Mag. Dr. Peter Lieberzeit and his research group for letting me perform several measurements in their facilities at the University of Vienna.
Abstract

Molecularly imprinted polymers have been a matter of research for several decades. However, their industrial implementation lags far behind. Inkjet printing could provide a way of low-cost fabrication of MIP thin-films. To test the feasibility of inkjet printed MIPs, two experimental routes were followed. One of them was, to prepare an imprinted polymer with a ready-made inkjet ink. The other approach was aimed at formulation of a MIP and subsequent optimisation for inkjet printing. The acrylate based ink VeroClear was chosen for the first approach, the second route was realised with methacrylic acid and 4-vinylpyridine as functional monomers. The antibiotic penicillin V was used as a template. For selectivity studies on the produced MIPs, they were deposited on quartz crystal microbalances and mass sensing measurements were carried out in penicillin V containing solutions.

The imprint in the ready-made ink showed only little selectivity. Better results were obtained with a formulation of methacrylic acid, ethylene glycol dimethacrylate and acetonitrile with the free acid of penicillin V as a template. The highest response was obtained with measurement solutions prepared in acetonitrile. Regarding inkjet printability, some promising formulations with a solvent mixture of water and 1-propanol were developed. In formulations with 4-vinylpyridine, penicillin V was rapidly destroyed. Their reaction was observed and described by means of FTIR spectroscopy. Moreover, several important improvements of the used mass sensing setup were achieved.
Contents

Abbreviations 3

1 Introduction 6
  1.1 Motivation and aim of the work ......................... 6
  1.2 Chemical sensors ......................................... 7
  1.3 The quartz crystal microbalance (QCM) .................. 8
  1.4 Molecularily imprinted polymers (MIPs) .................. 14
  1.5 Inkjet printing ............................................. 17
  1.6 Penicillin V - the target analyte ....................... 19

2 Experimental 21
  2.1 Methods and materials .................................... 21
    2.1.1 Chemicals and devices ............................... 21
    2.1.2 Quartz crystal microbalances ....................... 23
    2.1.3 QCM measuring setups ............................... 23
      2.1.3.1 Oscillator circuit ............................... 23
      2.1.3.2 Impedance analysis circuit ..................... 24
    2.1.4 UV lamps for photopolymerization ................... 25
    2.1.5 Precipitation of free penicillin V .................. 26
  2.2 Molecularily imprinted polymers ......................... 26
    2.2.1 VeroClear™ and free penicillin V ................... 28
    2.2.2 Methacrylic acid and free penicillin V .............. 28
    2.2.3 4-Vinylpyridine and free penicillin V ............... 28
    2.2.4 Methacrylic acid and penicillin V potassium in dimethyl sulfoxide 29
    2.2.5 Methacrylic acid and penicillin V potassium in water/alcohol mixtures 30
  2.3 Polymer deposition ........................................ 31
    2.3.1 Spin coating .......................................... 31
    2.3.2 Inkjet printing ....................................... 31
  2.4 Determination of layer thickness ........................ 31
  2.5 Oxidation of 4-vinylpyridine ............................ 32
3 Results and discussion

3.1 Introductory remarks
3.1.1 Roadmap
3.1.2 Polymer deposition via spin coating
3.1.3 Mass sensing measurements

3.2 Tests and modifications of the measuring setup
3.2.1 Different types of signal noise
3.2.2 Dissolution of gold electrodes
3.2.3 Pressure sensibility
3.2.4 Temperature sensibility
3.2.5 Tests with plain QCMs

3.3 Molecularly imprinted polymers
3.3.1 VeroClear™-MIP
3.3.2 MIP with free penicillin V and methacrylic acid
3.3.3 MIPs with penicillin V potassium and methacrylic acid
3.3.4 Miscellaneous MIPs

3.4 Inkjet compatibility of MIP formulations
3.5 Oxidation and polymerisation of 4-vinylpyridine
3.6 Degradation of penicillin V
3.7 Summary and outlook

List of Figures

List of Tables

Literature
Acronyms

3D  Three-dimensional
4-VPy  4-Vinylpyridine
ABCN  1,1’-Azobis(cyclohexanecarbonitrile)
AC  Alternating current
ACN  Acetonitrile
AIBN  Azobis(isobutyronitrile)
arb. u.  Arbitrary units
ATRP  Atom-transfer radical polymerisation
CHEMFET  Chemically sensitised field effect transistor
CIJ  Continuous inkjet
CRP  Controlled radical polymerisation
CTA  Chain transfer agent
DC  Direct current
DMF  Dimethylformamide
DMSO  Dimethylsulfoxide
DOD  Drop-on-demand
DVB  Divinylbenzene
EGDMA  Ethylene glycol dimethacrylate
EtOH  Ethanol
Acronyms

**FFG** Forschungsförderungsgesellschaft

**FRP** Free radical polymerisation

**FTIR** Fourier-transform infrared spectroscopy

**i-PrOH** 2-Propanol

**IR** Infrared

**IUPAC** International union of pure and applied chemistry

**KOAc** Potassium acetate

**LOD** Limit of detection

**LOQ** Limit of quantification

**MAA** Methacrylic acid

**MeOH** Methanol

**μf-MEMS** Microfluidic microelectomechanical systems

**MIP** Molecularly imprinted polymer

**NIP** Non-imprinted polymer

**n-PrOH** 1-Propanol

**OLED** Organic light emitting diode

**PDMS** Polydimethylsiloxane

**PenV** Penicillin V (free acid)

**PenVK** Penicillin V potassium

**PMMA** Poly(methyl methacrylate)

**PIJ** Piezoelectric inkjet

**PP** Polypropylene

**QCM** Quartz crystal microbalance

**RAFT** Reversible addition-fragmentation chain transfer polymerisation
Acronyms

**rpm** Revolutions per minute

**TIJ** Thermal inkjet

**TRL** Technology readiness level

**UV** Ultraviolet
1 Introduction

1.1 Motivation and aim of the work

Profactor GmbH is a company located in Steyr, Austria, which does research in the fields of additive micro/nano manufacturing, functional surfaces and industrial assistive systems.

The project AquaNOSE, which is funded by the Austrian Forschungsförderungsgesellschaft (FFG), was started to promote the development of commercial microfluidic microelectromechanical systems (µf-MEMS). The goal of the project is the fabrication of multi analyte nanosensors for biological targets [1].

Building on the work of a project partner, the Department of Physical Chemistry at the University of Vienna, sensors based on molecular recognition should be developed. The detection of various analytes, ranging from small molecules to bacteria, is enabled by deposition of a thin layer of a molecularly imprinted polymer (MIP) onto a quartz crystal microbalance (QCM).

Molecular imprinting has been known for several decades, but when it comes to industrial fabrication, there are only few successful examples in research and literature, like the ‘nanoMIPs’ from MIP Diagnostics Ltd [2]. The major drawback of MIPs, which prevents their industrial breakthrough, is their lack of reproducibility. Nevertheless, a cheap and easily automatable production process, like inkjet printing, could help to promote the industrial interest in MIPs.

This work should present a feasibility study on inkjet printability of MIPs. For the sake of convenience, the work was limited to directly printable MIP formulations (so-called bulk imprinting). However, there are some more promising approaches, like ATRP or RAFT polymerisation as well as core-shell polymers, which are potentially inkjet printable as well. Additionally, the selectivity and reproducibility of the MIPs should be investigated. Penicillin V, a commonly used oral antibiotic, was chosen as the target analyte.

In practice, most time was invested in establishing a newly developed QCM measuring setup, thus there were only few interpretable results in terms of MIP selectivity and reproducibility. Due to a temporary shortness of QCMs, auxiliary research was done concerning the chemical reactions of 4-vinylpyridine, one of the tested monomers, with penicillin V.
1.2 Chemical sensors

It was in the 1920s when the word sensor started to spread and since then it has become part of everyone’s vocabulary. With the increasing intelligence of machines the need of ‘artificial sensory organs’ had arisen and indeed, cameras can be seen as artificial eyes or microphones as artificial ears. Continuing this way of thinking, chemical sensors could be referred to as artificial noses or tongues. Similar to their biological analogues, they transform chemical information into an electric signal. Compared to other analytic methods, chemical sensors offer several advantages: They are usually rather small, cheap, selective and respond quickly. Good sensors also provide reversibility and are stable to long-term use [3].

According to IUPAC, a chemical sensor is a device, that transforms chemical information into any analytically useful signal. The chemical information of interest is commonly the concentration of one or several analyte molecules. A chemical sensor consists of two functional units: The receptor is sensitive to the chemical information of interest, and thus makes it measurable for the transducer that transfers the information of the receptor into a processable signal. Figure 1.1 shows a schematic drawing of a sensor in general. The receptor part can be based either on a chemical reaction that causes the signal or on any physical principle, which means that no chemical reaction takes place.

![Figure 1.1: Schematic drawing of a chemical sensor, consisting of receptor and transducer. The sensor is usually part of an analyser, that includes additional data processing.](image)

Sensors can be categorized by their sensing principle (according to IUPAC 1991) [4]:

**Optical devices** measure changes of optical phenomena caused by the analyte like absorbance, reflectance, luminescence, fluorescence, refractive index or light scattering.
Electrochemical devices make use of the electrochemical interaction of the analyte with an electrode. They can be subdivided into voltammetric and potentiometric sensors, chemically sensitised field effect transistors (CHEMFET) and potentiometric solid electrolyte gas sensors.

Electrical devices are based on analyte induced changes of electric properties with no electrochemical reaction taking place. They include metal oxide or organic semiconductor sensors as well as electrolytic conductivity and electric permittivity sensors.

Mass sensitive devices transform mass changes on a sensitive surface caused by analyte accumulation into a measurable change of the support material. They are usually either piezoelectric or surface acoustic wave devices.

Devices based on other physical properties contain for example magnetic oxygen sensors, thermometric devices or radiation-based sensors.

The sensors that are subject of this master thesis are mass sensitive or more precisely piezoelectric devices. They contain a thin layer of a molecularly imprinted polymer coated onto a quartz crystal microbalance that forms the receptor part. Since the mechanism of analyte adsorption at the specific molecular recognition sites is not fully understood, it can not unequivocally said if the sensor is based on chemical or physical interactions. Maybe, even both of them are present. The transducer part is taken either by an oscillator circuit or an impedance analysis circuit which transform the frequency change of the oscillating quartz into an electric signal that can be processed by a computer.

1.3 The quartz crystal microbalance (QCM)

When deforming certain non-conductive materials, an electric voltage is built up. This phenomenon is called ‘piezoelectric effect’. The effect occurs when an external force is applied to a crystal, which causes its centres of positive and negative charge ($Q^+$ and $Q^-$) to fall apart. Thus, an electric polarization forms between the two centres of charge with opposite sign and charges are built up at the crystal surfaces which can be measured as an electric voltage. Figure 1.2 shows a schematic drawing of the piezoelectric effect. The term ‘inverse piezoelectric effect’ is used when a voltage applied to a material leads to deformation of the same. When applying an AC voltage, the piezoelectric material is forced into vibration. The most important piezoelectric material is $\alpha$-quartz. Besides quartz crystal microbalances, the piezoelectric properties of quartz are for instance used in pressure and force sensors as well.
as in watches or inkjet printheads (see section 1.5) [5].

Figure 1.2: Visualisation of the piezoelectric effect. In the left picture the centers of positive and negative charge are canceling each other out. When a force is applied to the crystal (right picture) they fall apart and give rise to an electric voltage (adapted from [6]).

For their use as QCMs, quartzes are cut into wafers in a specific angle with respect to the crystallographic axis. The so-called 'AT-cut' gives a pure thickness shear mode resonator with a resonant frequency being quite stable against small temperature changes [7]. The thickness shear mode provides the lowest damping and thus the highest stability of all known vibration modes. In figure 1.3 the vibration pattern of a thickness shear mode resonator is visualised [8].

Figure 1.3: Schematic drawing of a thickness shear mode resonator. The direction of propagation is parallel to the quartz surfaces, the amplitude is orthogonal to the same (adapted from [8]).
At oscillating frequencies close to its resonant frequency, a piezoelectric quartz resonator can be described by the Butterworth van Dyke equivalent circuit, pictured in figure 1.4. The circuit consists of two branches, of which one is a pure capacitance $C_p$ and represents the dielectric properties of the quartz. The second branch, consisting of a capacitance $C_s$, an inductance $L$ and an ohmic resistance $R$, represents the piezoelectric oscillator [9]. Physically, $C_s$ is a measure for the restoring force, $L$ for the inertial mass and $R$ for the intrinsic damping of the oscillator [8].

