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Olivia Gatterbauer BSc

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Frequently used acronyms

ACN	Acetonitrile
AIBN	Azobis(isobutyronitrile)
DMSO	Dimethyl sulfoxide
DVB	Divinylbenzene
EtOH	Ethanol
HOAc	Acetic acid
MAA	Methacrylic acid
MeOH	Methanol
MIP(s)	Molecularly imprinted polymer(s)
NIP(s)	Non-imprinted polymer(s)
NP(s)	Nanoparticle(s)
PenV	Penicillin V
PenG	Penicillin G
QCM	Quartz crystal microbalance
SEM	Scanning electron microscopy
SF	Selectivity factor
TEA	Triethylamine
TRIM	Trimethylolpropane trimethacrylate

List of symbols

Δf	Frequency change
f_0	Fundamental frequency of the quartz
Δm	Mass change
A	Area of the electrodes
ρ_Q	Density of the quartz
μ_Q	Shear modulus of the quartz
ρ_L	Density of the liquid
η_L	Viscosity of the liquid
s	Standard deviation (baseline)
k	Slope of the calibration curve

Contents

1. Introduction.....	8
2. Theoretical Background	10
2.1. β -lactam Antibiotics.....	10
2.2. Chemical sensors	11
2.2.1. Quartz crystal microbalances.....	12
2.2.2. Molecularly imprinted polymers	15
2.3. Precipitation polymerization for preparing molecularly imprinted nanoparticles	17
2.4. Scanning electron microscopy	21
3. Experiments.....	22
3.1. Chemicals, materials and instruments	22
3.2. Preparation of QCM sensors for penicillin V	23
3.2.1. Preparation of MIP and NIP NPs	23
3.2.2. Different template removal techniques	25
3.2.3. Preparation of QCM sensors.....	26
3.2.4. QCM measurements	27
3.3. Preparation of penicillin G imprinted particles	28
3.4. Preparation of amoxicillin imprinted particles.....	30
3.5. Sample preparation for the characterization of the particles with SEM	31
4. Results and Discussion	32
4.1. QCM sensors for penicillin V.....	32
4.1.1. Characterization of MIP and NIP nanoparticles and the influence of different parameters on particle size.....	32
4.1.2. Influence of different solvents and extraction times on template removal	41
4.1.3. QCM Sensor characteristics	45
4.2. Penicillin G imprinted particles.....	53
4.3. Amoxicillin imprinted particles.....	54
5. Conclusion and Outlook	56
6. References.....	58
7. Appendix.....	62
7.1. Abstract.....	62
7.2. Zusammenfassung	63

1. Introduction

Penicillin was the first antibiotic, which was found by Alexander Fleming, who observed that bacteria colonies on an agar plate would not grow near mold. Fleming named this antibacterial acting agent produced by mold penicillin. Today there is a wide range of antibiotics, that play a special role in pharmaceuticals because they act selectively against bacteria cells. Being able to control infections caused by bacteria has been a great success in medicine; today antibiotics are used in almost all medical disciplines. [1]

However, due to the excessive use of antibiotics in the livestock sector and the misuse in human medicine, microorganisms can develop antibiotic resistance. In livestock production, for example, antibiotics such as penicillin and tetracycline are given in large quantities in order to promote growth or to prevent infections rather than just treating existing infections. In agriculture, crops are treated with antibiotics against bacteria on their surface. Finally, the prescription of antibiotics against viral infections is an example for the misuse of antibiotics in human medicine. This inevitably leads to the selection of antibiotic-resistant microorganisms. Infections with resistant bacteria strains cannot be treated with common antibiotics and pose a risk to public health. [2, 3] As a result, 33,000 people die each year in the European Union from infections with antibiotic-resistant bacteria. [4]

To determine the amount of antibiotics in water samples, analytical methods such as ultra-high-performance liquid chromatography high-resolution mass spectrometry (UHPLC-HRMS) are used. [5] These methods require expensive devices as well as highly trained staff. For specific analytical problems however, chemical sensors offer a cost-effective alternative to expensive and time-consuming methods. [6]

The aim of this master thesis is therefore to develop a sensor, which is able to detect penicillin V in aqueous solutions. The sensor system used in this work is based on a mass-sensitive quartz crystal microbalance (QCM) in combination with a selective layer of molecularly imprinted polymer nanoparticles (MIP NPs). Non-imprinted polymer nanoparticles (NIP NPs) are used as a reference, respectively. The particles are produced using precipitation polymerization because it is a simple and surfactant-free method that gives a good yield of spherical particles. [28] The recipe for preparing imprinted polymer particles with penicillin V as template was already established in the working group in an ongoing PhD-project. Therefore, this thesis focuses on optimizing the particles and evaluating their performance in QCM measurements.

One goal of this work is to adjust the size of the MIP and NIP NPs to under 200 nm, which is required for the AQUANOSE project in order to introduce the particles into the sensor production process. Therefore, the influence of parameters such as monomer concentration and amount of initiator on the particle size is investigated, in order to find the desired parameters to obtain MIP and NIP NPs with diameters under 200 nm.

To obtain cavities in the MIP NPs, that can selectively rebind the analyte in the sensing process, the template must be successfully removed. The conditions for extracting the template from the MIP NPs are optimized by testing different solvent mixtures, extraction modes and extraction times. Successful removal is confirmed using QCM measurements.

Furthermore, QCM measurements are carried out with the prepared sensors to evaluate the sensor response in terms of sensitivity, selectivity, reversibility and reproducibility.

As a side project, experiments are performed in order to find a method to produce penicillin G and amoxicillin imprinted particles by precipitation polymerization. These could then be used for preparing sensors for penicillin G and amoxicillin, respectively.

2. Theoretical Background

2.1. β -lactam Antibiotics

“Antibiotics are secondary metabolites or semi-synthetic or synthetic derivatives that can inhibit and kill pathogenic microorganisms.” [24] Depending on their chemical structure they are classified into four main groups: β -lactam antibiotics, tetracycline antibiotics, macrolides and aminoglycoside antibiotics. The group of the β -lactam antibiotics is most commonly used to treat bacterial infections in human and animals. This group includes penicillins, cephalosporins and monobactams. [24] All these compounds share the same structural feature, a beta-lactam ring. [25] Figure 1 shows the general structure of beta-lactam antibiotics with the lactam ring highlighted in red.

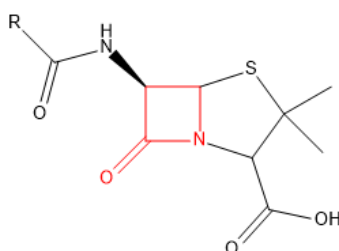


Figure 1: Chemical structure of β -lactam antibiotics. The lactam ring structure is highlighted in red. Similar to [25]

β -lactam antibiotics target the cell wall of bacteria by inhibiting the penicillin-binding protein (PBP), which is an essential enzyme needed for the biosynthesis of peptidoglycan for the cell wall of bacteria and therefore leads to lysis and death of the cells. [25]

Worldwide, 100,000-200,000 tons of antibiotics are used every year. The extensive use of antibiotics in human medicine, livestock and aquaculture caused bacteria to develop resistance against antibiotics. [26] The main defense mechanism against β -lactam antibiotics is the production of beta-lactamase. This enzyme is able to deactivate β -lactam antibiotics by hydrolyzing the lactam ring. [25] Other defense mechanisms include the mutation of PBP, so that β -lactam antibiotics are no longer affine to this enzyme, and the overexpression of efflux pumps, so that β -lactam antibiotics do not reach their target. [24]

In this work, the penicillins amoxicillin, penicillin V and penicillin G are used to generate molecularly imprinted polymer nanoparticles as recognition element for chemical sensors. Their chemical structure can be seen in Figure 2.

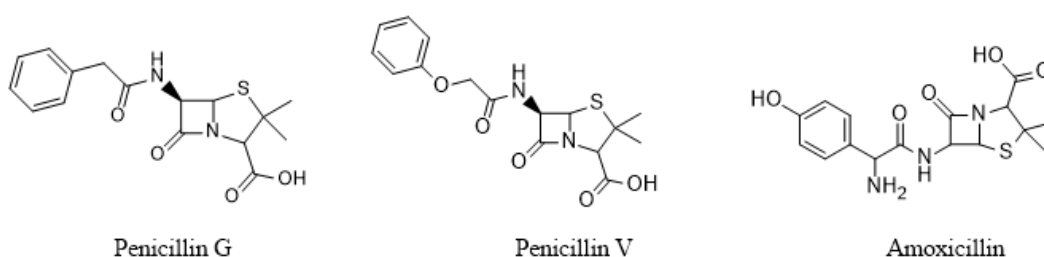


Figure 2: From left to right: Chemical structures of penicillin G, penicillin V and amoxicillin.

2.2. Chemical sensors

The International Union of Pure and Applied Chemistry (IUPAC) defines a chemical sensor as “a device that transforms chemical information, ranging from the concentration of a specific sample component to total composition analysis, into an analytically useful signal”. [8]

As shown in Figure 3, the setup of a chemical sensor essentially consists of a receptor, a transducer, the measuring electronics and the data processing unit. [9]

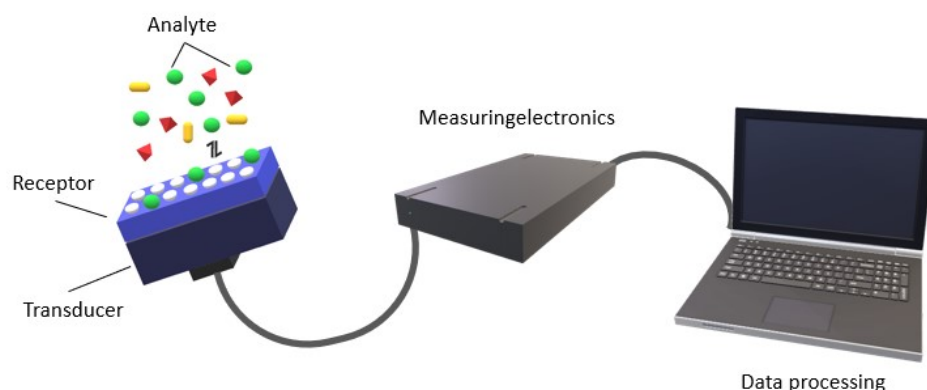


Figure 3: Schematic structure of a chemical sensor.

The receptor is an element that is supposed to interact with the analyte in a sensitive and selective manner. This interaction causes a change in chemical or physical properties (e.g. mass, temperature or conductivity), which can be transformed into a measurable quantity (preferably of electronic nature) by the transducer. [6, 9]

In case of the receptor, one can distinguish between biological and synthetic recognition elements. Biological receptors include catalytic receptors, such as enzymes, as well as affinity receptors, including antibodies, oligonucleotides and aptamers. Whereas synthetic receptors are for example macrocycles and molecularly imprinted polymers (MIPs). [9]

While biological receptors are superior to synthetic recognition materials in terms of selectivity, they lack stability and durability when exposed to certain conditions. The outstanding property of synthetic receptors, however, is their robustness. Combining the advantages of the two methods by mimicking biological binding sites with synthetic materials is therefore an interesting approach that is used for molecularly imprinted polymers (MIPs). [11] Since MIPs are used as receptors in this work, they are explained more detailed in chapter 2.2.2.

Depending on the working principle, transducers can be categorized into five different groups: electrochemical transducers, optical transducers, thermal transducers, mass sensitive transducers and those with other transduction mechanisms. [9] This work uses the quartz crystal microbalance, which is a mass sensitive transducer. Its working principle and function are described in detail in chapter 2.2.1.

Chemical sensor systems are used in a wide range of applications because there is a need for low-cost, miniaturizable and simple alternatives to expensive analytical instruments. In environmental applications for example, chemical sensors are used to measure air and water pollution. However, they are also used in healthcare and in defense applications for sensing explosives, toxic gases or pathogenic microorganisms. [6]

2.2.1. Quartz crystal microbalances

The working principle of most mass sensitive sensors relies on the piezoelectric effect or the inverse piezoelectric effect, which was first investigated by the Curie brothers in 1880. [6]

In principle, the direct piezoelectric effect means that subjecting a piezoelectric material to mechanical stress leads to an electric polarization on the surface. Figure 4 (A) shows the formation of surface charges in case of crystalline quartz (SiO_2). On the left side one can see an unloaded quartz crystal between two metal electrodes. The blue circles represent the positively charged silicon ions and the green circles stand for the negatively charged oxygen ions. How the application of mechanical stress (black arrows) leads to the deformation of the crystal structure is shown in the middle and on the right side of the figure. Subsequently, electric charges are formed on the crystal surface, which induce the opposite charge in the electrodes.