![Equivalent circuit (left) and wiring symbol (right) for an oscillating quartz crystal.](image)

The three most common methods to examine the behaviour of a quartz resonator are the oscillator circuit, impedance analysis and the transient dissipation technique. With this work, the chiefly used method was impedance analysis. In this method, the frequency of the applied voltage is varied in a range around the expected resonant frequency. Compared to the rather cheap and simple oscillator circuit, impedance analysis requires more sophisticated instrumentation but in return offers independence of damping and the possibility to observe the complete impedance spectrum of a QCM. A typical spectrum of absolute value and phase of the complex quartz impedance is shown in figure 1.5. [8]. There are two frequencies where the phase angle and consequently the imaginary part of the impedance is zero. These frequencies are at the same time the minimum and maximum of the absolute value. The frequency with minimal absolute value of the impedance approximately equals the series resonant frequency, which is the resonant frequency of the dynamic branch in the Butterworth van Dyke equivalent circuit [10].
When a thin layer of a rigid material is applied onto a quartz oscillator, its resonant frequency changes linearly with the oscillating mass. The corresponding mathematical relation was proposed by Sauerbrey in 1959 and is given in equation (1.1). Since small frequency changes can be measured very precisely, an oscillating quartz crystal provides a method to measure mass changes in the range of several ng cm\(^{-2}\). The resulting device is called ‘quartz crystal microbalance’ or simply QCM. The precision of the method is limited mainly by the temperature dependence of the quartz crystal’s resonant frequency. By carefully choosing the cutting angle, this effect can be minimized. An AT-cut quartz with a cutting angle of 35°10’ provides good temperature stability around room temperature, as shown in figure 1.6 [11]. Besides temperature, humidity or residual stress of the quartz can lead to undesirable frequency changes [12].
\[ \frac{\Delta f}{f} = -\frac{\Delta d}{d} = -\frac{\Delta m}{\rho_q \cdot A \cdot d} \] (1.1)

\( \Delta f \)  Frequency change [Hz]
\( f_0 \)  Resonant frequency [Hz]
\( \Delta d \)  Thickness change [cm]
\( d \)  Thickness of quartz plate [cm]
\( \Delta m \)  Change of mass [g]
\( \rho_q \)  Density of quartz (\( \rho_q = 2.684 \text{ g cm}^{-3} \))
\( A \)  Area of quartz plate [cm\(^2\)]

Figure 1.6: Temperature dependence of resonant frequency of AT-cut quartz for three different cutting angles (adapted from [11]).

A QCM does not only work under air or vacuum, but also in liquids. However, viscous damping has to be considered in this case. A quantitative relation between the viscosity and density of the used liquid and the shift of the fundamental frequency is given in equation (1.2). It was published by Kanazawa and Gordon in 1985 [13].
\[ \Delta f = -f_0^{3/2} \sqrt{\frac{\eta \rho_l}{\pi \mu_q \rho_q}} \] (1.2)

\(\Delta f\)  Frequency change [Hz]  
\(f_0\)  Resonant frequency [Hz]  
\(\eta_l\)  Viscosity of liquid [g cm\(^{-1}\) s\(^{-1}\)]  
\(\rho_l\)  Density of liquid [g cm\(^{-3}\)]  
\(\rho_q\)  Density of quartz (\(\rho_q = 2.684\) g cm\(^{-3}\))  
\(\mu_q\)  Shear modulus of AT-cut quartz (\(\mu_q = 2.947 \cdot 10^{11}\) g cm\(^{-1}\) s\(^{-2}\))

Figure 1.7 shows a typical QCM design which was also used in the present work. The black areas represent the gold electrodes applied onto an AT-cut quartz wafer. The unequal size of the two electrodes helps to focus the electric field in the region between them. Especially when measuring in liquids it is important to avoid fringing fields that would lead to less reliable results [12].

Figure 1.7: Design of the used electrodes (left: front side, right: back side). Black areas represent the gold electrodes. The gold layer was spread over the edges at both sides, to allow contacting of each electrode at the front as well as on the back side.
1.4 Molecularly imprinted polymers (MIPs)

The concept of molecular imprinting was for the first time published by Wulff and Sarhan in 1972 [14]. They called their discovery ‘polymers with enzyme-analogous structures’, which is actually a pretty accurate description of a MIP.

Indeed, the mechanism of recognition in molecularly imprinted polymers is thought to be very similar to biorecognition in antibodies and enzymes. It can involve all kinds of non-covalent interactions, like van der Waals forces, dipole-dipole interactions, hydrogen bonding, hydrophobic interactions as well as charge-transfer and coulombic interactions. Affinity of a (biological or chemical) receptor towards a certain molecule or epitope is achieved by appropriate orientation of functional groups as well as by shape selectivity [15]. The crucial difference is, that a chemical receptor is not produced in a biological way, but by chemical synthesis.

Although MIPs are frequently described by biological analogies, there is also the inverse idea, that the actual origin of biology lies in molecular imprinting. It was hypothesised, that molecular imprints could have played an important role in the formation of proto-enzymes, proto-ribosomes and proto-cells on early earth, and thus could have contributed to abiogenesis, the origin of life [16, 17].

Figure 1.8 shows a highly schematic visualisation of the molecular imprinting process. When the template molecule is combined with appropriate functional monomers, a self-assembling process starts, as functional groups of the monomers are (mostly non-covalently) attached to the template. Subsequently, the polymerisation is initiated in the presence of a cross-linker (a compound with more than one polymerisable group) to ‘freeze’ the template-monomer-complex. The template is removed from the polymer, usually by extraction in a solvent, to leave an imprint of its exact shape in the polymer matrix. Since they offer an exact sterical fit and functional groups with suitable affinity, these cavities can selectively rebind template molecules. This way of rebinding is referred to as molecular recognition.

Various kinds of templates have been reported for molecular imprinting. Besides small organic molecules like drugs [20], pollutants [21] or explosives [22], there are imprints for metal cations and inorganic anions [23], as well as for peptides and proteins [24, 25]. Bigger species like microorganisms from viruses up to yeasts or bacteria, can be applied as templates for imprinting too [26–28]. Even the use of man-made metal nanoparticles has been reported [29].

Originally, molecular imprinting was divided into a covalent and a non-covalent approach. In the former, reversible covalent bonds between template and polymer are present. However,
this approach only works with a very limited number of compounds [30]. The non-covalent approach is much easier to realise and offers almost infinite flexibility in the choice of template. Therefore, most of today’s research is focussed on non-covalent imprinting [31].

MIPs exist in various forms and shapes. Nanoparticles and thin-films are two widely-used examples. Both forms can be prepared by the simple and traditional bulk imprinting method, where templates, functional monomers, cross-linkers, initiators and solvents are just mixed together followed by subsequent curing of the monomers. Usually, template to functional monomer ratios of 1:4 or more are employed [31]. To obtain particles of a certain size, the resulting bulk product is ground and sieved [23]. To produce a MIP thin-film, the pre-polymerisation mixture has to be applied onto a flat substrate, e.g. by spin coating. Recently, the fabrication of a microstructured MIP thin-film via two-photon stereolithography has been reported [32]. Another recent trend is the application of MIP layers onto nanostructured carbon materials, that allow an improved binding capacity of the MIP due to the drastically increased surface area [33]. Concerning MIP particles, bulk imprinting leads to a quite irregular shape and size and poor reproducibility. Improved properties are obtained by precipitation and emulsion polymerisation as well as iniferter polymerisation. The latter
enables to fabricate MIP nanoparticles with controlled size by addition of a so-called ‘inifer-
ter’ (e. g. dithiocarbamate), which is initiator, transfer agent and terminator rolled into one [18]. Another way to get very homogeneous MIP nanoparticles, is the core-shell approach. It is realised by grafting thin-layers of MIPs onto the surface of existing nanoparticles equipped with immobilised templates[34]. A further advantage of core-shell nanoparticles is, that their core can have an additional function, like dictation of the particle shape. The use of Fe$_2$O$_3$ cores allows easy separation of the particles from the reaction mixture by a magnet [18]. A commonly used technique for comparatively big analytes, like proteins or microorganisms, is surface imprinting. In this method, the template is first assembled on a ‘stamp’, which is subsequently pressed onto a layer of pre-polymer. After curing, the stamp is removed together with the template and leaves a surface imprinted MIP [26]. A basic concept for surface imprinting on industrial scale has been patented by Profactor GmbH [35].

Most molecularly imprinted polymers are produced by radical polymerisation. The simplest form thereof is the free radical polymerisation (FRP). It includes four reaction steps: First, an initiator is decomposed (either by heat or radiation) to yield free radicals that in turn react with the double bond of a monomer, forming a monomer radical. The latter can react further with double bonds of other monomers in numerous propagation steps, leading to long polymer chains with high molecular weight. If two radical species hit on each other, they can recombine or disproportionate in a termination step, to form a non-radical species again. Due to its flexibility in choice of monomers, experimental conditions and reagent purity, FRP is still very attractive, especially for non-experts in polymer chemistry [31].

However, FRP is a statistical, kinetically driven process and can never lead to a homogeneously distributed affinity over the binding sites. Instead, the resulting cavities are very unequal in size and shape, leading to big differences in selectivity [36]. There are different types of controlled radical polymerisation that allow increased control over the reaction kinetics of MIP synthesis. In all CRP methods, a fast dynamic equilibrium between active free radical species and a majority of dormant (inactive) chains is established. Due to the reduced concentration of free radicals, termination reactions are largely suppressed [37]. One approach is the ‘reversible addition-fragmentation chain transfer’ polymerisation (RAFT). It needs only one additional ingredient, the chain transfer agent (CTA), which is often a dithiobenzoate or a trithiocarbonate. During the reaction, the CTA combines with an active polymer chain to form a radical species, that subsequently fragments into a new active chain and a dormant chain including the CTA itself. A rapid transfer of the CTA between the different polymer chains by repeated addition and fragmentation ensures their growth to equal length [38].
A second commonly employed type of CRP is the 'atom-transfer radical polymerisation' (ATRP). It requires a transition metal complex, functioning as a catalyst, and a organohalogenide initiator. The metal complex cleaves a halogen atom from the initiator (or dormant species), undergoing a one-electron oxidation. Thus, a new radical species is formed that can either propagate or rebind the halogen atom from the catalyst and return to dormant state. In ATRP, the fast transfer of the halogen atom is the crucial step, that ensures uniform chain growth [37].

Besides radical polymerisation, completely different polymer systems, like polyurethane, which is a product of polyaddition, are in use as well [29].

Potential applications for molecularly imprinted polymers are mainly found in analytics, catalysis and medicine [39]. Analytical separations are probably the most widespread application of MIPs. They are used as stationary phases in chromatography, capillary electrophoresis and solid-phase extraction. Moreover, they can replace biological receptors in immunoassays [18, 30]. Another wide field of application for MIPs is their use as highly selective sensitive layers or membranes in different kinds of sensors, including optical and electrochemical [40], as well as mass-sensitive devices [26, 27, 41, 42]. With their highly specific binding sites, MIPs are able to mimic biological enzymes and thus catalyse biochemical reactions [43].

In-vivo-applications of MIPs, like drug delivery, are a promising research field as well [18, 44].

Whilst there are some successful examples for the use of MIPs in analytical separations, the commercialisation of MIP sensors lags far behind. Existing MIP sensors demonstrate a maximum technology readiness level of TRL6, which means, that there are already functional prototypes and representative models, but they still lack a proof of concept in a real scenario [40]. To reach higher TRLs, various sensor metrics have to be improved, including sensor drift, accuracy, stability, repeatability, reproducibility and shelf life. Moreover, consistent material quality for a large batch of sensors must be ensured, which is still a problem [45].

1.5 Inkjet printing

As they offer very good printing quality at low cost, inkjet printers have become a part of standard equipment in almost every office and household. In contrast to most other printing techniques, inkjet is not dependent on a physical template of the printing pattern. It rather builds up a picture by jetting a large number of digitally directed droplets onto the substrate [46]. Therefore, it shows great flexibility in the choice of pattern and is employed for various applications apart from document printing. For example, inkjet offers a rapid,
1 Introduction

1.5 Inkjet printing

flexible and color-saving method for advanced colouration of textiles [47]. Another wide field of application are printed electronics, including thin-film transitors, OLEDs, solar cells and sensors. Conductive structures are realised by printing of either conductive polymers or metal nanoparticles [48]. Moreover, inkjet is used for 3D printing. Current research topics include the fabrication of biological tissues [49], microfluidic structures [50] and metal structures [51].

Inkjet technologies are usually divided in two fundamental classes: Continuous inkjet (CIJ) and drop-on-demand inkjet (DOD). In CIJ, the ink leaves the nozzle in a continuous jet, that shortly breaks up into single electrically charged droplets. Basically, the droplet jet leads into a gutter, from where the ink is recirculated. For printing, the droplets are deflected by an electric field in order to reach the substrate. DOD means, that every single drop is ejected from the nozzle by an individual pulse and droplet are only generated for actual printing. The pulse is usually generated either thermally or piezoelectrically (see figure 1.9). In thermal inkjet (TIJ), an electrical heating element inside the nozzle generates a vapour bubble, that pushes a droplet outside the nozzle. In piezoelectric inkjet (PIJ), the droplet is pushed out by a piezoelectric actuator, that quickly reduces the volume of the ink chamber. Typically, the piezoelectric element consists of lead zirconate titanate. It is either separated from the ink by a membrane, or it is part of the ink chamber itself [52].

In 2016, approximately three quarter of all inkjet printers were TIJ printers, which has mainly historical reasons. The rise of PIJ technology goes along with increasing use of inkjet in micromanufacturing. PIJ printers are highly favoured for this purpose, as they do not necessarily heat the ink and thus enable higher flexibility in choice of the ink material [53].

![Figure 1.9: Principle of drop-on-demand inkjet. Left: piezoelectric inkjet, right: thermal inkjet (adapted from [52]).](image-url)
The Dimatix DMP-2800, the inkjet printer which was used in this work, is a piezoelectric drop-on-demand device, that was designed for research and development applications, like testing of new ink formulations.

1.6 Penicillin V - the target analyte

Penicillins are the oldest class of β-lactam antibiotics. Their bactericidal effect is based on inhibiting the bacterial cell wall synthesis [54]. In the year 1951, penicillin V, the first acid-stable, and thus orally administrable, penicillin in history was discovered in Kundl (Tyrol) by E. Brandl and G. Margreiter [55]. It differs from the previously known penicillin G only in having a phenoxymethyl instead of a benzyl group in the α-position of the N-acyl side chain. The chemical structure of penicillin V (PenV) is depicted in figure 1.10. It is readily soluble in polar organic solvents, like alcohols, but insoluble in apolar solvents [56]. The antibiotic is commonly applied as one of its alkaline salts, which are freely water-soluble but poorly soluble in alcohols [57]. The carboxylic acid group of PenV has a pKₐ of 2.735 ± 0.05 [58].

![Chemical structure of phenoxymethylpenicillin, better known as penicillin V.](image)

Aqueous solutions of penicillin V are most stable at pH 6-7. Under basic conditions, the degradation is considerably faster than in acid environment [59]. In figure 1.11, the most common degradation pathways of penicillins are illustrated. In alkaline environment, as well as in the presence of a nucleophile or the enzyme penicillinase, ring-opening of the β-lactam leads to penicilloic acid, which is further decarboxylated to penilloic acid. At acidic pH, a new ring is formed by intramolecular nucleophilic attack of the N-acyl side chain. The resulting intermediate, pseudopenicillin, undergoes an isomerisation to form penicillenic acid. Therefore, the acid-stability of a particular penicillin depends on the nucleophilicity of its side chain carbonyl group. The electron-withdrawing phenoxymethyl group of PenV leads to a reduced electron density at the side chain carbonyl, which consequently is less nucleophilic [60].
Figure 1.11: Degradation pathways of penicillins in general. Left: reaction with nucleophiles, bases or penicillinase, right: reaction in acid environment (adapted from [60].)
2 Experimental

2.1 Methods and materials

2.1.1 Chemicals and devices

Table 2.1 shows a list of the used chemicals. In table 2.2 a list of all used devices is provided. Whenever water was used, it was ultra pure water from a MilliQ® water purification system. Ethanol was used at 2 purity levels. The less pure bioethanol (96 %) was used for template extraction and measuring solutions in mass sensing measurements. For all other purposes, water-free ethanol (99.9 %) was used.

Table 2.1: Chemicals used.

<table>
<thead>
<tr>
<th>Substance</th>
<th>Abbreviation</th>
<th>Purity / %</th>
<th>Manufacturer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetonitrile</td>
<td>ACN</td>
<td>99.9</td>
<td>Roth</td>
</tr>
<tr>
<td>1,1’-Azobis(cyclohexanecarbonitrile)</td>
<td>ABCN</td>
<td>98</td>
<td>Sigma Aldrich</td>
</tr>
<tr>
<td>Azobis(isobutyronitrile)</td>
<td>AIBN</td>
<td>98</td>
<td>Sigma Aldrich</td>
</tr>
<tr>
<td>Bioethanol</td>
<td>EtOH</td>
<td>96</td>
<td>Roth</td>
</tr>
<tr>
<td>D(+)−Glucose monohydrate</td>
<td></td>
<td>-</td>
<td>Roth</td>
</tr>
<tr>
<td>Dimethylsulfoxide</td>
<td>DMSO</td>
<td>99.9</td>
<td>Sigma Aldrich</td>
</tr>
<tr>
<td>Divinylbenzene</td>
<td>DVB</td>
<td>p. s.</td>
<td>Merck</td>
</tr>
<tr>
<td>Ethanol (abs.)</td>
<td>EtOH</td>
<td>99.9</td>
<td>Sigma Aldrich</td>
</tr>
<tr>
<td>Ethylene glycol dimethacrylate</td>
<td>EGDMA</td>
<td>98</td>
<td>Sigma Aldrich</td>
</tr>
<tr>
<td>Ethylphenyl(2,4,6-trimethylbenzoyl)phosphinate (Omnirad TPO-L)</td>
<td>-</td>
<td></td>
<td>IGM Resins</td>
</tr>
<tr>
<td>Formic acid</td>
<td>HCOOH</td>
<td>98</td>
<td>Roth</td>
</tr>
<tr>
<td>Hydrochloric acid (conc.)</td>
<td>HCl</td>
<td>37, extra</td>
<td>Roth</td>
</tr>
<tr>
<td>2-Hydroxy-4’-(2-hydroxyethoxy)-2-methylpropionophenone (Omnirad 2959)</td>
<td>-</td>
<td></td>
<td>IGM Resins</td>
</tr>
<tr>
<td>Methacrylic acid</td>
<td>MAA</td>
<td>98</td>
<td>Fluka</td>
</tr>
<tr>
<td>Methanol</td>
<td>MeOH</td>
<td>99.9</td>
<td>Sigma Aldrich</td>
</tr>
<tr>
<td>Penicillin V Potassium</td>
<td>PenVK</td>
<td>-</td>
<td>Sandoz</td>
</tr>
</tbody>
</table>
### Table 2.1: Chemicals used, continued.

<table>
<thead>
<tr>
<th>Substance</th>
<th>Abbreviation</th>
<th>Purity / %</th>
<th>Manufacturer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenylbis(2,4,6-trimethylbenzoyl) phosphine oxide (Omnirad 819)</td>
<td>-</td>
<td>-</td>
<td>IGM Resins</td>
</tr>
<tr>
<td>Potassium acetate</td>
<td>KOAc</td>
<td>extra pure</td>
<td>Merck</td>
</tr>
<tr>
<td>Potassium chloride</td>
<td>KCl</td>
<td>99</td>
<td>Roth</td>
</tr>
<tr>
<td>Potassium nitrate</td>
<td>KNO₃</td>
<td>99</td>
<td>Roth</td>
</tr>
<tr>
<td>1-Propanol</td>
<td>n-PrOH</td>
<td>99.5</td>
<td>Roth</td>
</tr>
<tr>
<td>2-Propanol</td>
<td>i-PrOH</td>
<td>99.5</td>
<td>Roth</td>
</tr>
<tr>
<td>Sodium hydroxide</td>
<td>NaOH</td>
<td>98</td>
<td>Sigma Aldrich</td>
</tr>
<tr>
<td>Sylgard® 184 silicone elastomer</td>
<td></td>
<td>-</td>
<td>Dow Corning</td>
</tr>
<tr>
<td>(base and curing agent)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tandil Eco</td>
<td>-</td>
<td></td>
<td>Tandil</td>
</tr>
<tr>
<td>VeroClear RGD810</td>
<td></td>
<td></td>
<td>Stratasys</td>
</tr>
<tr>
<td>4-Vinylpyridine</td>
<td>4-VPy</td>
<td>95</td>
<td>Sigma Aldrich</td>
</tr>
</tbody>
</table>

### Table 2.2: Devices used.

<table>
<thead>
<tr>
<th>Device</th>
<th>Manufacturer</th>
<th>Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Analytical balance</td>
<td>Sartorius</td>
<td>CPA225D</td>
</tr>
<tr>
<td>Centrifuge</td>
<td>Heraeus</td>
<td>Biofuge primo</td>
</tr>
<tr>
<td>Digital microscope</td>
<td>Keyence</td>
<td>VHX 5000</td>
</tr>
<tr>
<td>Digital thermometer</td>
<td>Lascar Electronics</td>
<td>EasyLog USB Data Logger</td>
</tr>
<tr>
<td>Drying oven</td>
<td>Memmert</td>
<td>UFB500</td>
</tr>
<tr>
<td>Frequency counter</td>
<td>Agilent Technologies</td>
<td>53131A</td>
</tr>
<tr>
<td>FTIR spectrometer</td>
<td>Bruker</td>
<td>Tensor 37</td>
</tr>
<tr>
<td>Heating plate</td>
<td>IKA</td>
<td>RCT basic</td>
</tr>
<tr>
<td>Inkjet Printer</td>
<td>Fujifilm</td>
<td>Dimatix DMP-2800</td>
</tr>
<tr>
<td>Multimeter</td>
<td>Fluke</td>
<td>83 III</td>
</tr>
<tr>
<td>Network analyser</td>
<td>Agilent Technologies</td>
<td>E5062A (300 kHz – 3 GHz)</td>
</tr>
<tr>
<td>Power supply</td>
<td>Elektro-Automatik</td>
<td>EA-PS-2032-025 (0 – 32 V, 0 – 2.5 A)</td>
</tr>
<tr>
<td>Profilometer</td>
<td>Bruker</td>
<td>Dektak 150</td>
</tr>
<tr>
<td>Shaker</td>
<td>Scientific Industries</td>
<td>Vortex Genie 2</td>
</tr>
<tr>
<td>Spincocoater</td>
<td>SPS-Europe</td>
<td>Polos Spin 150i</td>
</tr>
<tr>
<td>Ultrasonic bath</td>
<td>Elma</td>
<td>Elmasonic TI-H10 MF</td>
</tr>
<tr>
<td>UV lamp (2x)</td>
<td>Ushio</td>
<td>UVE 001044</td>
</tr>
</tbody>
</table>
2.1.2 Quartz crystal microbalances

During this work, different types of QCMs were used, all of them having a resonant frequency of approximately 10 MHz. The first share was received from the group of P. Lieberzeit at the University of Vienna. They were circle shaped AT-cut quartz plates with a cutting angle of 35° 15’ ± 3’ and a diameter of 13.8 mm. The surface of the quartzes was either smooth and transparent or turbid with a surface roughness of several hundreds of nm. Electrodes were applied by the group of Lieberzeit via screen printing of a colloidal gold paste and subsequent baking. The typically used electrode design is depicted in figure 1.7. Some of the transparent QCMs were sputtered with chromium before application of the gold paste. In this case, the front electrode covered the entire quartz surface. The back electrode was in accordance with the aforementioned design. In all cases, the gold layer was spread over the edges of the quartz in order to allow contacting of both electrodes at the front as well as at the back side.

The second share of the used QCMs was purchased from Krystaly, Hradec Králové (Model 3, Au+Cr, 10,000 MHz). They were the same size as the QCMs from the University of Vienna and their surface was turbid. A profilometer measurement of a new QCM revealed an average roughness of 263 nm. Again, the gold layer was spread over the quartz and both electrodes could be contacted from the front as well as from the back.

2.1.3 QCM measuring setups

2.1.3.1 Oscillator circuit

The oscillator circuit, which was used for some reference measurements, was designed by the group of P. Lieberzeit. It was based on a Colpitts oscillator and connected to a frequency counter which measured the oscillation frequency of the QCM. Two opposed diodes in the circuit were limiting the AC voltage to ±380 mV. Recording of the output frequency was achieved by a specially tailored software developed by the University of Vienna. The circuit was powered by a laboratory power supply, providing 12.5 V and 0.6 A. Figure 2.1 shows the used measurement cell which was basically made of PMMA. The QCM was sandwiched between two PDMS cylinders and pressed together by tightly screwing the PMMA top. Two
copper brushes were included between the lower PDMS cylinder and the QCM to contact its two electrodes and connect them to the oscillator circuit via a coaxial cable. The upper PDMS cylinder included two tubes for infilling and removal of liquid and about 200 µL of hollow space at the upper surface of the QCM. The cell was placed on a piece of rubber foam to damp vibrations of the table.

Although technically the QCM served as the frequency determining component, the output frequency had to be adjusted into a range where it could ‘jump’ to the QCM’s resonant frequency. Therefore, the resonant frequency of the QCM was determined by a network analyser before each measurement. Then, the oscillator circuit was trimmed to this frequency by a built-in rotary potentiometer. An Eppendorf pipette was used to fill the cell with liquid. Excess liquid was absorbed by a paper towel.

Figure 2.1: Measurement cell built by the group of Lieberzeit.