Vice versa applying electric voltage to the metal electrodes leads to mechanical deformation. This is known as the inverse piezoelectric effect. When an alternating electric field is applied, the quartz starts to oscillate (see part B of Figure 4). [6]

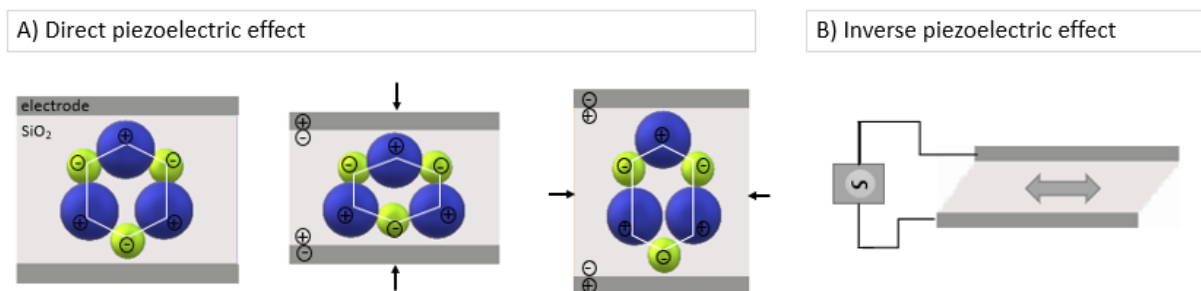


Figure 4: A) Schematic representation of the formation of surface charges in a quartz crystal after applying mechanical stress (direct piezoelectric effect). B) Oscillation of a piezoelectric material is induced by applying an alternating electric field because of the inverse piezoelectric effect. Similar to [6]

The induced acoustic wave propagates through the material. Depending on how this wave expands through the material, one can distinguish between surface acoustic wave resonators (SAW) and bulk acoustic wave resonators (BAW). The QCM is considered a thickness shear mode resonator, which is a type of BAW resonator. [6]

If the thickness of the quartz is a multiple of half the wavelength of the acoustic wave, the damping of the amplitude is minimal. This frequency is the resonant frequency of the quartz. The thinner the quartz disk, the higher is the resonant frequency and the higher is mass sensitivity. [12]

Günter Sauerbrey found that the frequency of an oscillating quartz plate changes when the oscillating mass is changed. [13] With formula (1), the Sauerbrey equation, he showed how the mass change is correlated to the change in frequency. [6]

$$\Delta f = - \frac{2 * f_0^2 * \Delta m}{A * \sqrt{\rho_Q * \mu_Q}} \quad (1)$$

Δf	<i>frequency change</i>
f_0	<i>fundamental frequency of the quartz</i>
Δm	<i>mass change</i>
A	<i>area of the electrodes</i>
ρ_Q	<i>density of the quartz</i>
μ_Q	<i>shear modulus of the quartz</i>

However, this equation is only valid for rigid layers. When a quartz is operated in a liquid medium, one has to use equation (2), which was derived by Kanazawa and Gordon in 1985. [14]

$$\Delta f = - f_0^{\frac{3}{2}} * \sqrt{\frac{\eta_L * \rho_L}{\pi * \rho_Q * \mu_Q}} \quad (2)$$

ρ_L	<i>density of the liquid</i>
η_L	<i>viscosity of the liquid</i>

When working in liquid samples this adaption of the Sauerbrey equation is needed because the movement of the liquid near the quartz surface has an influence on the oscillation frequency. Therefore, the density and viscosity of the liquid also have to be considered. [12]

The following description gives a general explanation of what information can be derived from QCM measurements.

Usually, one electrode on the QCM is coated with MIP NPs and one with NIP NPs as a reference. During QCM measurements, the frequency of these two channels is recorded. As example, Figure 5 schematically shows what happens during a QCM measurement on the MIP side of the quartz: The measurement starts with distilled water in the measuring cell until the baseline is stable. When a solution containing the analyte is injected one can observe a frequency drop on the MIP channel. As already described, the oscillation frequency of the QCM drops because binding of the analyte leads to an increase in the oscillating mass.

When the analyte is sufficiently removed from the recognition element by injecting distilled water, the frequency is expected to return to the baseline again.

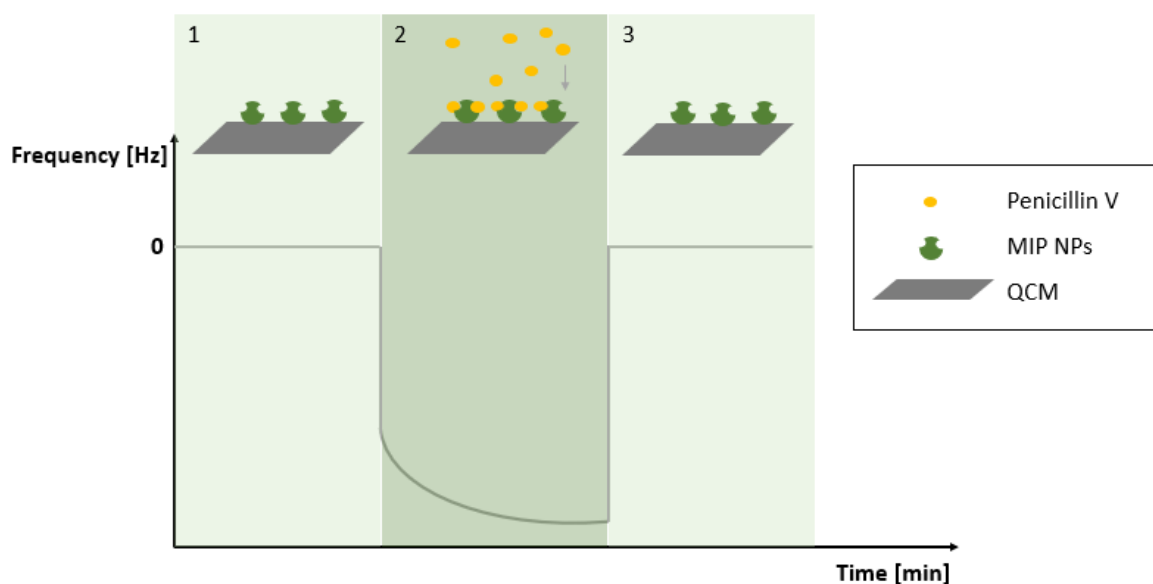


Figure 5: Schematic representation of a QCM measurement with MIP NPs on the electrode.

The sensor response of the NIP is considered a measure for non-specific binding. Since the NIP is prepared at the same conditions as the MIP except for the absence of the template, the functional groups from the monomer are arranged randomly and can bind, to some extent, the template in a weaker and less selective way. In the following, the difference between MIP and NIP can be calculated as a measure of the specific binding. [39]

In this case, another effect contributes to the measured unspecific signal. The QCM measurement starts with distilled water in the measuring cell until the signal is stable. When the sample is injected, the conductivity of the solution in the measuring cell is changed because PenV potassium salt is used to prepare the measuring solutions. When a QCM is operated in liquids, the conductivity of the liquid has an influence on the resonant frequency. [42]

2.2.2. Molecularly imprinted polymers

Molecularly imprinted polymers combine the high affinity and selectivity towards a target of biological receptors with the outstanding robustness of highly crosslinked polymers. Further advantages are the low costs in production, the straight-forward synthesis and reusability of these materials. [20]

Preparing specific binding sites for a template in a polymer matrix in principle involves three steps, as depicted in Figure 6. First, the functional monomers arrange themselves around the template molecule. The interactions that are formed between the functional monomers and the template can be either covalent or non-covalent. In the second step, these prearranged complexes are polymerized with a crosslinker to form a rigid polymer network around them. The final step is the extraction of the template from the polymer network in order to obtain three-dimensional cavities that are complementary to the template molecule in terms of shape, size and chemical functionality. These cavities can then be used to selectively rebind the template molecule.

[10, 11]

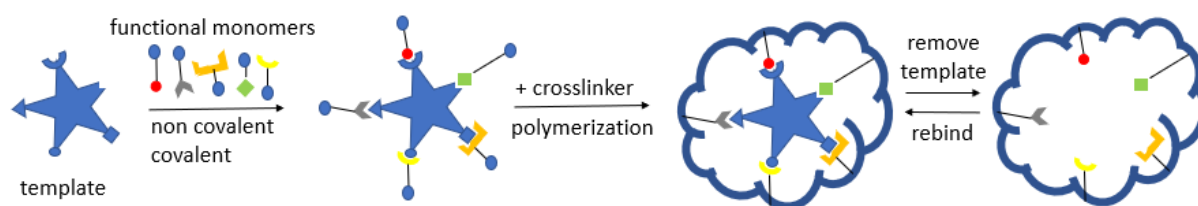


Figure 6: Schematic illustration of the processes during molecular imprinting. Adapted from [10]

Depending on the interactions between the template and the functional monomers during imprinting and rebinding one distinguishes between covalent-, noncovalent-, and semi-covalent imprinting. [10]

Since the template is covalently bound to the functional monomer during imprinting, the advantage of covalent imprinting is that the functional monomers are only found in the cavities after the template has been removed. However, the use of covalent imprinting is limited to reversible condensation reactions for example the formation of Schiff bases between amines and carbonyls or the formation of ketals between diols and ketones. In addition, the binding and dissociation take a long time. [10, 21]

Non-covalent imprinting is most commonly used in molecular imprinting and involves interactions such as hydrogen bonding, coulombic interactions, hydrophobic and Van der Waals forces, ion-pairing or π - π -stacking interactions. Binding and removing the template is much faster than in covalent imprinting. [10]

In semi-covalent imprinting, the imprinting step is covalent, but the rebinding of the template is non-covalent. This combines the advantages of the two previously mentioned methods. The

functional groups are only present in the binding site and the rebinding is only restricted by diffusion. [21]

There are two main imprinting techniques, bulk imprinting and surface imprinting. In bulk imprinting all components (template, functional monomer, crosslinking monomer and initiator) are dissolved in the solvent and the polymerization is initiated. However, this approach only works for small molecules that can diffuse into the polymer network. For macromolecules, bacteria or viruses surface imprinting is more suitable. [22]

MIPs can be prepared in different formats. On the one hand, MIPs can be prepared as thin films directly on the sensor. Thin films can either be coated onto a flat sensor surface or they are obtained by electropolymerization on a conducting surface. [23] On the other hand, one can prepare particles or beads, which are then fixed on the sensor surface. Particles can either be obtained by crushing a polymer monolith and sieving, which produces particles of irregular shape, or the beads are prepared directly, resulting in a more regular and spherical product. [22]

Applications for MIPs are, for example, solid-phase extractions or as packing materials for chromatography columns. [11] They are also used as recognition elements in chemical sensors, since not only small molecules with low molecular weight [15], but also macromolecular species, like proteins [16], or even microorganisms, like yeast [17], bacteria [18] and viruses [19] can be imprinted. Furthermore, MIPs are suitable for being coupled to a piezoelectric transducer, like a QCM, because a binding event at the MIP results in an increased mass on the oscillating quartz, which leads to a decrease in frequency. [20]

2.3. Precipitation polymerization for preparing molecularly imprinted nanoparticles

Nanostructured recognition materials have some outstanding properties, which is why there is increasing interest and research on this topic. MIPs within a nanometer size range have a high surface-to-volume-ratio, which means that per gram polymer they have a much higher total surface area than polymer thin films. This is especially of interest for sensor applications as it increases sensitivity. [27]

Besides that, the binding sites for the analyte are much better accessible in nanomaterials. Consequently, the template can be removed more efficiently from the polymer network, so that less template stays within the bulk. This is advantageous if MIPs are used as column materials because an efficient removal of the template prevents leaching. In addition, the rebinding of the target is faster, resulting in better binding kinetics and lower mass transfer resistance. [27, 29]

In the following, some applications are described, in which molecularly imprinted nanoparticles are used.

Traditional applications for MIP NPs are chromatography and electrophoresis, as well as chemical sensors. [31]

New applications also include:

- Drug delivery:

In drug delivery, NPs containing drugs are used to bring the pharmaceuticals to specific areas in the body and release the drug there. For example, the side effects of anticancer drugs can be minimized if the drug is only released in cancer tissue. [31] Madadian-Bozorg et al. prepared poly-(methacrylic acid)-based molecularly imprinted polymer NP around 65 nm, containing 5-fluorouracil, a drug against colon cancer. [32]

- Biomimetic Antibodies:

Research is done on MIP particles that can replace antibodies in the immune reaction. [31]

- Cell culture:

Studies showed that some cells in cell culture grow better on molecularly imprinted polymers. [31]

Preparation of MIP NPs:

MIP NPs can be prepared using a top-down approach, in which a bulk imprinted polymer monolith is crushed and grinded. However, this method leads to very irregular particles. To obtain particles of specific size they have to be sieved, which drastically reduces the yield. [27] Since monodisperse, spherical particles are desired for most of their applications, various methods have been developed to prepare MIP NPs of defined size and shape in good yield in a bottom-up approach. [28] Commonly used methods include precipitation polymerization, emulsion polymerization, interfacial polymerization and the preparation of core-shell particles, etc. [29] Their advantages and limitations have been reported in detail in several reviews on this topic [28, 29], as well as examples for their successful application [27, 30]. In this work, precipitation polymerization was used to prepare MIP NPs and is therefore explained in detail in the following section.

Precipitation polymerization:

The principle of precipitation polymerization is straightforward: All components (template, functional monomer, crosslinking monomer and initiator) are dissolved in an excess of solvent. Monomer and crosslinker typically have concentrations of <5 % w/v relative to the solvent. [28] When the polymerization is initiated, oligomers are formed and grow as they catch more monomers from the solution. When a critical molecular weight is reached, the polymer starts to precipitate from the solution because the used solvent is a nonsolvent to the polymer. [27]

Another approach is post-dilution polymerization, where a pre-polymerized solution is added to an excess of a non-solvent, yielding particles between 15 nm and 700 nm. [27, 29]

Selecting the right solvent is therefore important to obtain the desired particles. The solvent must be able to dissolve the template, the monomer and the crosslinker, as well as the oligomers to a certain extent. However, it must be a nonsolvent to the polymer chains. Solvents with these properties for a certain polymer system are called theta solvents. Acetonitrile is most commonly used in precipitation polymerization. But sometimes also mixtures of acetonitrile and toluene are used to increase the porosity of the formed particles. The higher the porosity of the particles, the easier the template molecule can diffuse into and out of the polymer network. [28]

Particle size, porosity and specific surface area strongly depend on the solubility parameter of solvent and polymer. [28] To select a suitable solvent or nonsolvent for a certain polymer system the Hildebrand solubility parameter δ is used. It is defined as the square root of the cohesive energy density and constitutes compatibility of a solvent and polymer based on the common principle “like dissolves like”. “The cohesive energy density of a condensed substance is a measure of the intramolecular bond strength [...]”. [33] If the difference in δ between the solvent and polymer is more than $\pm 2 \text{ MPa}^{1/2}$, the solvent is considered a nonsolvent, if it lies within $\pm 2 \text{ MPa}^{1/2}$ the solvent is a good solvent for the polymer, respectively. [33]

Another model to estimate the compatibility of a solvent and a polymer is the Hansen solubility parameter. It differentiates between the dispersion, the hydrogen bond and the polar components (δ_D , δ_H , δ_P) of the Hildebrand parameter. Good solvents for a polymer lie within a sphere with $r=8 \text{ MPa}^{1/2}$, non-solvents are outside this range. [33]

A graphical representation of solvents and non-solvents according to the Hildebrand and Hansen solubility parameters is given in Figure 7.

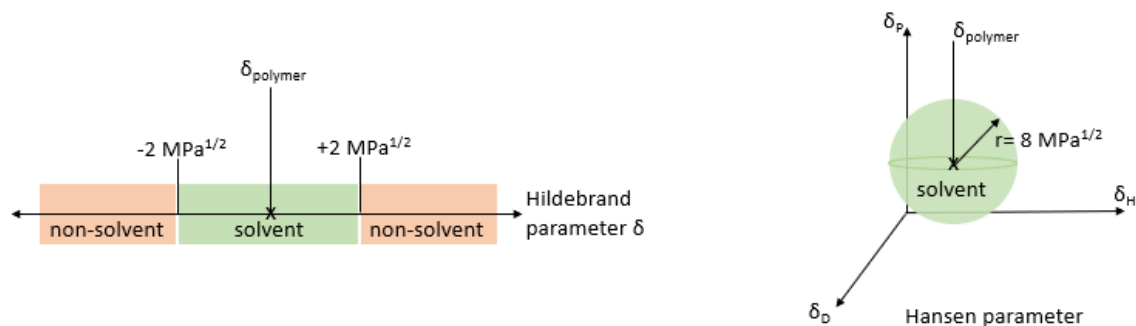


Figure 7: Depiction of solvents and non-solvents for a polymer by the Hildebrand (left) and the Hansen (right) parameter. Similar to [33]

Apart from the solvent, other parameters have a major influence on the size, morphology and size distribution of the particles. For example, the type and amount of template, the amount of initiator and the initiation method, the type of and ratio between functional monomer and crosslinker, as well as other parameters during synthesis, such as agitation or temperature. [27]

Template: Yoshimatsu et al. found that their non-imprinted particles were two times bigger than the propranolol imprinted ones and concluded that the template molecule strongly affects particle growth. [34] Shi et al. prepared gatifloxacin imprinted particles. They found that the MIP particles were smaller than the NIP particles and they reported that the size difference between MIP and NIP decreased with increasing the crosslinker content. They explained that presumably for high crosslinker concentrations, the interactions between the template and functional monomer are likely to be negligible relative to the abundant, covalent bonds between the crosslinks. [36]

Initiator: To start the polymerization, one can use initiators that form radicals either when exposed to UV radiation or to high temperatures. The latter can adversely affect the interactions between template and the functional monomers. [28] The influence of the initiator on the performance of MIPs has been intensively studied by Mijangos et al. They suggest that MIPs should be prepared using small amounts of initiator because when a high initiator concentration is used, the temperature during polymerization is much higher and reduces the imprint quality. [35]

Crosslinker: The crosslinker is a major reaction component and strongly affects the size of the precipitated particles and the obtained yield. Yoshimatsu et al. reported that they could precisely tune the size of propranolol imprinted particles in a range between 100 nm and 2.4 μm by varying the ratio of two crosslinker. Using only DVB as the crosslinker resulted in particles around 2.4 μm in low yield. When they used only TRIM they got particles with about 130 nm with high yield. A mixture of TRIM/DVB resulted in particles with intermediate size. [34]

Agitation: Gentle agitation during the polymerization by stirring or rotating results in more monodisperse particles. [27]

Temperature: If the temperature during the polymerization reaction is increased, the obtained particles have bigger diameters. On the one hand, the higher temperature leads to increased velocities of initiation; therefore, more nuclei are formed. On the other hand, critical polymer chain length increases, because solubility of the polymer is enhanced. The temperature also influences the viscosity of the solution so that the nuclei tend to aggregate more. Overall, these effects lead to the formation of bigger particles when higher temperatures are used during polymerization. [7]

Advantages and limitations of precipitation polymerization:

Precipitation polymerization is a simple, one-pot method for preparing spherical MIP NP with comparably high yield. The polymerization parameters can be adjusted to fine-tune the properties of the desired particles, such as size and size distribution. It is also advantageous that, in comparison to other methods (like emulsion polymerization), precipitation polymerization does not require using surfactants. This even makes molecular imprinting with biomolecules possible. [27, 29]

The limitation of precipitation polymerization is the high dilution of the polymer solution, since it can adversely affect the interaction between template molecules and functional monomers, which leads to reduced sensitivity and selectivity. [29] However, the high surface-to-volume ratio overcompensates this limitation. Another disadvantage is that the polymerization parameters have to be optimized for every template-copolymer system separately to obtain particles within the desired size range. [27]

Polymer-system used in this work:

In this work a polymer system with MAA as functional monomer and TRIM as crosslinker was prepared in acetonitrile with AIBN as the initiator. MIP particles were imprinted with PenV. In Figure 8 one can see the chemical structures of these compounds. The functional monomer (MAA) arranges itself around the template (PenV) by forming hydrogen bonds. The crosslinker (TRIM) and the initiator (AIBN) is added and the polymerization is thermally induced. The crosslinker then connects the functional monomers, forming a rigid polymer network around the template. Removing the template leaves behind a cavity that is complementary in shape, size and chemical functionality.

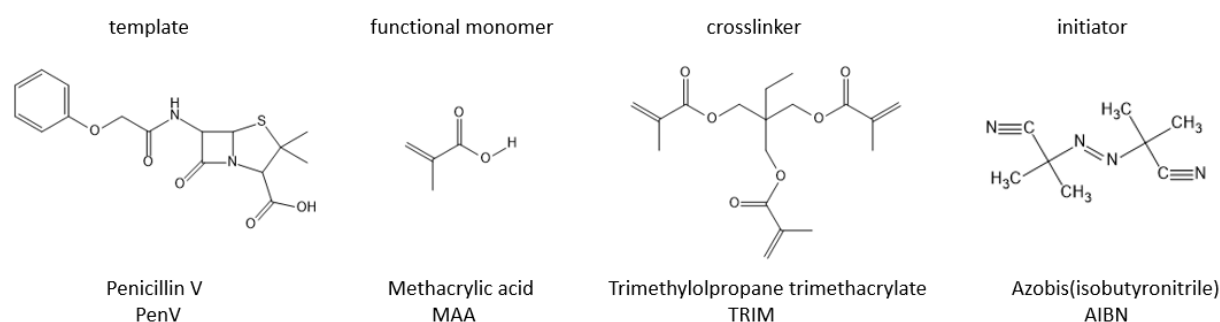


Figure 8: Chemical structures of template, functional monomer, crosslinker and initiator.

2.4. Scanning electron microscopy

Scanning electron microscopy was used in this work to characterize the prepared MIP and NIP NPs. In principle, an electron source emits an electron beam that is focused onto the sample by a system of lenses. The emitted signals are collected by the detector. The whole system requires high vacuum to prevent scattering of the electrons. [43, 44]

When an electron beam is shot at a solid sample, the interactions give rise to the following signals: Auger electrons, cathodoluminescence, X-rays, backscattered electrons and secondary electrons (see Figure 9).

Backscattered and secondary electrons are used to generate the images, while X-rays can be used for chemical analysis of the sample. [43, 44]

When the electron beam hits the surface of the sample, it can interact inelastically with loosely bound electrons from the conduction band of the solid. These electrons are then emitted with an energy of a few eV. These secondary electrons come from the first 5-50 nm under the surface, so they contain information about topography.

Other electrons are scattered elastically thus keeping their kinetic energy constant. They leave the surface as backscattered electrons. [43] Since scattering efficiency depends on the atomic number Z of the element, backscattered electrons result in “atomic number- or compositional contrast” in the images. [44]

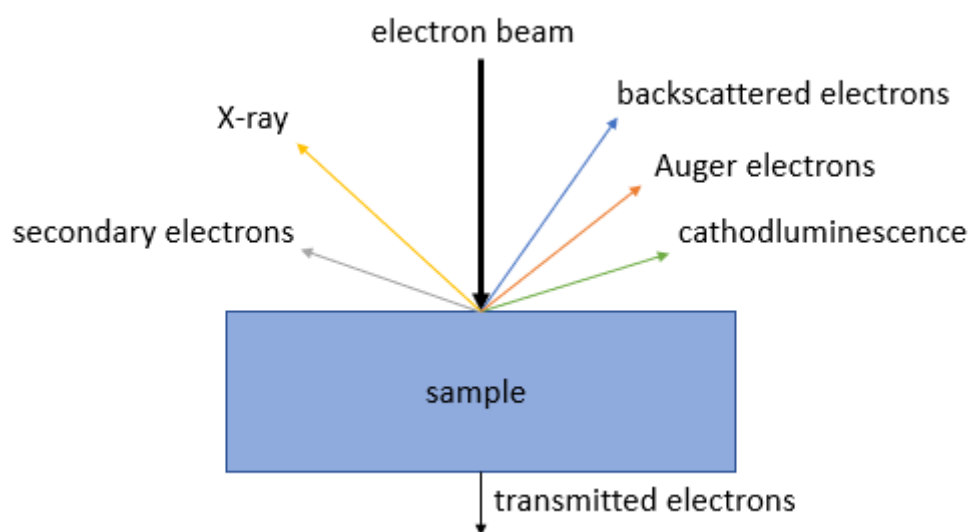


Figure 9: Schematic representation of the signals that are induced when an electron beam is shot at a sample in scanning electron microscopy. Similar to [43, 44]

The samples need to be electrically conducting to ensure that they do not become charged. If they are not conductive - like most biological samples – one coats them with a thin metal film by sputtering. [43]

3. Experiments

3.1. Chemicals, materials and instruments

Table 1 lists all used chemicals.

Table 1: Vendor and Purity (if given) of all used chemicals.