2.1.3.2 Impedance analysis circuit

Based on the installation described in the previous section, the team of Profactor GmbH developed a new setup, using an impedance analysis circuit to determine the QCM’s resonant frequency. In contrast to the oscillator circuit, the impedance analysis method allows to sweep the applied AC voltage over a defined frequency range. Thus, a complete impedance spectrum is recorded and the obtained resonant frequency of the QCM is regarded to be more reliable. The setup was designed to simultaneously investigate two QCMs in the same solution, therefore the circuit contained two separate channels which were independently read out by a software made by Profactor GmbH. Besides the AC voltage, there was a DC
voltage of 2.47 V applied to each channel. The newly designed measurement cell (figure 2.2) was printed by a Stratasys PolyJet 3D printer using the UV curable acrylate ink VeroClear. The bottom part was equipped with two spring-loaded gold pins in each cell, to contact the electrodes bottom-up. A specially invented mechanism allowed to move the pins up and down by turning a wheel. After inserting a QCM into the cell, a ring-shaped PDMS sealing was put onto it before the top of the cell was fixed by four screws. After injecting liquid through the small entrance hole to the first cell, it reached the second cell via a tunnel inside the top before taking the exit through the bigger hole. Usually, measurement solutions were inserted by a pipette or syringe and excess liquid was simultaneously sucked off by a second pipette.

![Measurement cell for 2 QCMs](image)

**Figure 2.2: Measurement cell for 2 QCMs, built by Profactor GmbH. Left: cell during measurement, centre: insertion of QCMs, right: open cell with QCMs, sealings and removed top part.**

### 2.1.4 UV lamps for photopolymerisation

For curing and pre-curing of the polymers, two Hg vapour lamps of the same type (Ushio UVE 001044) were used. The first one was part of the so-called ‘curing stage’, a software-controlled device, which was self-assembled by Profactor GmbH. It was designed to house different UV and IR light sources and allowed exact adjustment of exposure time and distance to each lamp. The second Hg vapour lamp was covered by a simple metal housing and exposure time was controlled by a manual shutter.

The intensity of each lamp was determined by means of a UV sensor which recorded the radiation dose in the wavelength range of 230-410 nm. The dose was measured for 10 s at a distance of about 1 cm at the centre of each lamp. Repeated measurements revealed a radiated power of $3038 \pm 112 \text{ W m}^{-2}$ for the curing stage lamp and $5194 \pm 112 \text{ W m}^{-2}$ for the lamp in the metal housing. The uncertainty of $112 \text{ W m}^{-2}$ equals the smallest measuring
interval of the sensor. Besides the radiant energy, both lamps emitted a considerable amount of heat as well.

2.1.5 Precipitation of free penicillin V

In accordance with a procedure by Aumsuwan et al. the free acid of PenV was yielded from the potassium salt [61]. 1.5 g (4 mmol) of PenVK were dissolved in 15 mL of water. 50 mL of 0.1 M HCl (5 mmol) are added. Precipitated PenV was filtered off and washed with water several times. Subsequently, it was dried over silica gel in a desiccator at room temperature overnight. 1.29 g of dry PenV were obtained (95 % yield). It was permanently stored in the freezer.

2.2 Molecularly imprinted polymers

Different MIP formulations were tested with PenV as well as with PenVK as a template. The choice was mainly directed by solubility of the template in the monomers. The employed monomers, cross-linkers and initiators are depicted in figures 2.3 and 2.4, respectively. In table 2.3, the used ratios of template to functional monomer to cross-linker are listed.

![Structures of the used functional monomers and cross-linkers.](image)

Figure 2.3: Structures of the used functional monomers and cross-linkers.
2 Experimental 2.2 Molecularly imprinted polymers

Azobisisobutyronitrile (AIBN)

1,1'-Azobis(cyclohexanecarbonitrile) (ABCN)

2-Hydroxy-4'-(2-hydroxyethoxy)-2-methylpropiophenone (Omnirad 2959)

Phenyl bis(2,4,6-trimethylbenzoyl)-phosphine oxide (Omnirad 819)

Ethyl phenyl(2,4,6-trimethylbenzoyl)phosphinate (Omnirad TPO-L)

Figure 2.4: Structures of all used thermal and photo initiators.

Table 2.3: Ratios of template to functional monomer to cross-linker for all prepared MIP formulations. The ratio for the MIPs with PenVK, where no literature is cited, was taken from a recipe used by the group of Lieberzeit at the University of Vienna.

<table>
<thead>
<tr>
<th>Template</th>
<th>Functional monomer</th>
<th>Cross-linker</th>
<th>Molar ratio</th>
<th>Section(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PenV</td>
<td>VeroClear</td>
<td>VeroClear</td>
<td>unknown</td>
<td>2.2.1</td>
</tr>
<tr>
<td>PenV</td>
<td>MAA</td>
<td>EGDMA</td>
<td>1:13:67 [62]</td>
<td>2.2.2</td>
</tr>
<tr>
<td>PenV</td>
<td>4-VPy</td>
<td>EGDMA</td>
<td>1:15:19 [63]</td>
<td>2.2.3</td>
</tr>
<tr>
<td>PenVK</td>
<td>MAA</td>
<td>EGDMA</td>
<td>1:6:12</td>
<td>2.2.4 and 2.2.5</td>
</tr>
</tbody>
</table>
2.2 Molecularly imprinted polymers

2.2.1 VeroClear™ and free penicillin V

A very simple MIP was prepared by dissolving 7 mg (0.02 mmol) of PenV (free acid) in 100 µL of the acrylate based UV curable ink VeroClear and 900 µL of EtOH (abs.). The exact composition of VeroClear remains unknown. The amount of PenV was chosen to match the maximum solubility of PenV in VeroClear (70 g L⁻¹). For the NIP, the same mixture was prepared omitting PenV. PenVK showed little if any solubility in VeroClear. The mixture was applied onto QCMs via spin coating or inkjet printing and cured under the more powerful Hg vapour lamp for 4 min. To remove the template, the QCMs were leached with EtOH in a stirred beaker overnight.

Mass sensitive measurements with the impedance analysis circuit were carried out in water, in aqueous solutions of KCl, KOAc and PenVK, as well as in EtOH and PenV in EtOH. Reference measurements with the oscillator circuit were done in aqueous solutions of KCl and PenVK as well as in EtOH and PenV in EtOH.

2.2.2 Methacrylic acid and free penicillin V

The used recipe was based on the work of Cederfur et al. [62]. For the MIP, 10 mg (0.03 mmol) of PenV, 34 µL (0.40 mmol) of MAA and 377 µL (2.00 mmol) of ethylene glycol dimethacrylate (EGDMA) were used, representing a ratio of template to functional monomer to cross-linker of 1:13:67. They were dissolved in 400 µL ACN in a PP vial and 58 mg (0.24 mmol, 0.1 equiv.) 1,1’-azobis(cyclohexanecarbonitrile) (ABCN) were added. The NIP was prepared in the same way omitting PenV. After purging with nitrogen or argon for 10 min, 200 µL of the mixture were pre-polymerised by the less powerful Hg vapour lamp for 30 s. 5-10 µL of the pre-polymerised mixture were spin coated onto a QCM and cured by the stronger UV lamp. For template removal, the QCMs were leached with EtOH in a stirred beaker overnight..

Mass sensitive measurements with the impedance analysis circuit were carried out in water, in aqueous solutions of KCl, KOAc and PenVK, as well as in EtOH, PenV in EtOH, ACN and PenV in ACN. One single reference measurement with the oscillator circuit was done in EtOH and PenV in EtOH.

2.2.3 4-Vinylpyridine and free penicillin V

Building on the work of Skudar et al., different formulations with 4-vinylpyridine (4-VPy) as functional monomer and PenV as template were developed [63]. In a basic recipe for a MIP, 10 mg (0.03 mmol) of PenV, 48 µL (0.44 mmol) of 4-VPy and 106 µL (0.56 mmol) of EGDMA were used, representing a ratio of template to functional monomer to cross-linker of 1:15:19. Together with 17 mg of ABCN (0.07 mmol, 0.1 equiv.), they were dissolved in 100 µL ACN

28
in a PP vial, purged with nitrogen for 5 min and pre-polymerised for 30-60 s using the less powerful UV lamp. 10 μL of the mixture were spin coated onto a QCM and cured by the more powerful UV lamp. The QCMs were leached with EtOH in a stirred beaker overnight to remove the template. The NIP was prepared the same way, omitting PenV.

Modified formulations with 12 μL (0.11 mmol) and 24 μL (0.22 mmol) of 4-VPy were prepared. Furthermore, some MIPs were prepared with reduced solvent volume (56 μL). Another recipe was tested where EGDMA was replaced by 80 μL (0.56 mmol) of divinylbenzene (DVB).

Mass sensitive measurements with the impedance analysis circuit were carried out with each of the aforementioned formulations in water as well as in aqueous solutions of KCl and PenVK.

### 2.2.4 Methacrylic acid and penicillin V potassium in dimethyl sulfoxide

In accordance with a recipe by the group of Lieberzeit, a thermally cured MIP with PenV potassium salt (PenVK) was tested. 8 mg (0.02 mmol) of PenVK, 10 μL (0.12 mmol) of MAA and 45 μL (0.24 mmol) of EGDMA were used, representing a ratio of template to functional monomer to cross-linker of 1:6:12. They were dissolved in 800 μL of dimethyl sulfoxide (DMSO) in a PP vial and 7 mg (0.04 mmol, 0.1 equiv.) of azobis(isobutyronitrile) (AIBN) were added. The mixture was purged with argon for 10 min and subsequently put into a 50 °C water bath for 15 min. For the NIP formulation, PenVK was replaced by 1.5 mg (0.02 mmol) of KCl. 5 μL of the mixture were spin coated onto a QCM and placed in a drying oven at 80 °C overnight. To extract the template, the QCMs were leached with water in a stirred beaker overnight.

AIBN was generously provided by the University of Vienna. Prior to that, experiments were carried out replacing AIBN by 1,1’-Azobis(cyclohexanecarbonitrile) (ABCN). Since ABCN has a lower thermal decomposition rate constant than AIBN, the recipe was modified using the Arrhenius equation to calculate the reaction parameters for a similar decomposition rate of ABCN [64]. Consequently, AIBN was replaced by 16 mg (0.07 mmol, 0.2 equiv.) of ABCN, the temperature of the pre-polymerisation water bath was raised to 75 °C and the curing in the drying oven was performed at 85 °C for 20 h.

Additional experiments were carried out to test photopolymerisation of this formulation. For this purpose, AIBN was replaced by 10 mg (0.04 mmol, 0.1 equiv.) of ABCN. For curing, 10 μL of the mixture were placed on a glass slide and irradiated with a Hg vapour lamp for up to 11 min.
2 Experimental

2.2 Molecularly imprinted polymers

2.2.5 Methacrylic acid and penicillin V potassium in water/alcohol mixtures

For all tested MIP formulations, 8 mg (0.02 mmol) of PenVK, 10 µL (0.12 mmol) of MAA and 45 µL (0.24 mmol) of EGDMA, representing a ratio of template to functional monomer to cross-linker of 1:6:12, were dissolved in a water/1-propanol mixture with a photoinitiator (for further explanations see section 3.3.3). For the NIP, PenVK was replaced by 1.5 mg (0.02 mmol) of KCl. After purging with argon for 10 min, 200 µL of each formulation were pre-polymerised by means of the less powerful UV lamp, spin coated on QCMs and cured under the more powerful UV lamp for 5 min. Template removal was done by leaching with water in a stirred beaker overnight. An overview of all tested formulations is given in table 2.4. A mixture of 56.8 % water and 43.2 % 1-propanol matches the azeotropic composition of the two solvents [65].

Mass sensitive measurements with the impedance analysis circuit were carried out with selected MIPs in water and in aqueous solutions of KCl and PenVK.

<table>
<thead>
<tr>
<th>No.</th>
<th>Photo initiator (PI)</th>
<th>m / mg</th>
<th>$V_{H_2O} / \mu$L</th>
<th>$V_{n-PrOH} / \mu$L</th>
<th>Pre-polymerisation time / s</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Omnirad 2959</td>
<td>10</td>
<td>600</td>
<td>400</td>
<td>10</td>
</tr>
<tr>
<td>2</td>
<td>Omnirad 2959</td>
<td>10</td>
<td>200</td>
<td>800</td>
<td>10</td>
</tr>
<tr>
<td>3</td>
<td>Omnirad 2959</td>
<td>10</td>
<td>568</td>
<td>432</td>
<td>7</td>
</tr>
<tr>
<td>4</td>
<td>Omnirad TPO-L</td>
<td>19</td>
<td>600</td>
<td>500</td>
<td>2</td>
</tr>
<tr>
<td>5</td>
<td>Omnirad TPO-L</td>
<td>19</td>
<td>200</td>
<td>800</td>
<td>5</td>
</tr>
<tr>
<td>6</td>
<td>Omnirad 819</td>
<td>17</td>
<td>200</td>
<td>800</td>
<td>20</td>
</tr>
<tr>
<td>7</td>
<td>AIBN</td>
<td>7</td>
<td>400</td>
<td>800</td>
<td>5</td>
</tr>
</tbody>
</table>

In another experiment, the water/1-propanol mixture was replaced by methanol. The corresponding MIP recipe consisted of 8 mg (0.02 mmol) of PenVK, 10 µL (0.12 mmol) of MAA and 45 µL (0.24 mmol) of EGDMA, representing a ratio of template to functional monomer to cross-linker of 1:6:12. They were dissolved in 800 µL of MeOH together with 7 mg (0.04 mmol, 0.1 equiv.) of azobis(isobutyronitrile) (AIBN). For the NIP, PenVK was replaced by 1.5 mg (0.02 mmol) of KCl. After purging with argon for 10 min, a considerable amount of MeOH was evaporated. Hence, the vial was refilled with 500 µL of MeOH. 200 µL of the mixture were pre-polymerised under the less powerful Hg vapour lamp. 5 µL of the pre-polymerised solution were spin coated onto a QCM and cured under the strong UV lamp for 5 min. The QCM was leached with water in a stirred beaker overnight to extract the template.
The MIP was tested in mass sensitive measurements with the impedance analysis circuit in water and aqueous solutions of KCl and PenVK.