Chemical	Vendor	Purity
Acetic acid (glacial)	Merck	100 %
Acetonitrile	ROTH	>99.5 %
AIBN	Sigma Aldrich	98 %
Amoxicillin sodium salt	^a	
Dimethyl sulfoxide	VWR	100 %
Ethanol	VWR	96 %
Gold Paste	Heraeus	
Methacrylic acid (MAA)	Sigma Aldrich	*
Methanol	VWR	99.9 %
Methyl acrylate	Merck	*
Penicillin G sodium salt	^a	
Penicillin V potassium salt	^a	
Triethylamine	Merck	99.0 %
Trimethylolpropane trimethacrylate (TRIM)	Sigma Aldrich	*

*stabilized with hydroquinone monomethylether.

^a provided by the AQUA NOSE project.

In the following, details to all used instruments are given.

Characterization of Penicillin:

- IR-Spectrometer Spectrum 100 from Perkin Elmer

Preparation of MIP and NIP NP:

- Magnetic stirrer
- Ultrasound Bath
- Eppendorf Centrifuge (5702)

Characterization of MIP and NIP NPs:

- Electron microscope Zeiss Supra 55 VP (at the Center for Nanostructure Research, Faculty of Physics, University of Vienna)

Preparation of QCM sensors:

- Oven, Heraeus
- Spin coater (G3P-8), SCS Specialty coating systems

QCM measurements:

- Network Analyzer (ENA Series Network Analyzer, E5062A), Agilent Technologies
- Laboratory Power Supply (EA-PS 2032-025)
- Oscillator (manufactured in house)
- Measuring cell (designed and manufactured in house)
- Frequency Counter (53131A, Universal Counter), Agilent
- Measuring software: FreqMeas5313Xa 1.4

3.2. Preparation of QCM sensors for penicillin V

3.2.1. Preparation of MIP and NIP NPs

PenV free acid, which was used as a template for the MIP NPs, was prepared from PenV potassium salt using the following protocol.

1.5 g PenV potassium salt had to be dissolved in 15 mL distilled water in a beaker with a magnetic stirrer. Then 50 mL 0,1 M hydrochloric acid was added dropwise using a Pasteur pipette while stirring vigorously. White crystals of PenV free acid precipitated from the solution. The white powder was filtered with vacuum using a sintered glass filter and an Erlenmeyer flask. It was then washed with 100 mL distilled water and dried in the desiccator for 24 hours. An IR-spectrum was taken to ensure that the potassium salt was successfully converted to the free acid. Figure 10 shows IR-spectra of both the potassium salt (blue line) and the free acid (orange line). One can clearly see the band of the C=O beta lactam stretch at 1775 cm^{-1} in both spectra. This means that the lactam ring was still intact in the free acid; the molecule did not decompose. It was assumed that the formation of the free acid was successful, due to the broader band around 3000 cm^{-1} of the OH stretch.

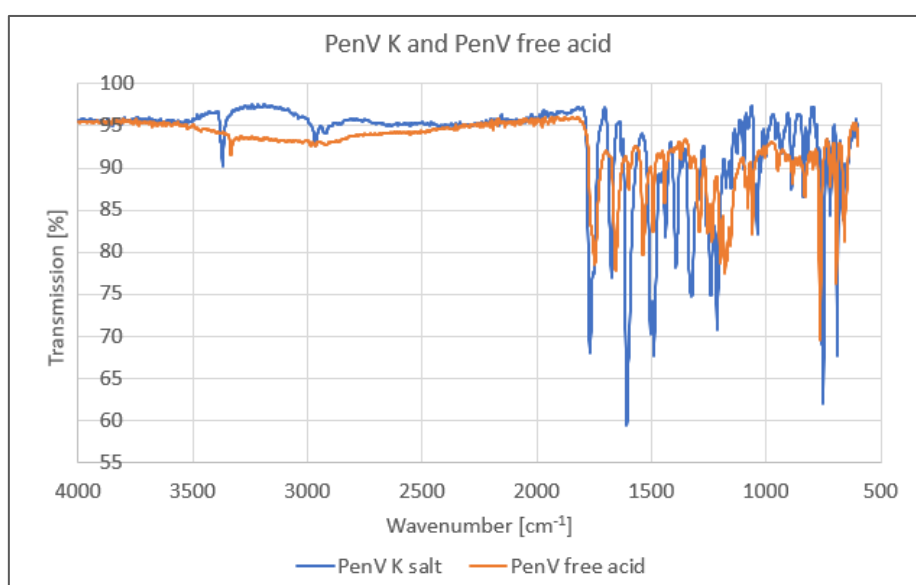


Figure 10: ATR-IR Spectra of PenV potassium salt (black line) and PenV free acid (orange line).

In the following a general description for the preparation of MIP and NIP NPs is given. Tables 2-4 list the exact compositions of all the prepared MIP and NIP NPs.

To prepare MIP NPs, the template (Penicillin V free acid) and the functional monomer (methacrylic acid, MAA) had to be added to the solvent (acetonitrile, ACN) in a 25 mL Duran bottle. A small round magnetic stirrer was added and followed by sealing the bottle with parafilm.

To ensure that the components were completely dissolved and mixed, the mixture was sonicated in an ultrasonic bath for 20 minutes. Then the crosslinker (Trimethylolpropane trimethacrylate, TRIM) was added, the bottle was sealed again with parafilm and the mixture was sonicated again for 10 minutes. For prepolymerization, the Duran bottle was placed in an oil bath at 60°C for 30 minutes, followed by stirring at room temperature for another 30 minutes.

To remove oxygen, the mixture was purged with argon gas for 10 minutes using two needles, one for gas inlet and one as gas outlet. The inlet needle was pierced through the parafilm and into the solution so that the gas bubbled all the way through the solution. The gas outlet needle just reached straight into the headspace allowing the gas to escape from the vessel.

Then the initiator (Azobis(isobutyronitrile), AIBN) was added. After that, the sealed vessel was placed in an oil bath at 60°C and stirred for 24 hours.

In order to collect the NPs, one had to centrifuge at 4400 rpm for 20 minutes and discard the supernatant. The remaining particles were washed three times with 5 mL acetonitrile and three times with 5 mL ethanol by resuspending the particles in the solvent using the ultrasonic bath for 5 minutes followed by centrifugation and decanting. The washed NPs were then dried under vacuum at room temperature for 24 hours.

The same protocol was used to make NIP NPs, except that no template was added in the first step.

The following tables show the exact composition of all the prepared MIP and NIP NPs.

Table 2: Composition of MIP and NIP 1-3 using different dilutions.

MIP/NIP	PenV free acid [mg]	MAA [μ L]	TRIM [μ L]	AIBN [mg]	ACN [mL]
MIP 1	150	150	150	70	15
NIP 1	-	150	150	70	15
MIP 2	150	150	150	70	25
NIP 2	-	150	150	70	25
MIP 3	150	150	150	70	30
NIP 3	-	150	150	70	30

Table 3: Composition of MIP and NIP 4-6, varying the amount of initiator.

MIP/NIP	PenV free acid [mg]	MAA [μ L]	TRIM [μ L]	AIBN [mg]	ACN [mL]
MIP 4	150	150	150	50	25
NIP 4	-	150	150	50	25
MIP 5	150	150	150	70	25
NIP 5	-	150	150	70	25
MIP 6	150	150	150	90	25
NIP 6	-	150	150	90	25

Table 4: Composition of MIP and NIP 7, aiming for NPs with a diameter under 200 nm.

MIP/NIP	PenV free acid [mg]	MAA [μ L]	TRIM [μ L]	AIBN [mg]	ACN [mL]
MIP 7	75	75	75	40	30
NIP 7	-	150	150	80	30

3.2.2. Different template removal techniques

In order to obtain selective binding sites in the MIP NPs, the template molecule had to be removed from the cavities. Different combinations of solvents, extraction modes (sonication or stirring) and extraction times were used. Their respective efficiencies were tested using QCM measurements.

Method 1: Sonication-assisted template removal with ethanol

Briefly, 50 mg MIP NPs were dispersed in 5 mL Ethanol in a 15 mL tube and sonicated for 1 hour. The NPs were then collected by centrifugation (4400 rpm) and the supernatant was discarded. Table 5 shows the number of times this procedure was repeated for each sample, and if, and how long the particles were stored in ethanol prior to use.

Table 5: Details of template extraction technique 1.

Sample	Sonication in ethanol	time stored in ethanol
1	3x1h	-
2	3x1h	14 d
3	6x1h	-

Method 2: Batch-mode incubation with acidic pH

50 mg MIP NPs, 9 mL methanol, 1 mL acetic acid (glacial) and a magnetic stirrer were added to a 25 mL Duran bottle, which was sealed with parafilm and placed on a magnetic stirrer for 24 or 72 hours, respectively. The particles were collected using centrifugation and decantation, as described in method 1. Then the particles were washed with 10 mL distilled water five times by resuspending and pelleting. Table 6 summarizes how long each sample was incubated, if, and how long it was stored in ethanol prior to use.

Table 6: Details of template extraction technique 2.

Sample	Incubation in MeOH:HOAc 9:1	time stored in ethanol
1	24 h	-
2	24 h	6 d
3	24 h	14 d
4	72 h	-

Method 3: Batch-mode incubation with alkaline pH

For the incubation with alkaline pH, the protocol was the same as described in method 2, except that the solvent for the incubation in the first step consisted of 9 mL methanol and 1 mL of triethylamine (TEA). In Table 7, the incubation details are given.

Table 7: Details of template extraction technique 3.

Sample	Incubation in MeOH:TEA 9:1	time stored in ethanol
1	24 h	-
2	24 h	6 d
3	24 h	14 d
4	72 h	-

3.2.3. Preparation of QCM sensors

Preparing a QCM sensor started from a blank quartz wafer (10 MHz, 168 μm thick) and applying gold electrodes by screen printing onto its surface. For that purpose, the blank wafer was fixed with vacuum and the stencil for printing the two round electrodes was placed on top. A piece of rubber was used to apply the gold paste. The quartz wafers were placed in the oven at 400°C for 4 hours. When cooled down, the side that was later exposed to the sample, was completely covered with gold paste, using a sieve without a template. Again, the quartzes were placed in an oven at 400°C for 4 hours.

In Figure 11 on the left-hand side one can see the geometry of the two round gold electrodes, which are used to connect the quartz in the measuring cell. The other side of the quartz is shown on the right. The entire surface is completely metallized and on this side the MIP and NIP NPs are applied. This side is later exposed to the sample in the measuring cell during the QCM measurements.

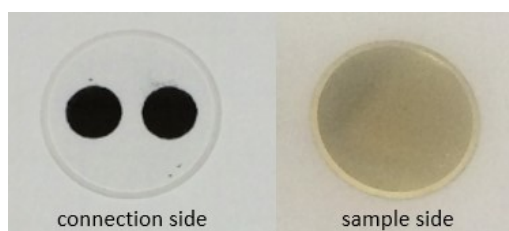


Figure 11: Screen-printed electrode design on the two sides of the quartz wafer.

In the next step, the MIP particles had to be attached as the selective recognition element to one side of the quartz and the NIP particles as reference to the other side. Therefore, 5 mg NPs were dispersed in 1 mL ethanol in an Eppendorf tube. 2 μ L methyl acrylate were added and the mixture was sonicated for 10 minutes.

2 μ L of the MIP suspension was distributed on one side of the quartz and the NIP suspension on the other side. Then a spin coater was used to distribute the particles evenly on the surface of the electrodes. A rotation speed of 500 rpm for 50 seconds was used.

The spin-coated quartz was then dried in the oven at 80°C for 24 hours. Then the quartz wafers were placed in a Petri dish along with a magnetic stirrer and distilled water. They were stirred for 2 hours to remove all particles that did not stick to the electrodes. The quartz was then dried again in the oven at 80°C for 24 hours.

3.2.4. QCM measurements

Figure 12 shows the setup used to perform the QCM measurements. One can see the measuring cell that contains the prepared quartz and is connected to the oscillator. The measuring cell and the oscillator are manufactured in house. The oscillator is connected to a laboratory power supply (voltage: 14,7 V; current: 0,07 A) and a frequency counter. The measurements are managed using a measuring software (FreqMeas5313Xa 1.4) on a computer.