2.3 Polymer deposition

2.3.1 Spin coating

To remove contaminations from the surface of the QCMs and prepare them for spin coating, various cleaning protocols were tried, comprising one or several of the listed steps.

- Washing in aqueous detergent solution (Tandil Eco)
- Rinsing with water and isopropyl alcohol
- Blow-drying with compressed air
- Spinning with ethanol or 2-propanol

Rotation speed, acceleration and spin coating time were optimised in order to get a homogeneous polymer layer with a height of 100-500 nm. Depending on the polymer and the surface properties of the QCM, spinning for 30 s at 1000-2000 rpm led to the desired result. To remove droplets at the borders of the QCM, a second spinning step at 4000 rpm for 1 s was added.

2.3.2 Inkjet printing

Inkjet printing was tested with a MIP based on the commercial ink VeroClear (see section 2.2.1). A Dimatix DMP 2800 printer was used for that purpose. For optimisation of the printing parameters, printing was tested on glass slides. Drop spacing, jetting voltage, printing distance as well as cartridge and substrate temperature were varied in order to get the printed layer as homogeneous as possible. The best results were achieved using a drop spacing of 35 μm, a jetting voltage of 16 V, a printing distance of 1 mm and keeping cartridge and substrate temperature at room temperature. The same parameters were used to print the MIP and NIP on QCMs.

2.4 Determination of layer thickness

Layer height was determined either by profilometer measurements or by evaluation of the change of resonant frequency. Profilometer measurements were only possible with smooth QCMs. In case of QCMs with a turbid surface, the thickness of the coated layers vanished under their surface roughness.
For the other method, the resonant frequency of a QCM in air was recorded by the impedance analysis setup before and after coating with a polymer. By means of the Sauerbrey equation (1.1), the mass change was calculated. Assuming that the density of the polymer is $1 \text{ g cm}^{-3}$, the rough estimation can be made that 1 kHz of frequency drop corresponds to 40 nm layer thickness [66]. Although this assumption is commonly made for different polymers, it is very speculative as long as the porosity of the respective polymer is unknown.

### 2.5 Oxidation of 4-vinylpyridine

To investigate the reaction of pure 4-VPy with air, 3 mL were distilled under vacuum. Subsequently, the distillate was stirred at 100 °C for 4 h. To investigate a possible connection between the arising red colour and the acid properties of PenV, 100 µL of 4-VPy were mixed with 23 µL of formic acid and with 10 µL of HCl (conc.), respectively.

### 2.6 Degradation of penicillin V

Several experiments were carried out to investigate the degradation of PenV in combination with 4-VPy. As in the recipes described in section 2.2.3, 10 mg of PenV were mixed with 48 µL of 4-VPy. FTIR spectra were recorded after 0, 3, 5, 11 and 26 days. Further FTIR measurements were carried out with solutions of PenV in ACN (42 g L$^{-1}$), PenV in EtOH (100 g L$^{-1}$) and PenVK in water (500 g L$^{-1}$) directly after mixing, after 24 h and 5 days. A saturated solution of PenV in 4-VPy (420 g L$^{-1}$) was measured directly after mixing, after 4 days at room temperature and after subsequent heating to 100 °C for 1 h and 5 h, respectively.
3 Results and discussion

3.1 Introductory remarks

3.1.1 Roadmap

In order to develop an inkjet printable MIP, two different routes were followed within this work. Figure 3.1 presents an overview of the two approaches. The left side, the formulation of a MIP using a ready-made ink, was realised with the commercial acrylate ink VeroClear. On the right side, methacrylic acid and 4-vinylpyridine were tested as functional monomers and free PenV as well as PenVK were used as templates. The last rectangle, ’optimisation for printing’, is dashed, because this step was, for a lack of time, only dealt with in a theoretical way.

Figure 3.1: Roadmap for the development of an inkjet printable MIP

Besides this simple, straightforward plan, there were several additional issues to be solved. Especially the newly developed impedance analysis setup, that was used for the mass sensing
measurements, needed some special care to overcome its teething problems. Section 3.2 provides more information on that issue. In section 3.3, the core of this work, the MIPs and their performance in QCM measurements, is presented, while section 3.4 deals with inkjet compatibility of the formulated MIPs. Additional experiments were carried out to investigate the compatibility of PenV with 4-vinylpyridine as a functional monomer. The results thereof are found in section 3.5 and 3.6.

### 3.1.2 Polymer deposition via spin coating

Since printing cartridges are rather expensive, polymers (also VeroClear) were mainly deposited via spin coating. To ensure a rigid polymer layer, which is required for validity of the Sauerbrey equation, the layer height should preferably not exceed 500 nm. As expected, for a given formulation, the height of a spin coated layer was mainly defined by the spinning speed. On a smooth QCM, layers of several hundreds of nm were obtained at a speed of 1000 rpm. However, on QCMs with a rough surface the same speed led to a considerably higher layer thickness up to 2 \( \mu \text{m} \).

A second important issue was the homogeneity of the spin coated layer. Often, the QCM surfaces were not perfectly even and the polymer could not be equally distributed on the surface. Various cleaning steps did not lead to a visible change in the result. Therefore, the cleaning procedure was mostly reduced to 2-3 short spinning steps with EtOH or \( \text{t-PrOH} \). Sometimes, big droplets formed at the borders of the QCM. They were successfully removed by adding a short spinning step at elevated speed.

### 3.1.3 Mass sensing measurements

The following pages include selected curves from mass sensitive measurements of the impedance analysis setup. Within this work, several improvements were made at the setup (see section 3.2). All of the presented curves were recorded after implementation of these modifications, using industrially produced QCMs from Krystaly, Hradec Králové. The measurement solutions were consciously prepared at relatively high concentrations, compared with the intended future use of the sensor. In order to spot any selective response of the MIPs, solutions with minimum 2 mg L\(^{-1}\) PenV or PenVK were used. To eliminate ionic effects of potassium, the solutions of PenVK were prepared in 50 mM KCl. For solubility reasons, the measuring solutions with free PenV had to be prepared in an organic solvent. Usually EtOH or ACN were used and no salt was added.

The measured frequency data was prepared for graphical interpretation in several steps. First, the frequencies of both QCMs were scaled to the frequency measured in the respective
results and discussion 3.2 tests and modifications of the measuring setup

background solution. Since the absolute frequency of the QCMs is of minor importance, the graphs show the difference to the background solution (baseline) only. A simple drift correction was performed by subtraction of a linear function. The slope of this function was adjusted in order to get a horizontal baseline. A percentile filter was applied to reduce noise and remove spikes. The shape of the curves was not affected by this filter. Frequency jumps were quantified by taking the average of a time interval (approx. 1 min) of the unfiltered data with more or less stable frequency. Subsequently, the jumps from the baseline and back to the baseline were averaged. If there was more than one injection of the same measuring solution, only the overall average of all jumps was determined.

3.2 Tests and modifications of the measuring setup

3.2.1 Different types of signal noise

When the impedance analysis setup was placed into operation for the first time, the output signal was overlaid by a random noise in the range of 100 Hz and more. Since the expected response was in the same frequency range, this noise prevented sensitive measurements for several months. The exact source of the noise could not be found, but most likely it was caused by some electromagnetic field, generated by other devices within the same room. The noise disappeared, when the whole setup was moved to another room.

The impedance analysis circuit produced a complete impedance spectrum for each measuring point, which was fed to the computer. Figure 3.2 shows an example of these raw data. The resonant frequency of the QCM was then determined by an algorithm that detected the minimum of the absolute value of the impedance. Sometimes, the peak in the raw data was just missing. In that case, the algorithm detected the resonant frequency somewhere else. This led to giant spikes in the output curve. The reason for these irregularities could not be figured out yet, but when the time interval between the measuring points was raised to a minimum of 3 s, the occasional spikes did not show up again.

A third type of noise was caused by an odd-looking disturbance in the raw data, that is shown in figure 3.3. The disturbance was permanently present for both measurement channels and was shaped like a bump, whose amplitude maximum always appeared at exactly 10.000 MHz. Fortunately, most measurements were not affected at all by this 'bump'. However, when the resonant frequency came very close to 10.000 MHz, the noise of the output signal was strongly increased.
3 Results and discussion 3.2 Tests and modifications of the measuring setup

Figure 3.2: Absolute value $|Z|$ and phase angle $\phi$ of the complex impedance spectrum of an uncoated QCM (unscaled raw data).

Figure 3.3: Detail of the impedance spectrum of two simultaneously measured QCMs (unscaled raw data).
3.2.2 Dissolution of gold electrodes

In the first months, gold electrodes were frequently peeled off the quartz during a measurement. Their dissolution was a big mystery at the beginning, because the fact that a DC voltage of 2.47 V was applied to the measuring cell remained unknown for a long time. However, it was only observed in conductive solutions (mainly aqueous KCl), but never in pure water or ethanol. Figure 3.4 shows two of the electrolysis victims. On the left, a chromium sputtered QCM is shown, where almost the whole electrode dissolved. In other experiments, only a narrow band of the electrode (which was adjacent to the PDMS seal) dissolved, like in the right picture.

![Figure 3.4: Microscope pictures of dissolved gold electrodes. Left: Smooth QCM (University of Vienna) with damages all over the surface, Right: detail of turbid QCM (Krystaly, Hradec Králové) with only small damage.](image)

When the magnitude and especially the direction of the voltage was finally measured, it became clear that the dissolved electrodes had always been the anodes. It turned out, that with the mostly used design (see figure 1.7), both electrodes of a QCM were in contact with the solution. However, the worst case was, when the upper electrode of one QCM was attached to the positive and the other one to the negative pole of the circuit. In this arrangement, the electrolysis current was flowing from one QCM to the other. The polymer coating was obviously not sufficient to isolate the electrodes from the solution.

Figure 3.5 shows a measurement curve where the mass loss of the anode is as well visible as the gold deposition on the cathode. Unfortunately, this curve with its almost equal frequency change at both electrodes could not be reproduced. In other experiments, the electrolysis did not go as smooth. Sometimes, big pieces of gold or polymer became loose and often, the dissolution just led to a loss of contact at the anode.
Mass sensitive experiments with blank QCMs were carried out in 50 mM KCl and 50 mM KNO$_3$ to investigate the effect of chloride ions. In the chloride containing solution, the dissolution of the respective anode always started immediately, whereas in KNO$_3$, dissolution was only observed at yet damaged gold electrodes. The anodic dissolution of gold in chloride containing electrolytes has been reported earlier and is industrially exploited in the Wohlwill gold refining process [67]. Firstly, chloride functions as a complexing agent for gold ions and secondly it can dissolve the passive layer of gold oxide which is built on the electrode surface at potentials above 1.4 V [68]. Admittedly, the cited literature refers exclusively to acid media. However, the applied potentials came not even close to the 2.47 V in the present work. Under these violent conditions, an oxidation of gold seems not so unlikely, even in neutral media.

An effective way to prevent QCM destruction was to avoid current flow across the measurement solution. Consequently, it was important to insert both QCMs in a way, so that the electrodes in contact with the solution were always cathodes. In this arrangement, they could definitely not be oxidised. However, the solution was also in contact with a small gold coated segment on each QCM, that was connected to its counter electrode. In figure 1.7, the
used electrode design is depicted. The electrodes were designed this way, to enable to contact them at both the front and the back side.

For this reason, these parts of the QCM surface had to be isolated from the measuring solution. Thus, new o-ring shaped seals, that covered everything but the gold in the middle of a QCM, were stamped out of hardened PDMS (Sylgard 184). To prevent the measuring solution from reaching the back side of the QCMs, a second type of seals was designed for the bottom of the cell. They featured two notches for the contact pins, as well as a circular pit in the centre, that enabled free oscillation of the QCM. These seals were cast from PDMS (Sylgard 184) in a mould, that was specially printed via stereolithography.