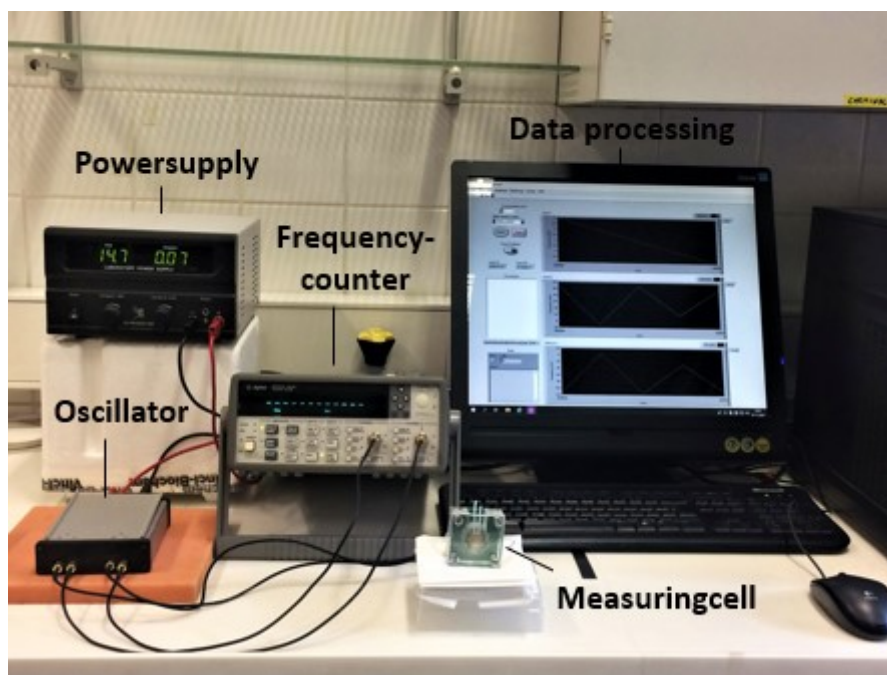


Figure 12: Setup for QCM measurements.

Both, sensitivity and selectivity measurements were performed using this setup. First, the measuring cell was filled with distilled water and the measurement was started. After about half an hour, the baseline was stable and one could start with the actual QCM measurements.

For sensitivity measurements, five solutions of PenV potassium salt in distilled water with concentrations from 10 mM to 50 mM were prepared. When the baseline was stable, the measurement was paused and 200 μ L of PenV solution were injected into the measuring cell using a piston pipette. The pause was stopped and the frequency drop was recorded for a few minutes. The measurement was again paused to flush the measuring cell with 1 mL distilled water. If needed this washing step was repeated until the baseline was reached again. After a few minutes, the next injection could be performed. Each concentration was injected twice and it was started with the highest concentration (50 mM) first.

Selectivity measurements were performed directly after the sensitivity measurements. Prior to measuring, 50 mM, 25 mM and 12.5 mM solutions of PenV potassium salt and PenG sodium salt were prepared in distilled water. The injection and washing steps were performed as described for the sensitivity measurements. First the 50 mM PenV solution was injected and the sensor response was recorded. After washing, the 50 mM PenG solution was injected. The same was done for the 25 mM PenV and PenG solutions followed by the solutions with a concentration of 12.5 mM.

3.3. Preparation of penicillin G imprinted particles

To prepare PenG free acid from PenG sodium salt the same protocol as described for PenV was used. The product was not a fine powder as expected, but a sticky substance, as can be seen in Figure 13.



Figure 13: Obtained product for the preparation of PenG free acid from the sodium salt.

The assumption that PenG decomposed at the harsh acidic conditions was confirmed with an IR spectrum as can be seen in Figure 14. The blue line is the IR spectrum of PenG sodium salt and the orange line is the spectrum of the glue-like substance. None of the specific bands for PenG can be observed in the orange spectrum anymore.

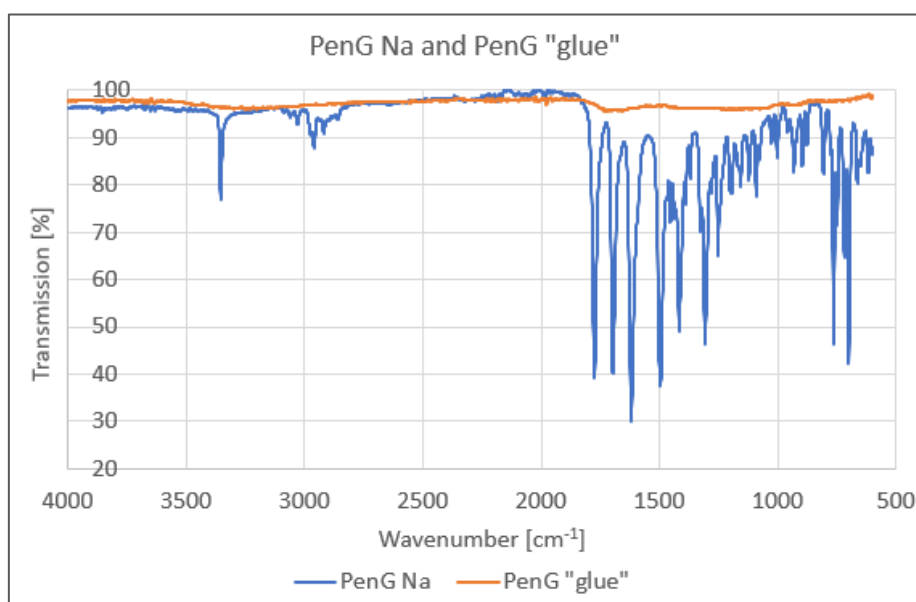


Figure 14: IR-spectra of PenG sodium salt (blue line) and the glue-like substance obtained from the preparation of the free acid (orange line).

The protocol was therefore adapted: 1.5 g PenG sodium salt was dissolved in 30 mL distilled water. While stirring vigorously with a magnetic stirrer, 0,1 M hydrochloric acid was added until the solution reached a pH of 2.5. After stirring for 10 minutes a white powder precipitated. It was filtered, washed with 100 mL distilled water and dried in the desiccator for 24 hours. An IR spectrum of the product can be seen in Figure 15 (orange line) in comparison to the spectrum of the PenG sodium salt (blue line). One can see that in this case the molecule was not completely decomposed, but still the band at 1775 cm⁻¹, belonging to the C=O beta lactam stretch, cannot be found in the orange spectrum, which means that the lactam ring was opened anyway.

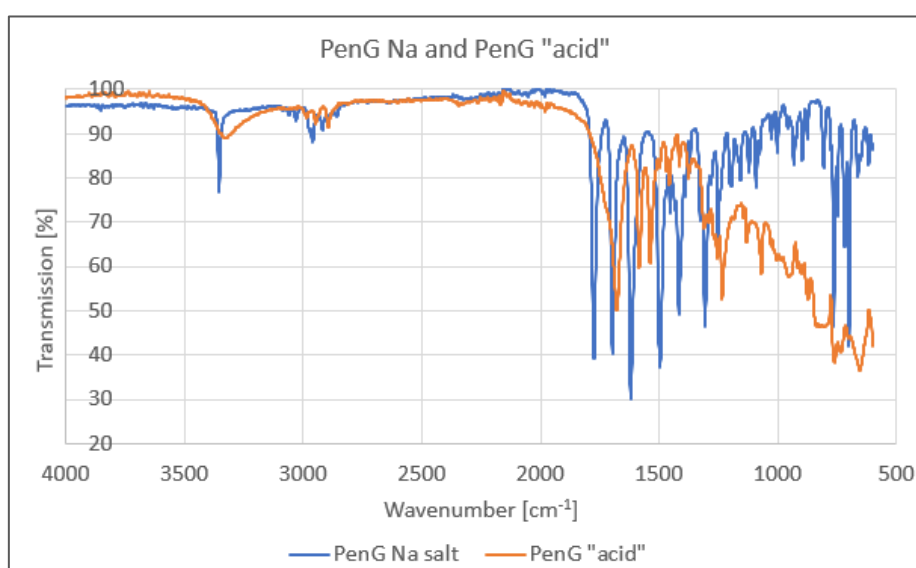


Figure 15: IR-spectra of PenG sodium salt (blue line) and PenG "free acid" (orange line).

Since PenG free acid could not be prepared from PenG sodium salt due to the instability of the molecule during acidic conditions, MIP NPs were prepared with PenG sodium salt as template using the following protocol.

In a 25 mL Duran bottle 100 mg PenG sodium salt was dissolved in a mixture of 10 mL ACN and 4 mL EtOH using sonication. Then, 100 μ L MAA and a magnetic stirrer were added and the vessel was sealed with parafilm. After 20 minutes of ultrasound treatment, 100 μ L TRIM was added and the Duran bottle was sealed again with parafilm. Sonication was used again for 10 minutes and then the bottle was placed in an oil bath at 60°C for 30 minutes. Then the mixture was stirred for an additional 30 minutes at room temperature.

The oxygen was removed from the solution by purging with argon gas in the same way as described in chapter 3.2.1. 70 mg AIBN was added and the sealed vessel was placed in an oil bath at 60°C for 24 hours for polymerization.

The particles were collected, washed and dried according to the protocol described in chapter 3.2.1 for the PenV imprinted NPs.

3.4. Preparation of amoxicillin imprinted particles

Amoxicillin free acid was prepared from amoxicillin sodium salt using the same protocol as for preparing PenV free acid (see chapter 3.2.1). An IR spectrum of the sodium salt and the free acid was taken and can be seen in Figure 16. One can see that the molecule was not decomposed because the C=O beta lactam stretch at 1775 cm^{-1} appears in the spectrum of the sodium salt (blue line) as well as in the free acid (orange line). The broad band around 3000 cm^{-1} indicates that the formation of the free acid was successful.

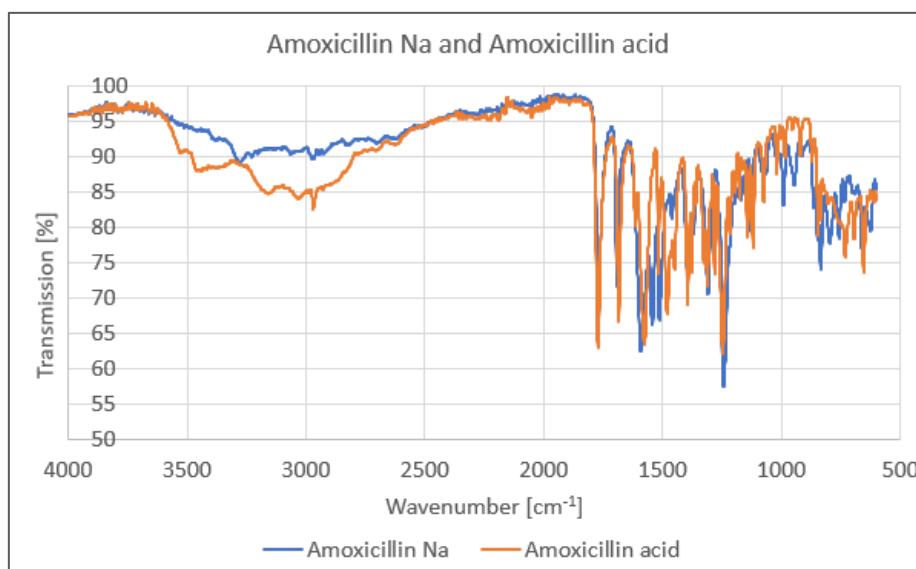


Figure 16: IR-spectra of amoxicillin sodium salt (blue line) and amoxicillin free acid (orange line).

Amoxicillin – MIP 1:

Since amoxicillin free acid is not soluble in acetonitrile, methanol had to be used as a solvent for precipitation polymerization instead.

25 mL MeOH, 100 mg amoxicillin free acid, 150 μ L MAA and a magnetic stirrer were placed into a 25 mL Duran bottle, which was then sealed with parafilm. After sonicating for 20 minutes, 150 μ L TRIM was added followed by continuing sonicating again for 10 minutes. The vessel was heated to 60°C in an oil bath for 30 minutes, followed by stirring at room temperature for another 30 minutes. Oxygen was removed by purging with argon for 10 minutes. Then 50 mg AIBN were added and the sealed vessel was placed in an oil bath at 60°C for 24 hours for polymerization.

The precipitated particles were collected by centrifugation at 4400 rpm for 10 minutes. The particles were washed three times with 5 mL ACN and three times with 5 mL EtOH by resuspending and centrifugation. Then the particles were dried in the desiccator for 24 hours.

Amoxicillin – MIP 2:

In a glass vial 25 mg amoxicillin free acid were dissolved in 700 μ L DMSO. 15 μ L MAA and 50 μ L TRIM were added and the vial was sealed with parafilm. After sonicating for ten minutes, the solution was purged with argon for 2 minutes. 5 mg AIBN were added and polymerization was initiated in an oil bath at 60°C for 20 minutes. The prepolymerized mixture was then added dropwise to 10 mL ACN while stirring vigorously. The particles were collected, washed and dried as described for MIP 1.