### 3.2.3 Pressure sensibility

Whenever a new solution was injected into the measurement cell, the frequency curve of the QCMs exhibited a peak with a height of up to 1 kHz. The peaks were not problematic, as long as the frequency returned to its original baseline. Unfortunately, this was not always the case and often, multiple injections of the same solution led to a new baseline with each injection. The frequency was also heavily influenced by the pressure applied via the seals. Thus, torquing down the screws that fix the cell top had a high impact on the frequency. Interestingly, it also helped to reduce the injection peaks. It is thought, that the injection of a liquid into the cell somehow affects the pressure of the seals on the QCM. In order to avoid any undesired force effects on the QCMs, the cell was filled via a flexible tube and a syringe that was fixed on a laboratory stand. In order to allow refilling of the syringe without bringing air bubbles into the solution, a simple valve was realised by a pinch cock. Adapters for connection of tube, syringe and cell were made from pipette tips. Pictures of the installation are shown in figure 3.6.

The installation helped to stabilise the QCM frequency during injections, but certain effects were still observed. The high pressure sensitivity of the setup becomes tangible, while opening the pinch cock. The only force applied to the QCM during this step is the additional hydrostatic pressure of the 3 mL of liquid inside the syringe. It leads to a frequency drop of 10-20 Hz. However, not all of the observed force effects are as well-understood yet, and since they are overlaying the response of the MIPs, they can easily lead to misinterpretations. To avoid these, the setup was tested before each measurement with multiple injections of the respective background solution and intermediate adjusting of the screws. Analyte solutions were only injected, if the baseline had repeatedly returned to its original frequency. Since this procedure was extremely time-consuming, deviations of less than 20 Hz were usually tolerated.
3.2 Tests and modifications of the measuring setup

3.2.4 Temperature sensibility

The remaining frequency shifts for multiple injections of the same solution are maybe partially caused by temperature effects. Indeed, according to figure 1.6, the resonant frequency of an AT-cut quartz is very stable to temperature changes. However, the Kanazawa-Gordon equation predicts a rather strong dependence on density and viscosity of the surrounding liquid, which are definitely changing with temperature. Using viscosity and density data from literature, the frequency increase with rising temperature was calculated for a 10 MHz QCM in all used solvents [69]. Around room temperature, it was found to be about 20 Hz K$^{-1}$ in water and EtOH and 6 Hz K$^{-1}$ in ACN.

3.2.5 Tests with plain QCMs

To ensure that the whole measurement setup was working properly, a few test measurements were carried out using blank QCMs without any coating. Figure 3.7 shows the result of a measurement in glucose solutions at different concentrations. The used QCMs showed approximately equal frequency shifts and thus, only their averaged shifts are visualised in figure 3.8. The error bars represent the standard deviation of this average, the red line is a linear regression curve.

Obviously, the frequency shift shows a linear dependency on the glucose concentration within the tested range. According to the Kanazawa-Gordon equation, the frequency of a QCM strongly depends on the viscosity and density of the measurement solution which of course changes with glucose concentration. Viscosity and density values for aqueous solutions
3 Results and discussion 3.2 Tests and modifications of the measuring setup

Figure 3.7: Mass sensitive measurement of two blank QCMs in solutions of D(+)-glucose at different concentrations.

Figure 3.8: Average frequency shift of the two blank QCMs against glucose concentration. The black line represents the frequency shift predicted by Kanazawa-Gordon.

The black line represents the frequency shift predicted by Kanazawa-Gordon.
of glucose (at 25 °C) were taken from literature [70]. Using the Kanazawa-Gordon equation, a prediction of the frequency shift caused by density and viscosity differences can be made. It is represented by the black line in figure 3.8.

Since there is a clear difference between the measured values and those predicted by Kanazawa and Gordon, the frequency changes cannot result from density and viscosity of the solution alone. Probably, the excess frequency drop is caused by adsorption of glucose on the gold surface. Similar results with glucose solutions on gold coated 10 MHz QCMs were reported in literature [71]. Interestingly, Kanazawa and Gordon tested their equation with glucose solutions as well. However, their experimental data were pretty well matching the prediction, implying that the frequency shifts were caused by density and viscosity changes alone. They did not report the exact type of quartzes they used, but apparently there was no adsorption of glucose on them [13].

![Figure 3.9: Mass sensitive measurement of two blank QCMs in solutions of PenVK at different concentrations.](image)

In another experiment, the response of blank QCMs to PenVK was tested. The measured curve is depicted in figure 3.9. The frequency drop was $26 \pm 5$ Hz for 5 mM PenVK and $128 \pm 16$ Hz for 50 mM PenVK. Thus, the frequency change of the blank QCMs is in the same order of magnitude as that of most NIP-coated ones. These results imply that the non-specific affinity of the tested polymer layers to PenVK is not bigger than that of the plain
Results and discussion

3.3 Molecularly imprinted polymers

3.3.1 VeroClear™-MIP

The commercial 3D printing ink VeroClear consists of 70% mostly not specified acrylic monomers and oligomers as well as a photo initiator. The rest of the components remains completely unknown. Nevertheless, it was tested in a MIP formulation, because there are numerous acrylic monomers which are suitable for molecular imprinting and the generally high cross-linking of a 3D printing ink is also favourable for MIPs. The use of PenVK as a template was prevented by its very poor solubility in VeroClear. However, free PenV was soluble with about 70 g L$^{-1}$. By dilution with EtOH, the height of inkjet printed and spin coated layers was effectively reduced to less than 500 nm. Like all tested formulations, VeroClear required an extraordinarily long curing time. For complete curing, the layers with several hundreds of nm thickness needed 4-5 min under the powerful Hg vapour lamp. The reason is probably a drastically increased oxygen inhibition compared to the layers printed by a 3D printer, that usually have a considerably higher thickness of about 20 µm. With decreasing layer height, the surface-to-volume ratio increases, leading to a higher exposure of the uncured film to air.

Since free PenV was hardly soluble in water, the respective mass sensing measurements had to take place in an organic solvent. Figure 3.10 shows the result of a measurement with PenV in EtOH. MIP and NIP were deposited via spin coating. The layer height of the MIP and the NIP was 1693 nm and 1430 nm, respectively (calculated via Sauerbrey equation).

Both MIP and NIP responded to PenV with a linear concentration dependency, but the difference is rather small and can only be seen at very high concentrations. The responses of MIP, NIP and the difference of MIP and NIP responses are plotted in figure 3.11. The polymer layer on these particular QCMs is relatively thick, because the quartzes had a rough surface, while the used spin-coating procedure was originally optimised for smooth QCMs. For this reason, the layer is maybe not rigid enough to vibrate properly in unity with the quartz crystal. However, there were no noticeable differences to other measurements with thinner layers.
3 Results and discussion

3.3 Molecularly imprinted polymers

Figure 3.10: Mass sensitive measurement of VeroClear/PenV-MIP. Different concentrations of PenV in EtOH.

Figure 3.11: Linear regression of the concentration dependent frequency change. VeroClear/PenV-MIP measured in solutions of PenV in EtOH.
Further measurements of the VeroClear-MIP in 5.5 mM PenV in EtOH were carried out at the impedance analysis setup as well as at the oscillator circuit. They all gave responses between 100 and 200 Hz and no significant difference of MIP and NIP. On a smooth QCM, a layer height of less than 500 nm was measured by the profilometer.

The VeroClear-MIP was as well tested in aqueous solutions of PenV potassium salt (PenVK). One of the resulting curves is depicted in figure 3.12. 5 mM PenVK induced a frequency shift of 10-20 Hz, 50 mM PenVK leads to 120-130 Hz frequency drop for both MIP and NIP. Probably, the response increases more or less linearly with concentration.

![Figure 3.12: Mass sensitive measurement of VeroClear/PenV-MIP in aqueous solutions. Different concentrations of PenVK.](image)

In conclusion, the VeroClear-MIP shows no response to PenVK in aqueous KCl and very little to PenV in EtOH, which indicates the presence of at least some imprints. The best results were achieved with EtOH as a porogenic solvent as well as in the measuring solutions. These results can be regarded as evidence for the presence of a solvent effect in molecular imprinting [30, 63]. Since the composition of VeroClear is for the most part unknown, the reasons for the low performance of these MIPs can only be guessed. At least, the appearance of the VeroClear-MIP layers was always very homogeneous and there was no visible precipitation of the template. Problems could possibly arise from the monomer/template ratio, the cross-
linking ratio, the monomers themselves or insufficient template removal during the washing step.

### 3.3.2 MIP with free penicillin V and methacrylic acid

The formulation with methacrylic acid (MAA) as a functional monomer and free PenV as the template was developed based on the work of Cederfur et al. that presented MIP particles with penicillin G sodium as template [62]. The proportions of the components were not changed, except for the photo initiator ABCN, of which the tenfold amount was used. Increasing the amount of photo initiator was the easiest way to reduce oxygen inhibition and ensure complete curing. Unfortunately, curing under inert atmosphere was not practicable, but the reaction mixture were purged with N₂ or Ar to get rid of dissolved oxygen. Generally, complete curing was only possible with the more powerful of the two identically constructed Hg vapour lamps, that emitted about 5000 W m⁻².

The reaction mixture was deposited on QCMs via spin coating. Prior to that, it was pre-polymerised for 30 s to increase the viscosity. For this purpose, the weaker of the two UV lamps was used, because the powerful one rapidly cured the whole mixture inside the PP vial. Although the pre-polymerisation time was the same for each prepared vial, the degree of polymerisation was probably not. There was no feasible way to determine it routinely, since IR spectra did not show any difference to the fresh mixture, even when the polymer already started to precipitate from the solution. Viscosity measurements were only feasible with unreasonably high sample wastage, and were therefore avoided.

The mass loss during extraction of two exemplary MIP coated QCMs was determined by mass sensing measurements at the impedance analysis circuit and use of the Sauerbrey equation. Both layers had a mass of approximately 100 µg before the extraction step. However, one MIP lost 48 µg, whereas the other one lost 14 µg. The amount of PenV in the 5 µL droplet that was applied for spin coating was about 25 µg. Considering the fact, that the applied volume is partially thrown away during spin coating, the mass loss of 48 µg can never arise from template extraction alone. Possibly, some uncured monomers were extracted as well.

As for the VeroClear-MIP, mass sensing measurements with the MAA/PenV formulation were as well carried out in the respective imprinting solvent, acetonitrile. Figures 3.13-3.15 show the results of one measurement. The curve was spread to three separate graphs for better readability. In figure 3.13 and figure 3.15, reproducibility was tested by multiple injections of 5.5 mM PenV. Figure 3.14 shows the response to different concentrations. Between
PenV injections, the cell was mostly flushed with ACN twice, hence the additional injection peaks in the curve. The small frequency drops before most injections arise from the increased hydrostatic pressure on the QCM, caused by opening the pinch cock. The layer thickness was calculated via the Sauerbrey equation. It was 611 nm for the MIP and 127 nm for the NIP. For this reason, the QCMs are actually not absolutely comparable (see page 51).

Figure 3.13: Mass sensitive measurement of MAA/PenV-MIP in solutions of PenV in ACN.

Among all formulations, the MAA/PenV-MIP showed the best selectivity towards PenV. Again, there seems to be a solvent effect, that enables higher selectivity when the MIP is tested in the imprinting solvent. It should not be a surprise, that the best performance of a MIP is obtained in ACN, as it was the only aprotic solvent in use. When it comes to molecular imprinting, aprotic and apolar solvents are highly favourable, as they support the electrostatic interactions between template and monomers [72]. However, the presence of a polar solvent, especially water, can disturb these interactions [73].
3 Results and discussion

3.3 Molecularly imprinted polymers

Figure 3.14: Mass sensitive measurement of MAA/PenV-MIP in solutions of PenV in ACN. Different concentrations of PenV.

Figure 3.15: Mass sensitive measurement of MAA/PenV-MIP in solutions of PenV in ACN.
In figure 3.16 the responses of MIP and NIP as well as the difference between MIP and NIP response are plotted against PenV concentration. The data points and error bars represent the average and standard deviation of the response, respectively. While the NIP response increases linearly with the concentration, the MIP exhibits a non-linear behaviour. The linear regression line for the MIP response is shown, to make the non-linear trend better visible. Its poor coefficient of determination underlines this observation. Looking at the difference curve, the non-linear response becomes even clearer, as the data points are far off the regression line and the coefficient of determination decreases to 0.92. The decreasing slope of the MIP curve at high concentrations matches the expectation of a saturation behaviour, as the MIP has a finite number of cavities and thus a limited capacity for selective PenV adsorption.

If all external effects like temperature, hydrostatic pressure and the pressure of the seal onto the QCM could be eliminated, the theoretical limit of detection would be predetermined only by the signal noise, which was usually around 1 Hz. Since the concentration dependency of the MIP response is clearly non-linear, it does not make any sense to calculate LOD and LOQ from the present data. Prior to that, the trend of the curve at lower concentrations has to be determined.

Figure 3.16: Linear regression of the concentration dependent frequency change. MAA/PenV-MIP measured in solutions of PenV in ACN.
The same MAA/PenV-MIP coated QCMs were additionally tested in aqueous solutions of PenVK. The resulting curve is depicted in figure 3.17. The response of the NIP was not remarkably higher than on a blank QCM, namely $23 \pm 2$ Hz for 5 mM PenVK and $127 \pm 3$ Hz for 50 mM PenVK. However, the MIP showed a slightly increased frequency drop of $35 \pm 6$ Hz for 5 mM PenVK and $180 \pm 10$ Hz for 50 mM PenVK. Thus, the difference of MIP and NIP was $11 \pm 4$ Hz for 5 mM PenVK and $52 \pm 7$ Hz for 50 mM PenVK, respectively. This leads to the conclusion, that the MAA/PenV-MIP, although imprinted with free PenV has a slight sensitivity towards PenVK.

The potassium salt of PenV differs from the free acid in having a negatively charged carboxylate group instead of the carboxylic acid. This, and maybe the presence of potassium ions as well, should technically lead to a slightly different molecular imprint. Perhaps, this difference contributes to the low response of the MIP in solutions of PenVK. However, it is doubtlessly to a certain extent caused by solvation of the PenV$^-$ ions in water. To examine the real difference between PenV and PenVK, it would be interesting, to measure both of them in the same solvent. Methanol or ethanol would be appropriate for this purpose. DMSO or DMF could be used as aprotic solvents, that potentially dissolve both species.

Figure 3.17: Mass sensitive measurement of MAA/PenV-MIP in aqueous solutions of PenVK. 5 mM PenVK was prepared in 45 mM KCl and 50 mM PenVK in pure water in order to achieve a constant concentration of potassium ions during the measurement.
Admittedly, the difference of MIP and NIP response of these particular QCMs could be owed to their different layer height. There was not enough time for further investigations on this issue, but one additional measurement was performed with the same MIP on different QCMs. Their layer heights were 2456 nm and 1751 nm for the MIP and the NIP, respectively. Tested in solutions of 5.5 mM PenV in ACN, the MIP responded with approximately 300 Hz and the NIP with about 200 Hz. Apart from the fact, that the layer heights are again not equal and way to high for a reliable conclusion, the difference between MIP and NIP is the same as in figure 3.16.

### 3.3.3 MIPs with penicillin V potassium and methacrylic acid

The MIP formulations with PenVK were based on a recipe of the group of P. Lieberzeit at the University of Vienna. Since AIBN was not available at the beginning, it was replaced by ABCN, an initiator with a slightly lower decomposition rate constant. Despite the carefully adjusted reaction parameters, neither thermal nor photo-curing was successful with ABCN. For the photopolymerisation, the failure is easy to explain, as the used solvent, dimethyl sulfoxide (DMSO), could of course not evaporate within the short photo-curing step.

Due to the thermal curing procedure and the use of the high-boiling solvent DMSO, this recipe was rather unsuitable for inkjet printing. Hence, a water-based, photo-curable MIP formulation was developed. The ratios of monomers and template were not changed, in comparison to the recipe from the University of Vienna. Whilst PenVK and MAA are perfectly water-soluble, a second, less polar solvent was needed to dissolve the cross-linker ethylene glycol dimethacrylate (EGDMA). 1-propanol was chosen because of its vapour pressure curve, which is very similar to that of water [69]. Among the tested photoinitiators, Omnirad 2959 is explicitly suitable for aqueous systems and Omnirad TPO-L is at least water-soluble, according to the manufacturer. Thus, in formulations with these initiators, 1-propanol was only needed for miscibility with EGDMA. Taking a higher ratio of 1-propanol enabled to use non-water-soluble initiators like Omnirad 819 or AIBN as well. As mentioned before, the reproducibility of the pre-polymerisation could not be determined by FTIR spectroscopy or viscosity measurements. However, the high polarity of the water/1-propanol mixture offered a new possibility. When the mixture was exposed to UV radiation, the oligomers always started precipitating from the solution after a certain time. The reaction continued for a couple of minutes after exposure and led to a further increase of turbidity. Consequently, the exposure time was adjusted to the highest possible value, at which the solution was still clear a
few minutes after irradiation. These values were reproducible with a deviation of less than 1 s.

In contrast to free PenV, which was soluble in the used monomers, PenVK usually precipitated during spin coating. Apparently, this caused a segregation of the water/1-propanol mixture on the gold surface. In formulations with a lower percentage of 1-propanol, water droplets formed, and as they evaporated, PenVK precipitated from them. As an example, figure 3.18 shows a smooth QCM, spin coated with the formulation no. 3 (according to table 2.4), in which an azeotropic mixture of water and 1-propanol was used as the solvent.

![Figure 3.18: Spots of precipitated PenVK on a spin coated smooth QCM. Aqueous MIP formulation no. 3 (56.8 % n-PrOH). Left: whole QCM, right: detail.](image)

In the recipes with 80 % 1-propanol, after spin coating, the gold surface was covered with lots of small droplets, most likely consisting of 1-propanol. PenVK crystallised in the space
between the droplets and formed a pattern like in figure 3.19. The pictures show a smooth QCM, spin coated with formulation no. 6 (according to table 2.4). The assumption, that the white precipitate was indeed PenVK, and not polymer, was confirmed by its water solubility. Due to the crystallisation, it is highly questionable, if there was enough PenVK left in the polymer matrix to build a sufficient amount of molecular imprints.

Mass sensitive measurements were carried out with two of the MAA/PenVK-MIPs, namely the water/1-propanol-based formulation no. 3 (see table 2.4) and the recipe with methanol as a solvent. The MAA/PenVK-MIP no. 3 did not show any notable difference in the behaviour of MIP and NIP, when tested in 5 mM PenVK. Figure 3.20 shows the corresponding measurement curve. However, the response seemed to decrease with each injection, which could indicate permanent binding of PenVK. The phenomenon could as well arise from a temperature difference of the used solutions. Possibly, the PenVK solution was about 2 °C colder than the KCl solution and was warmed to room temperature over time. The KCl solutions were stored at room temperature over weeks, whereas the PenVK solutions were always prepared freshly. Usually, they were diluted with the same KCl solution that was used for the measurements, but no special attention was paid to that. Temperature differences of the solutions could as well come from touching the injection syringe. The calculated thickness of the MIP and NIP layers was 192 nm and 159 nm, respectively.

![Figure 3.20: Mass sensitive measurement of MAA/PenVK-MIP in aqueous solutions of PenVK. The PenVK solutions were prepared in 50 mM KCl.](image-url)
In an additional experiment, the water/1-propanol mixture was replaced by a single solvent with intermediate polarity, which was methanol. However, with respect to a future use as an inkjet ink, methanol is less favourable, due to its toxicity and its relatively low boiling point.

The methanol-based MAA/PenVK-MIP was as well tested in aqueous solutions of PenVK (see figure 3.21). Both MIP and NIP responded to 5 mM PenVK with about 20 Hz frequency drop. In 50 mM PenVK, the NIP showed a higher response than the MIP. This difference cannot be caused by specific adsorption. Maybe the unspecific adsorption was different on the two QCMs, but probably the effect was caused by an external effect, like unequal pressure on the two QCMs. The calculated layer height was 81 nm for both the MIP and the NIP.

![Figure 3.21: Mass sensitive measurement of MAA/PenVK-MIP in aqueous solutions of PenVK.](image)

In conclusion, imprinting with PenVK did not yield any positive results. Since PenVK was poorly soluble in the used monomer mixture, it started precipitating on the QCM surface as soon as the solvent had evaporated (see figures 3.18 and 3.19). Perhaps, a small amount of PenVK was still dissolved in the monomers, but probably the crystallisation prevented the formation of an sufficient number of cavities.
3.3.4 Miscellaneous MIPs

Several experiments were carried out with MIPs containing 4-vinylpyridine as the functional monomer. Concerning oxygen inhibition and pre-polymerisation, the observations were similar to those with the MAA/PenV-MIP (section 3.3.2). The 4-VPy-MIPs were also investigated in mass sensitive measurements, but at that time, there were still various unsolved issues with the measuring setup. Thus, the measured data does not allow a reliable interpretation of the results.

3.4 Inkjet compatibility of MIP formulations

The VeroClear-MIP was successfully inkjet printed on several QCMs, using a Dimatix DMP-2800 printer. Beyond that, especially the water-based MIP formulations were optimised with a focus on inkjet printability. The major demands on an inkjet ink are its viscosity and surface tension that should be within a certain range. Moreover, all ingredients must be compatible with the cartridge and printhead materials. The solvent should be volatile enough to evaporate after printing. However, a too volatile solvent is undesirable as well, as it could volatilise directly from the nozzle. For the used Dimatix printer, the requirements for printable fluids are given in a data sheet, which can be downloaded on the manufacturer’s website [74]. This printer was specifically designed for feasibility testing of new printing fluids and serves as a research model of an industrial inkjet printer.

The MAA/PenVK-MIP no. 3 (according to table 2.4) was prepared with an azeotropic composition of water and 1-propanol with a boiling point of 88 °C [65]. Thus, the boiling point is around 10 °C lower than that of the pure solvents, which is advantageous for solvent evaporation. Another advantage concerning inkjet printing is, that this composition is very close to the viscosity maximum of a water/1-propanol mixture (approx. 50 wt%, 2.65 mPa s at 25 °C) [75]. The Dimatix printer requires a minimum viscosity of 2 mPa s, but its working optimum is at 10-12 mPa s. For a better printing result, the viscosity of the MIP could be increased by pre-polymerisation. If it is too viscous, an increased working temperature could put things right. Regarding surface tension, the solvent mixture alone has, according to literature, about 25 mN m$^{-1}$ [65]. Thus, it is only slightly off the required 28-33 mN m$^{-1}$. Of course, the surface tension can be strongly affected by addition of monomers, initiator and the template PenVK. With respect to material compatibility, water and 1-propanol are perfectly suitable for the Dimatix printer. In conclusion, the water-based MIP formulation no. 3 is most likely inkjet-printable.
As described in section 3.3.2, the most promising MIP was prepared with free PenV using the aprotic solvent ACN. As ACN could attack the printhead materials, it is clearly not the first choice for inkjet printing. Luckily, free PenV is, in contrast to the highly polar potassium salt, well soluble in various organic solvents. Therefore, it shall be possible to find a solvent or solvent mixture that dissolves all components and fulfills the demands of the printer at the same time. Printing without a solvent is probably not feasible, as the printed layers would be too thick.

3.5 Oxidation and polymerisation of 4-vinylpyridine

All MIP formulations containing PenV and 4-VPy developed a bright red colour after some hours. After 2 months, they contained some slimy red precipitate (see figure 3.22 (left)). NIPs without PenV were slightly reddish after 2 months. The used 4-VPy contained 100 ppm of hydroquinone as a stabiliser. Heating freshly distilled 4-VPy to 100 °C for 4 h led to a blood-red colour, as pictured in figure 3.22 (centre). Since the stabiliser hydroquinone should have remained in the sump, it is definitely not responsible for the red colour. When 100 µL of 4-VPy were mixed with 23 µL of formic acid, the mixture turned red and became highly viscous after a few seconds. Mixing 100 µL of 4-VPy with 10 µL of HCl (conc.) led to heating and immediate polymerisation, but only a slight red colour. After complete polymerisation, the colour does not change anymore. Glass slides with polymerised MIP layers, prepared with undistilled 4-VPy, did not turn red for at least 4 months. The right picture in figure 3.22 shows a mixture of PenV and 4-VPy that was completely hardened after 2 months at room atmosphere. FTIR spectra of oxidised 4-VPy did not show any difference to those of colourless 4-VPy.

Figure 3.22: Oxidation and polymerisation of 4-VPy. Left: MIP with PenV and 4-VPy, Centre: thermally oxidised 4-VPy, Right: polymerised PenV/4-VPy mixture.
3 Results and discussion 3.6 Degradation of penicillin V

The red coloured product most likely originates from the oxidation of 4-vinylpyridine by air, that has been previously reported in literature [76]. The oxidation is obviously accelerated by heating as well as by the presence of an acid. PenV possesses a carboxylic acid group with a pKₐ of 2.74 [58]. Acids also speed up the polymerisation of 4-VPy. Since no further efforts were put into the separation of the oxidation product(s), the exact structure of the red-coloured substance(s) remain(s) unknown. Since there was almost no difference in the FTIR spectra, the assumption was made that the majority of the red substance was still 4-VPy and there should be no major effect on imprinting behaviour.

3.6 Degradation of penicillin V

FTIR investigations revealed, that PenV was not stable in solutions containing 4-vinylpyridine. For a better understanding of the involved reactions, the different carbonyl bands in the FTIR spectra were assigned to the corresponding functional groups with the aid of literature [77]. Figure 3.23 shows the carbonyl region of an FTIR spectrum of penicillin V dissolved in ACN (42 g L⁻¹). ACN hardly shows any absorption in that region, thus all visible bands must belong to PenV.