3.5. Sample preparation for the characterization of the particles with SEM

A glass slide was cut into small squares of about 1x1 cm. They were cleaned from any residues or traces of grease by stirring them in acetone in a glass petri dish for 30 minutes. Then they were dried in the oven at 80°C. Meanwhile, 5 mg of dry NPs were dispersed in 1 mL acetonitrile in an Eppendorf tube and sonicated for 20 minutes. 10 μ L of the particle suspension was pipetted onto the glass slide and spread. The slides with the samples were then dried in the oven at 80°C overnight. The slides were sputtered with gold prior to characterization with SEM.

4. Results and Discussion

4.1. QCM sensors for penicillin V

4.1.1. Characterization of MIP and NIP nanoparticles and the influence of different parameters on particle size

As already explained in chapter 2.3, one needs to adapt the polymerization parameters for every template-polymer system individually to obtain the desired particles during precipitation polymerization. The size and size distribution depend on many parameters, such as solvent, type and amount of template, crosslinker, initiator, temperature etc. [27, 28, 33-36]

Project goals required MIP and NIP particles being below 200 nm in diameter. The reason is that this makes it possible to introduce them into the production processes of the sensor foreseen by the AQUANOSE project.

Subsequently, one can see the results of the experiments that were performed to evaluate the influence of the concentration of monomer and crosslinker and the amount of initiator on the size and size distribution of MIP and NIP particles. To find the right parameters to obtain the desired particles the monomer concentration and the amount of initiator were systematically varied in order to derive the trends that lead to the formation of smaller particles.

In the following set of experiments, three different MIP and NIP NPs were prepared. All parameters, such as template, functional monomer, crosslinker and initiator, were kept constant and only the amount of solvent was varied, resulting in different monomer concentrations. SEM was then used to evaluate how the diameter of MIP and NIP NPs alters with changing dilution.

Influence of monomer concentration on particle diameters

Table 8 gives the exact compositions of MIP 1-3. Furthermore, it also contains the diameters of the respective particles as determined from the SEM images given in Figure 17.

In general, the MIP particles were all spherical in shape with a smooth surface and monodisperse size distribution. One can see that in the case of MIP particles, particle diameters decreased with increasing dilution with acetonitrile. Going from MIP 1 to MIP 3 meant doubling the amount of solvent, which resulted in a decrease in the particle diameter of about 66 %. However, MIP 1-3 were all much larger in diameter than the desired 200 nm.

Table 8: Composition of MIP NPs 1-3 and their diameter (determined with SEM).

MIP	PenV	MAA	TRIM	AIBN	ACN	size (SEM)
1	150 mg	150 μ L	150 μ L	70 mg	15 mL	2,1-2,3 μ m
2	150 mg	150 μ L	150 μ L	70 mg	25 mL	730-850 nm
3	150 mg	150 μ L	150 μ L	70 mg	30 mL	670-720 nm

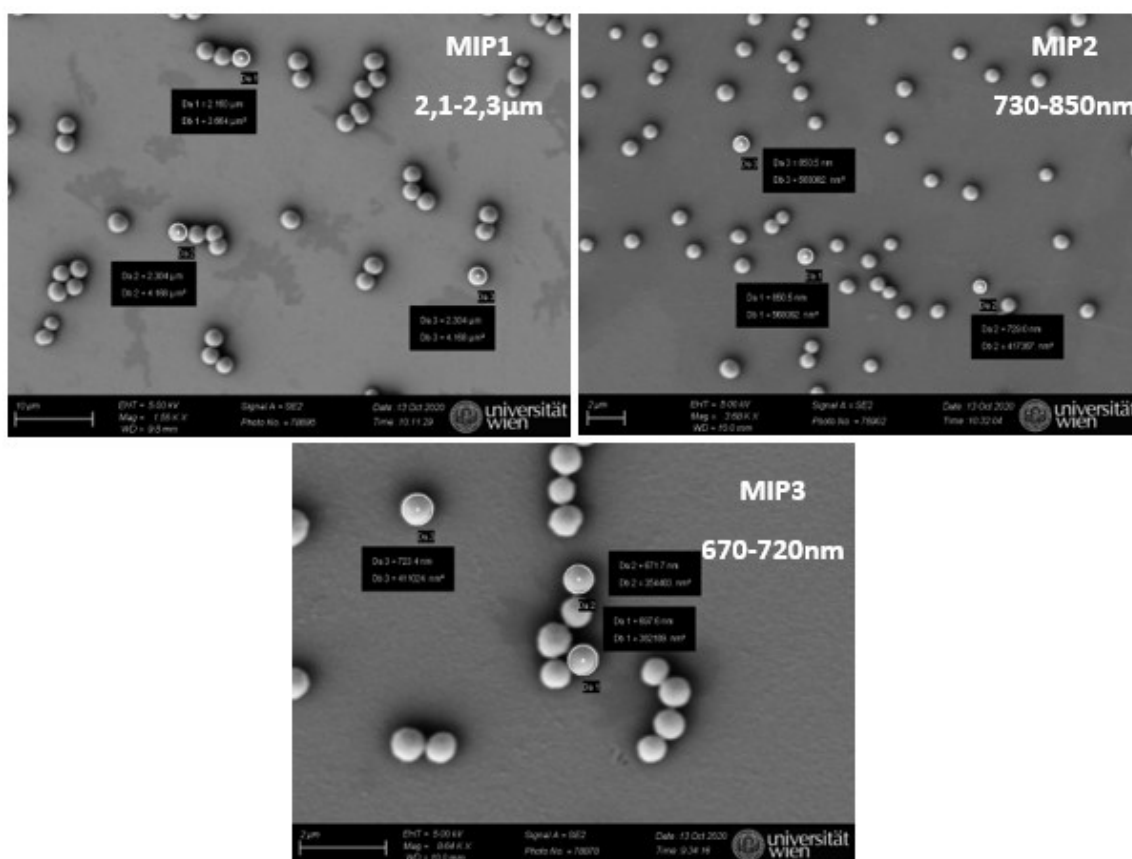


Figure 17: SEM images of MIP 1, MIP 2, and MIP 3, respectively.

For NIP 1-3 basically the same preparation protocol and composition was used as for the MIP particles, except that they did not contain the template. Table 9 gives the exact composition of the NIP NPs and their diameters, which were determined with SEM. Figure 18 shows SEM images of these particles.

Table 9: Composition of NIP NP 1-3 and their diameter (determined with SEM).

NIP	PenV	MAA	TRIM	AIBN	ACN	size (SEM)
1	0 mg	150 μ L	150 μ L	70 mg	15 mL	800 nm
2	0 mg	150 μ L	150 μ L	70 mg	25 mL	85-140 nm
3	0 mg	150 μ L	150 μ L	70 mg	30 mL	64-130 nm

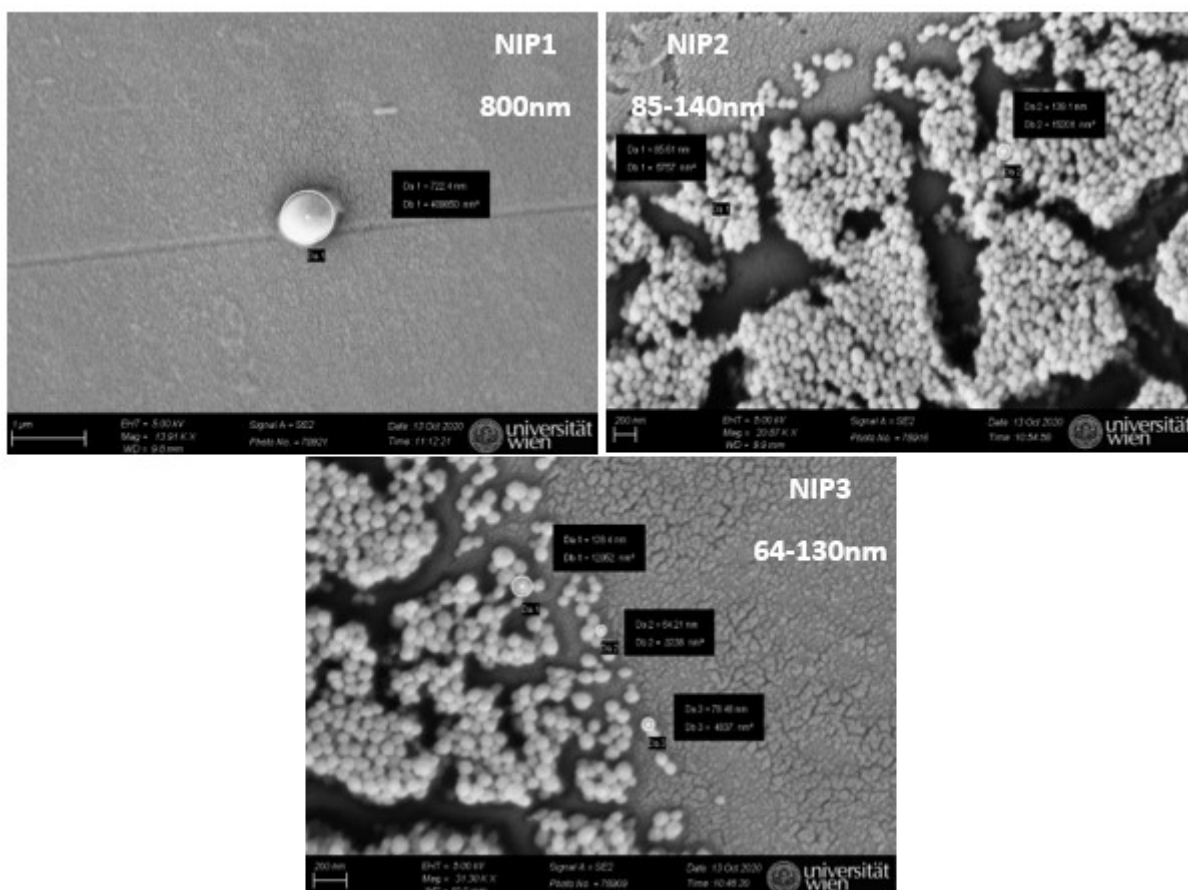


Figure 18: SEM images of NIP 1, NIP2 and NIP 3.

It immediately hits the eye that the NIP particles are generally much smaller compared to MIP particles, even though polymer composition is the same. NIP NPs 1, which were prepared with the lowest dilution, had a diameter of around 800 nm. This is much smaller than MIP NPs 1 at around 2 μm . Since all parameters were the same except for the addition of template in the MIP NPs, one can assume that the template somehow affects polymerization.

One way the template can affect the polymerization is by scavenging radicals that are formed by the initiator. If fewer radicals are present during polymerization, one would expect that the overall reaction is slower and that less but larger particles are formed.

To see if penicillin V had radical scavenging activity, an IR spectrum of PenV free acid in acetonitrile (blue line) was taken and compared with a spectrum of PenV with AIBN in acetonitrile, which was initiated at 70°C for 30 minutes (orange line), as can be seen in Figure 19.

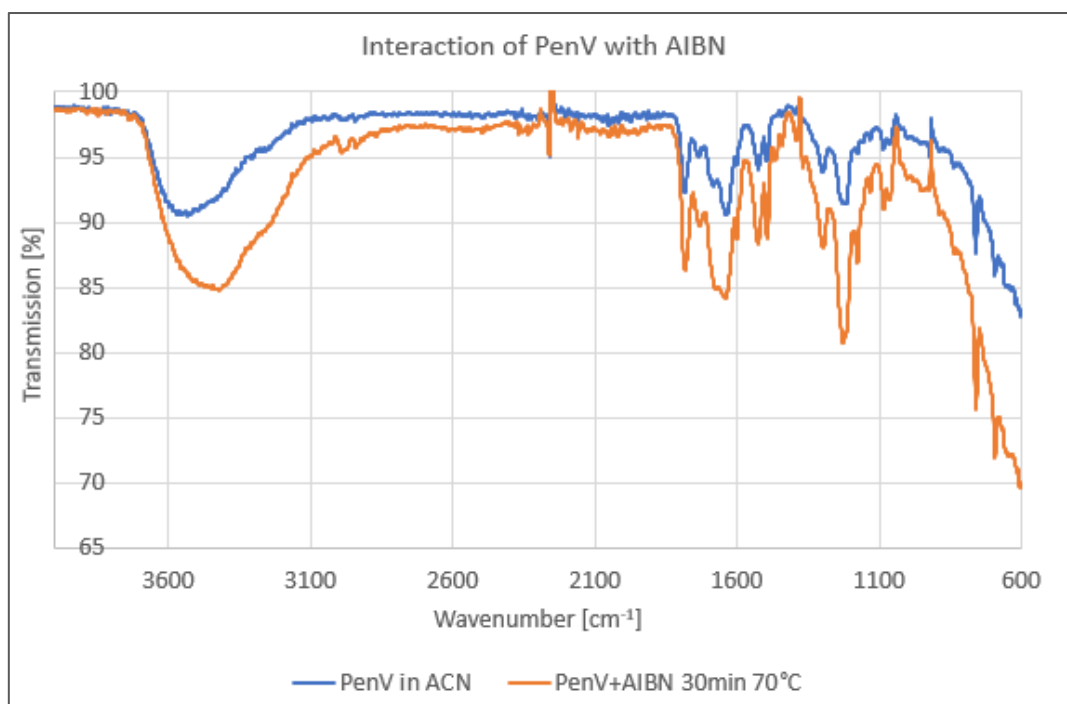


Figure 19: IR spectra of PenV free acid dissolved in acetonitrile (blue line) and PenV free acid and AIBN in acetonitrile incubated at 70°C for 30 minutes (orange line).