Figure 3.23: FTIR spectrum of penicillin V in ACN (42 g L⁻¹).

An FTIR spectrum of free PenV could not be found, but in figure 3.24 a reference spectrum of PenV potassium salt (recorded in a KBr wafer) is depicted [78]. Certainly, the two spectra are not fully comparable, since they were recorded under very different conditions. The
different aggregate state, the presence of potassium ions or ACN as well as the differences between a carboxylic acid and a carboxylate group should explain, that not every band occurs at the exact same position in both spectra.

Figure 3.24: FTIR spectrum of penicillin V potassium (KBr wafer) [78].

In table 3.1, a list of the relevant bands is given. Due to the high strain in a four-membered ring, the C=O band of the \( \beta \)-lactam (1) is shifted to a much higher wave number compared with a regular amide. Thus, it appears at the very left end of the carbonyl region. The second band at 1751 cm\(^{-1}\) most likely belongs to the carboxylic acid group (2a) of PenV. It is clearly visible in the spectrum of free PenV but also to a certain extent in the potassium salt. The third band probably comes from the amide (3). In the deprotonated carboxylic acid (2b), conjugation of the negative charge lengthens the C=O bond and it appears at the right end of the region.

<table>
<thead>
<tr>
<th>No.</th>
<th>Functional group</th>
<th>( \tilde{\nu} ) (PenV) / cm(^{-1})</th>
<th>( \tilde{\nu} ) (PenVK) / cm(^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>( \beta )-Lactam</td>
<td>1788</td>
<td>1770</td>
</tr>
<tr>
<td>2a</td>
<td>Carboxylic acid (attached to thiazolidine ring)</td>
<td>1751</td>
<td>1751</td>
</tr>
<tr>
<td>3</td>
<td>Amide</td>
<td>1697</td>
<td>1676</td>
</tr>
<tr>
<td>4</td>
<td>Carboxylate (penicilloic acid)</td>
<td>1632</td>
<td>1630</td>
</tr>
<tr>
<td>2b</td>
<td>Carboxylate (attached to thiazolidine ring)</td>
<td>1601</td>
<td>1608</td>
</tr>
</tbody>
</table>
3 Results and discussion

3.6 Degradation of penicillin V

The small band at 1632 cm$^{-1}$ in all probability does not belong to PenV, but to its common degradation product phenoxymethylpenicilloic acid, that features a second carboxylic acid (or carboxylate) group (4). A spectrum of the very similar benzylpenicilloic acid is shown in figure 3.25. It differs from phenoxymethylpenicilloic acid only in having a benzyl side group instead of a phenoxyethyl side group. The strong carboxylate band at 1632 cm$^{-1}$ suggests, that the compound to a certain extent exists as a zwitterion (see figure 3.26). The band at 1717 cm$^{-1}$ most likely belongs to one (or both) of the carboxylic acids in their protonated form and at 1668 cm$^{-1}$ the amide is visible.

![Figure 3.25: FTIR spectrum of benzylpenicilloic acid (degradation product of penicillin G, KBr wafer) [79].](image)

The degradation of PenV in presence of 4-VPy was studied at room temperature as well as at 100 °C. In accordance with literature and FTIR measurements, the reactions shown in figure 3.26 were suggested to take place [60]. Phenoxymethylpenicillin (PenV) is hydrolysed to phenoxymethylpenicilloic acid. When heated, the latter is decarboxylated to phenoxymethylpenilloic acid.
Figure 3.26: Suggested reactions during PenV degradation. Under alkaline conditions, the amine group of penicilloic acid is probably not protonated. The numbers correspond with table 3.1.

The basic and nucleophilic properties of 4-VPy clearly accelerate the opening of the β-lactam ring. Small amounts of water, which are omnipresent under normal atmospheric conditions, should suffice to transform the lactam into a carboxylate. This reaction was monitored via FTIR (see figure 3.27). The most interesting features in the spectrum are the vanishing β-lactam band at 1780 cm\(^{-1}\) and the growing penicilloic acid band at 1641 cm\(^{-1}\). Since the pH is still rather alkaline, both carboxylic acids are largely deprotonated and only appear as carboxylates. However, the carboxylate attached to the thiazolidine ring (2b) overlays with the very strong band of the C=C bond of 4-VPy at 1595 cm\(^{-1}\). Apart from that, the spectrum of 4-VPy does not interfere with the carbonyl region of PenV. Besides, the amide band (1697 cm\(^{-1}\)) seems to shift to lower wave numbers over time.
In another experiment, the follow-up reaction was tested by heating the yet aged PenV/4-VPy mixture for several hours. In figure 3.28, the decrease of the β-lactam band (1780 cm\(^{-1}\)) as well reoccurs as the shift of the amide (1697 cm\(^{-1}\)) and the newly arising carboxylate (1649 cm\(^{-1}\)). When the mixture is heated to 100 °C, the new carboxylate band at first continues growing, but after a few hours, it gets smaller again. This could give at least some evidence for an actual decarboxylation of phenoxymethylpenicilloic acid to phenoxymethylpenilloic acid. Since the concentration of PenV was twice as high than in the previous experiment, another band appears at 1724 cm\(^{-1}\) which could possibly be the carboxylic acid band of phenoxymethylpenicilloic acid.

Further degradation studies were carried out with PenV in ACN and EtOH as well as with PenVK in water. After 5 days, the β-lactam band had become weaker in all experiments, but to a lesser extent than at the presence of 4-VPy. In ACN, an arising penicilloic acid band (4) was spotted as well, whereas in EtOH and water the band was covered by the very broad amide band (3). The degradation of PenV happens on a time scale of hours to days. Since the whole imprinting process, from mixing the ingredients to photopolymerisation, is usually done within 15 min, it should technically not be affected by PenV degradation.
3 Results and discussion

3.7 Summary and outlook

Among the tested MIP formulations, only those with free penicillin V as a template, MAA and EGDMA as monomers and ACN as a solvent showed selectivity towards PenV (see section 3.3.2). However, a sufficient response was only obtained with measurement solutions of PenV in ACN. In aqueous solutions, the response was considerably lower. Obviously, there is a solvent effect in the molecular imprinting process. Probably, the solvatisation of PenV\(^{-}\) ions in water prevents their recognition by the cavities, that were originally built in aprotic ACN. In order to validate these results, further experiments have to be carried out, especially focussing on the relation between MIP layer thickness and sensor response.

Experiments with the commercial acrylate based ink VeroClear did not yield a really selective MIP. Mass sensitive measurements of the MIPs with 4-VPy as the functional monomer did not produce any reliable results, due to diverse problems with the measuring setup. The imprint of MAA/EGDMA with PenV potassium was probably prohibited by the precipitation of the template during spin coating.

The sensitivity of the MIPs could possibly be improved by trying new monomers and adjusting the ratios of monomers and template. However, there are more promising approaches than crude bulk imprinting. A change to MIP particles could as well achieve improvement as the implementation of controlled radical polymerisation techniques, like ATRP or RAFT polymerisation.

Figure 3.28: FTIR spectra of a solution of 420 g L\(^{-1}\) PenV in 4-VPy. Thermal degradation.
With respect to inkjet printing, ACN should be replaced by a less aggressive aprotic solvent. Moreover, the viscosity and surface tension of the MIP-ink should fulfill the requirements of the used printer. For an example, see section 3.4. MIP particles could be inkjet printed as a suspension in a supporting ink.

Concerning the impedance analysis setup, some severe problems could already be solved, so that, with some effort, reliable results can be obtained (see section 3.2). Firstly, the signal noise was reduced to an acceptable value. Further improvement of noise could possibly be achieved by electromagnetic shielding of the setup. Secondly, the electrolysis of gold electrodes was effectively banned. Additional work is necessary with respect to the pressure and temperature sensibility of the setup. The former has already been improved by indirect injection of the measuring fluids via a flexible tube. To ensure a constant pressure of the cell top onto the QCM, the entire closing mechanism maybe needs to be rethought. Possibly, a continuous flow through the cell could as well contribute to eliminate undesired force effects during the injection process. The temperature sensitivity could probably be handled by tempering the measurement solutions or, if required, the whole setup.
## List of Figures

1.1 Schematic drawing of a chemical sensor, consisting of receptor and transducer. 7
1.2 Visualisation of the piezoelectric effect. 9
1.3 Schematic drawing of a thickness shear mode resonator. 9
1.4 Equivalent circuit (left) and wiring symbol (right) for an oscillating quartz crystal. 10
1.5 Typical spectrum of absolute value $|Z|$ and phase $\theta$ of the complex quartz impedance against oscillation frequency $f$ (adapted from [8]). 11
1.6 Temperature dependence of resonant frequency of AT-cut quartz for three different cutting angles (adapted from [11]). 12
1.7 Design of the used electrodes (left: front side, right: back side). 13
1.8 Schematic representation of the general molecular imprinting process (adapted from [18, 19]). 15
1.9 Principle of drop-on-demand inkjet. 18
1.10 Chemical structure of phenoxyethylpenicillin, better known as penicillin V. 19
1.11 Degradation pathways of penicillins in general. 20

2.1 Measurement cell built by the group of Lieberzeit. 24
2.2 Measurement cell for 2 QCMs, built by Profactor GmbH. 25
2.3 Structures of the used functional monomers and cross-linkers. 26
2.4 Structures of all used thermal and photo initiators. 27

3.1 Roadmap for the development of an inkjet printable MIP. 33
3.2 Absolute value $|Z|$ and phase angle $\phi$ of the complex impedance spectrum of an uncoated QCM (unscaled raw data). 36
3.3 Detail of the impedance spectrum of two simultaneously measured QCMs (unscaled raw data). 36
3.4 Microscope pictures of dissolved gold electrodes. 37
3.5 Mass sensitive measurement of gold electrolysis. 38
3.6 Installation for force-free injection of measuring solutions. 40
3.7 Mass sensitive measurement of two blank QCMs in solutions of D(+)-glucose at different concentrations. ............................................. 41
3.8 Average frequency shift of the two blank QCMs against glucose concentration. ............................................ 41
3.9 Mass sensitive measurement of two blank QCMs in solutions of PenVK at different concentrations. ............................................. 42
3.10 Mass sensitive measurement of VeroClear/PenV-MIP. ................................................. 44
3.11 Linear regression of the concentration dependent frequency change. ............... 44
3.12 Mass sensitive measurement of VeroClear/PenV-MIP in aqueous solutions. ....... 45
3.13 Mass sensitive measurement of MAA/PenV-MIP in solutions of PenV in ACN. .... 47
3.14 Mass sensitive measurement of MAA/PenV-MIP in solutions of PenV in ACN. .... 48
3.15 Mass sensitive measurement of MAA/PenV-MIP in solutions of PenV in ACN. .... 48
3.16 Linear regression of the concentration dependent frequency change. ............... 49
3.17 Mass sensitive measurement of MAA/PenV-MIP in aqueous solutions of PenVK. .. 50
3.18 Spots of precipitated PenVK on a spin coated smooth QCM. ............................... 52
3.19 Areas of precipitated PenVK on a spin coated smooth QCM. ............................... 52
3.20 Mass sensitive measurement of MAA/PenVK-MIP in aqueous solutions of PenVK. .. 53
3.21 Mass sensitive measurement of MAA/PenVK-MIP in aqueous solutions of PenVK. .. 54
3.22 Oxidation and polymerisation of 4-VPy. ................................................................. 56
3.23 FTIR spectrum of penicillin V in ACN (42 g L$^{-1}$). ............................................. 57
3.24 FTIR spectrum of penicillin V potassium (KBr wafer) [78]. ............................... 58
3.25 FTIR spectrum of benzylpenicilloic acid (degradation product of penicillin G, KBr wafer) [79]. ............................................. 59
3.26 Suggested reactions during PenV degradation. ......................................................... 60
3.27 FTIR spectra of a solution of 208 g L$^{-1}$ PenV in 4-VPy. ........................................ 61
3.28 FTIR spectra of a solution of 420 g L$^{-1}$ PenV in 4-VPy. ........................................ 62
# List of Tables

2.1 Chemicals used. .................................................. 21
2.2 Devices used. .................................................... 22
2.3 Ratios of template to functional monomer to cross-linker for all prepared MIP formulations. ........................................ 27
2.4 Formulations of water-based polymers with MAA. .................... 30
3.1 Wave numbers of different C=O bands. ............................ 58
Literature


Literature


Literature


Literature


Eidesstattliche Erklärung

Ich erkläre an Eides statt, dass ich die vorliegende Masterarbeit selbstständig und ohne fremde Hilfe verfasst, andere als die angegebenen Quellen und Hilfsmittel nicht benutzt bzw. die wörtlich oder sinngemäß entnommenen Stellen als solche kenntlich gemacht habe. Die vorliegende Masterarbeit ist mit dem elektronisch übermittelten Teildokument identisch.

Linz, am 4. Dezember 2019

............................................