One can see in Figure 19, that the bands of PenV in the orange spectrum were unaffected by the radicals of the initiator. The higher intensity of the bands in the orange spectrum compared to the blue spectrum can be explained by the evaporated solvent during incubation, which slightly increased PenV concentrations in the solution. It was therefore concluded that there must have been a different reason for this result.

Different sizes of MIP and NIP particles have been reported several times in the literature. [36-38] All papers trace this back to interactions of the template with the functional monomers and the nature of the template in general. Shi et al. suspected that their gatifloxacin imprinted particles are smaller than the NIP because of the different interactions in the pre-polymer solution. In the NIP, MAA is present as free molecule or as a dimer, while in the MIP there is additional interaction between MAA and the template causing differences in the particle growth. [36] Yoshimatsu et al. and Long et al. explained that their MIP particles imprinted with propranolol are smaller than the NIP particles because the interaction between MAA and the template changes the solubility parameter of the polymer chains and therefore influences particle growth. However, they also showed through NMR that the template had an influence on the polymerization rate. Furthermore, they reported that the particle size depends on the nature of the template. When preparing propranolol-imprinted particles, they found that the MIP was smaller in diameter than the NIP, while for testosterone imprinted particles the MIP particles were significantly larger than the NIP particles. They attributed this to the neutral and hydrophobic nature of testosterone, while propranolol is amphiphilic. [37, 38]

Overall, the nature of the template and its interactions with the functional monomer seem to affect polymerization in MIP particles. In this case, probably the hydrophobicity of the phenoxyacetate side chain of PenV enhances the solubility of the growing polymer chains in the solution, resulting in larger diameters of MIP particles compared to the NIP NPs.

One can also see that there is the same trend as with the MIP NPs: The particle diameter decreases the higher dilution. When the amount of solvent is doubled for the MIP particles, as already mentioned, the diameter decreases by about two thirds. Whereas the diameter of NIP 2 and 3 are only about one tenth of the diameter of NIP 1. So, the dilution affected the diameter of the NIP particles more than that of the MIP particles.

The trend that the particle size increases with decreasing dilution of the polymer system can be explained as follows: When the monomer concentration is increased, solubility of the polymer in the continuous phase is enhanced. This in turn results in an increase of the critical polymer chain length, at which the polymer precipitates from the solution. All in all, fewer but larger particles are formed as a result, since the nuclei formed during polymerization aggregate. [7]

For NIP particles, the goal of diameters below 200 nm could already be accomplished by selecting a suitable dilution, while MIP particles were still too large.

In the next two sets of experiments all experimental parameters except for the amount of initiator were kept constant to evaluate how it affects the size and size distribution of the particles in the imprinted and non-imprinted system.

Influence of initiator concentration on particle size

Table 10 shows the compositions of NIP 4-6 and the corresponding particle diameters. From NIP 4 to NIP 6 the amount of initiator increased from 50 mg to 90 mg per batch. SEM images are given in Figure 20.

Clearly, the lowest initiator concentration leads to very large particles: diameter range from 590 nm to 1 μ m. The size distribution is also very broad.

For NIP 5 and 6, a higher concentration of AIBN was used in the polymerization. As a result, the diameters decreased dramatically to around 100 nm. Size distribution of the particles turned out monodisperse.

Table 10: Composition of NIP NP 4-6 and their diameters (determined with SEM).

NIP	PenV	MAA	TRIM	AIBN	ACN	size (SEM)
4	0 mg	150 μ L	150 μ L	50 mg	25 mL	590-1000 nm
5	0 mg	150 μ L	150 μ L	70 mg	25 mL	85-140 nm
6	0 mg	150 μ L	150 μ L	90 mg	25 mL	85-150 nm

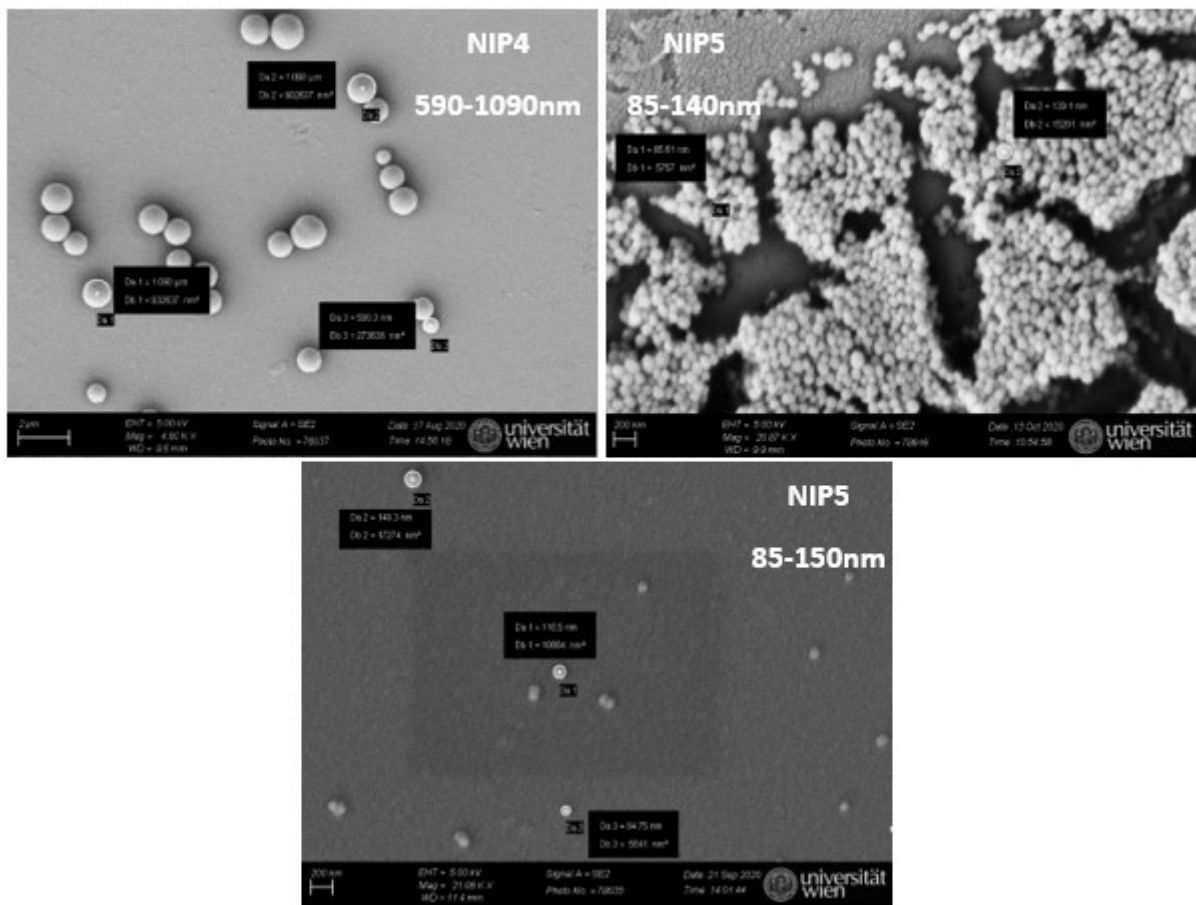


Figure 20: SEM images of NIP 4, NIP 5 and NIP 6.

When using higher initiator concentration, more free radicals form in solution. Therefore, there are more growing nuclei and more polymer spheres with smaller diameter precipitate from the solution. [35] This aligns with the results found for NIP particles 4-6.

Table 11 gives the compositions of MIP 4-6, together with the diameters of the respective particles, as determined by SEM. The corresponding SEM images are given in Figure 21.

For the MIP particles, however, the results unexpectedly showed the opposite effect. The diameter of the particles decreased by about half when going from a medium or high initiator concentration to the low AIBN concentration.

Table 11: Composition of MIP NP 4-6 and their diameter (determined by SEM).

MIP	PenV	MAA	TRIM	AIBN	ACN	size (SEM)
4	150 mg	150 μ L	150 μ L	50 mg	25 mL	316-460 nm
5	150 mg	150 μ L	150 μ L	70 mg	25 mL	740-870 nm
6	150 mg	150 μ L	150 μ L	90 mg	25 mL	680-720 nm

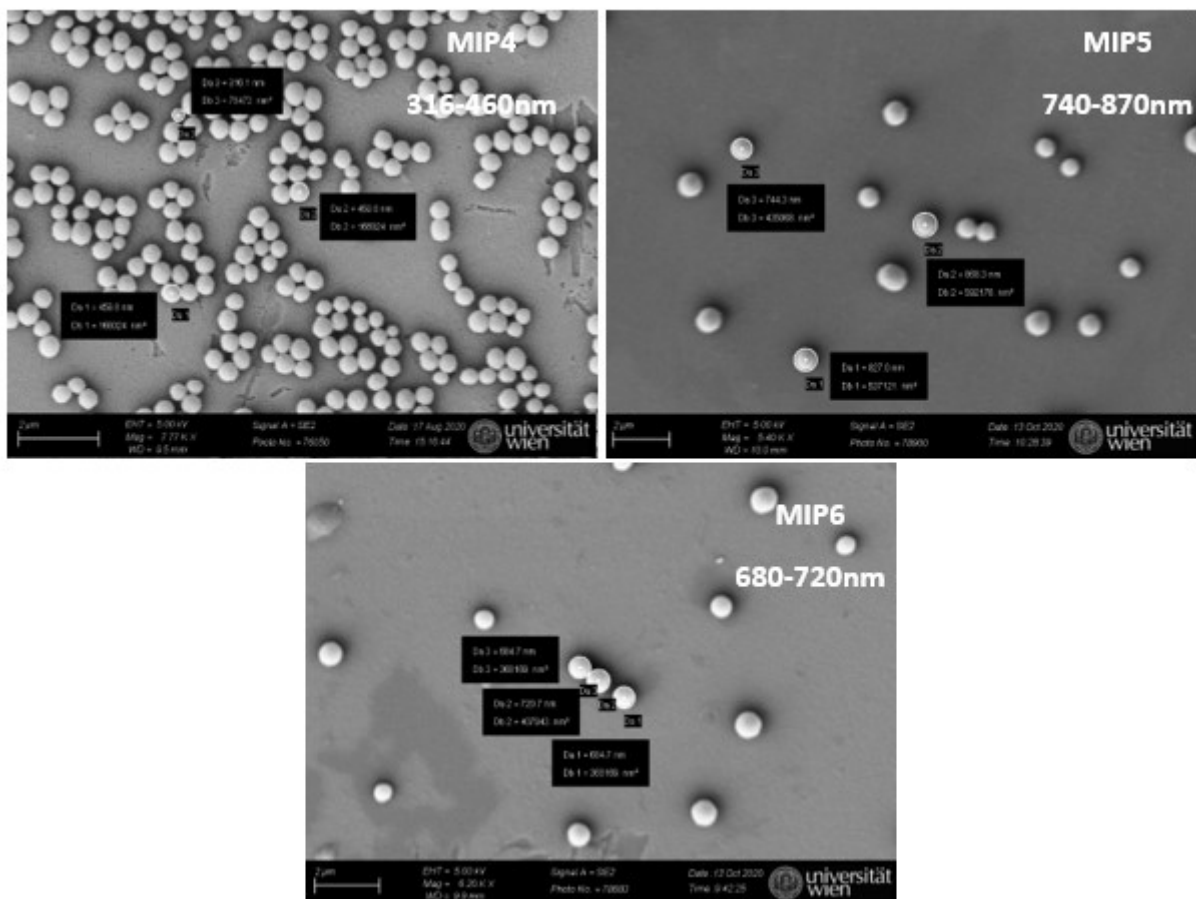


Figure 21: SEM images of MIP 4, MIP 5 and MIP 6.

Given the explanation from before for the NIP NPs this result seems counterintuitive. One would expect that more initiator forms a higher number of smaller particles. However, this is not the case for PenV imprinted particles.

A possible explanation for this result can be found in the literature by Li et al., who prepared MIP particles by precipitation polymerization with L-2-chloromandelic acid as template and also found that the particle diameter was larger with higher initiator concentrations. The higher the initiator concentration used during polymerization, the more exothermic the reaction. Therefore, the temperature in the reaction vessel is higher, which on the one hand increases the initiation and forms more nuclei. On the other hand, however, it also enhances the solubility of the polymer and therefore increases critical chain length. Together with the effect that the viscosity of the polymerizing system decreases and therefore the nuclei tend to aggregate more, as a result, larger particles are formed. [7]

However, the increased temperature in systems with high amounts of initiator does not only affect the size of the particles. Mijangos et al. showed that the higher temperature adversely affects complex formation between template and functional monomer and therefore leads to reduced affinity and selectivity of the MIP NPs. They conclude that it is advantageous to prepare MIP NPs with low initiator concentrations, low temperatures and with longer polymerization time. [35]

Overall, the influence of monomer concentration and the amount of initiator can be summarized as shown in Figure 22. The blue arrows indicate how the diameter of MIP particles is influenced by the monomer and initiator concentration and the yellow arrows stand for the NIP particles, respectively. One can see that the particle diameter decreases for MIP and NIP with decreasing monomer concentration, whereas the effect of the initiator concentration is inverse for MIP and NIP particles.

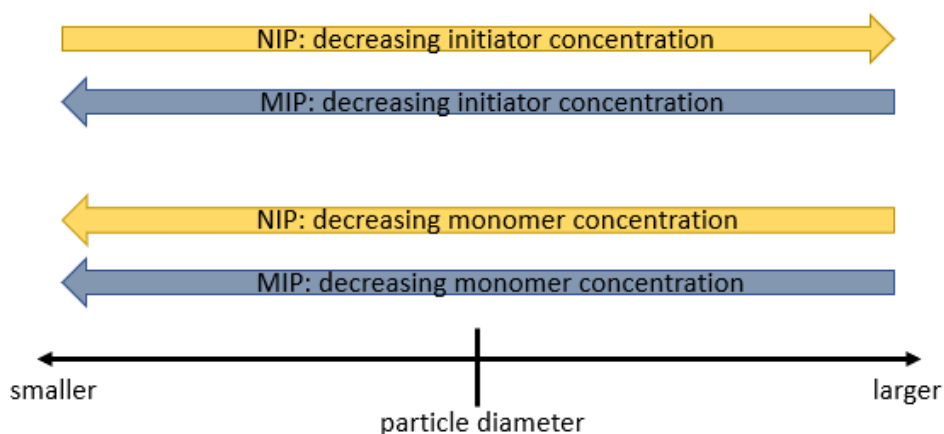


Figure 22: Influence of the monomer and initiator concentration on the particle diameter of MIP (blue arrows) and NIP (yellow arrows) NPs.

Aiming for MIP and NIP nanoparticles below 200nm

In order to prepare MIP and NIP NPs with diameters below 200 nm it seemed most feasible to combine the findings from Figure 22. As can be seen in Table 12, high dilution with ACN was used for both MIP and NIP, while low initiator concentration was used for MIP NPs and high initiator concentration for NIP NPs. In Figure 23, one can see the SEM images of MIP and NIP 7.

NIP 7 yielded very small and monodisperse nanoparticles with diameters between 60 nm and 80 nm. Batch MIP 7 did not contain particles with diameters below 200 nm. Most particles seemed to have diameters around 300 nm, but SEM images also reveal small fractions of smaller (~ 250 nm) and larger particles (~ 450 nm). Hence it turned out necessary to further optimize the polymerization parameters of MIP in order to obtain particles within the desired size range. However, due to the time limit of the master's thesis project, this could not be pursued. Further approaches could involve varying the polymerization temperature, using low temperature conditions during the prepolymerization, and, of course, implementing photoinitiation of the polymerization. It is also expected that these measures will be beneficial to the performance of the particles in terms of affinity and selectivity.

Table 12: Composition of MIP and NIP NPs 7 and their diameter (determined with SEM).

MIP/NIP	PenV	MAA	TRIM	AIBN	ACN	size (SEM)
MIP7	75 mg	75 μ L	75 μ L	40 mg	30 mL	250-450 nm
NIP7	0 mg	150 μ L	150 μ L	80 mg	30 mL	60-80 nm

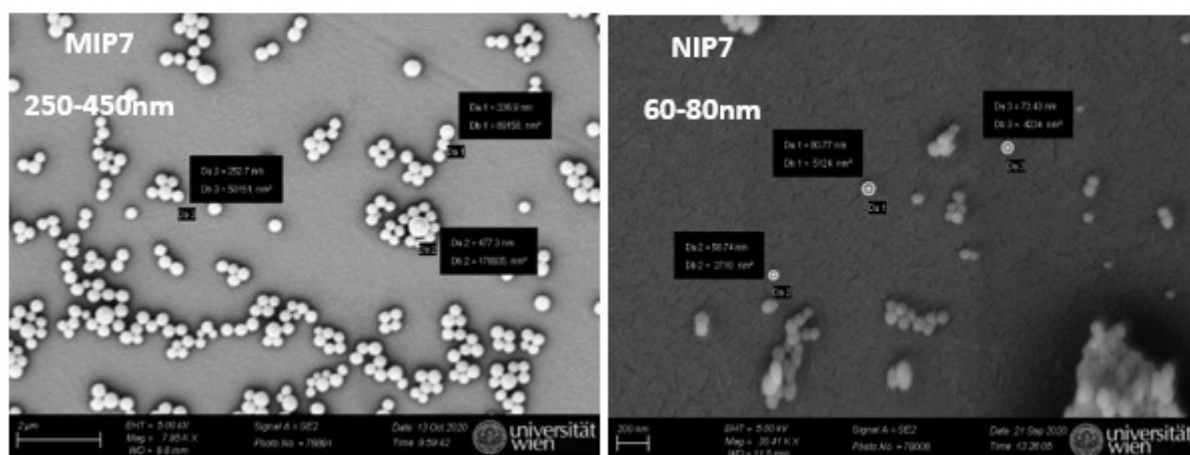


Figure 23: SEM images of MIP and NIP 7.

4.1.2. Influence of different solvents and extraction times on template removal

Removing the template from the cavities is a challenging and the least cost-effective step in the production of MIPs, because the optimization is time-consuming and often achieved by trial and error. However, it is essential for MIP to function. If template removal is insufficient, fewer binding sites are available and sensitivity is decreased. Bleeding of the template from the MIP is also problematic when applying them.

Because of the high crosslinking degree of the polymers and affinity of the template to the binding site, template extraction often requires harsh conditions, including high temperatures and comparably extreme pH values for extended periods of time. However, this can lead to distortion, rupture or collapse of the cavity, resulting in reduced sensitivity and selectivity.

There are basically three approaches for removing the template. The most straightforward method is extracting with solvents either by using Soxhlet extraction or just incubation. Then there are physically assisted extraction methods, such as ultrasound assisted extraction, microwave assisted extraction and pressurized liquid extraction. The third method involves subcritical or supercritical solvents. [40]

To remove PenV from the prepared polymer particles three approaches were tested. The first method involved ultrasound to physically assist extraction in ethanol. The second method comprised stirring the particles in methanol as a solvent with acetic acid to create acidic conditions. In the third attempt, alkaline conditions were created by adding triethylamine. After the extraction, all particles were stored in EtOH. Figure 24 summarizes the extraction conditions and extraction times.

Template removal techniques		
Batch-mode incubation with acidic pH MeOH:HOAc 9:1	Batch-mode incubation with alkaline pH MeOH:TEA 9:1	Ultrasound-assisted extraction EtOH
24 h	24 h	3x1 h
72 h	72 h	6x1 h
stored in EtOH		
6 d	6 d	-
14 d	14 d	14 d

Figure 24: Summary of template removal techniques.

In the following section, one can see the QCM measurements that show which of the template removal techniques were successful and which were not. For a detailed explanation about the interpretation of QCM measurements in general, the reader is referred to chapter 2.2.1, which describes the processes on the MIP and NIP side and outlines the information that can be derived from this.

Ultrasound-assisted template removal:

Ultrasound waves travel through matter by expansion and compression. In liquids, this leads to the formation of bubbles which collapse by cavitation. Subsequently, this generates very high temperatures and pressures. Therefore, ultrasound can be used to accelerate extraction processes. [41]

In Figure 25, one can see that ultrasound-assisted washing was not sufficient to remove the template from the cavities in the MIP. After sonicating in ethanol three times for one hour, the change in frequency for MIP is not higher than for NIP NPs. Therefore, one can conclude that removing the template was not successful, meaning that no specific binding sites were available during the measurement and thus one can only see unspecific interaction for MIP and NIP. In addition, it can be seen that sonicating for a longer time (six times for one hour) did not result in better extraction yield. After storing the particles for 14 days in EtOH no further extraction of the template could be determined.

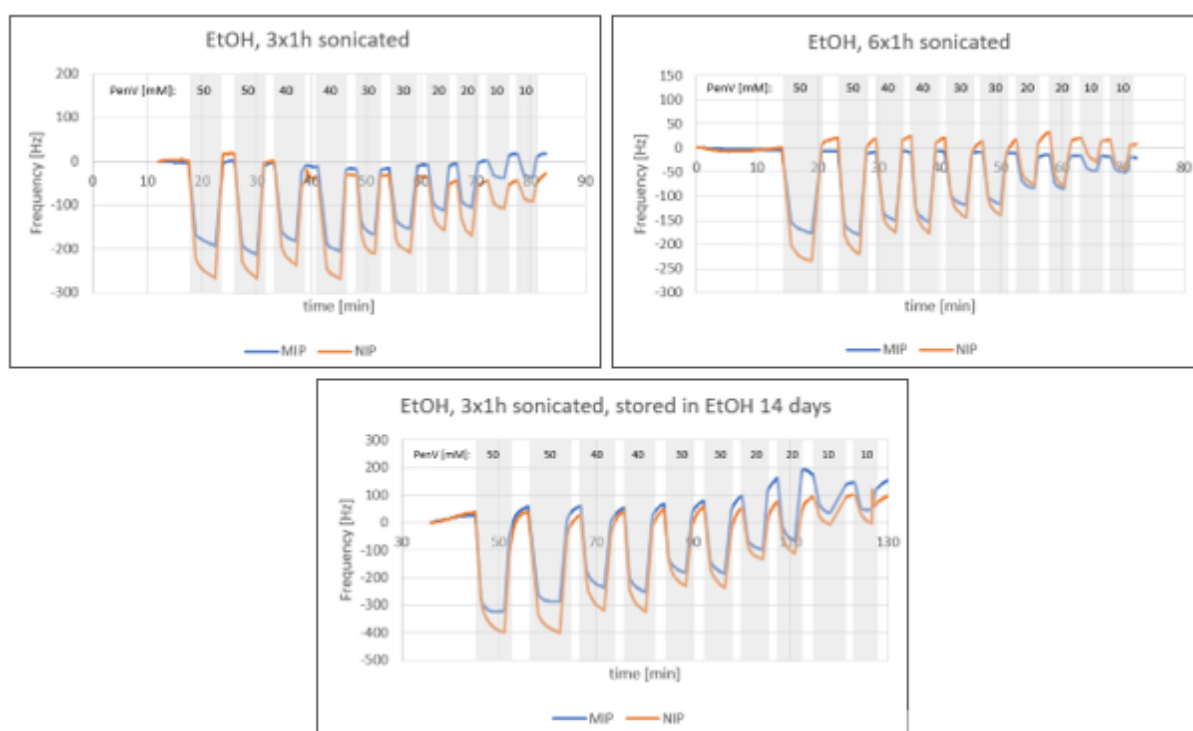


Figure 25: QCM measurements with MIP particles that were extracted with ethanol in an ultrasound bath.

Batch-mode incubation with acidic pH (MeOH:HOAc 9:1):

Extracting MIP NPs with methanol and acetic acid led to interesting results:

First the particles were extracted under acidic conditions for 24 hours. Again, there was no difference in frequency shifts for MIP and NIP in the QCM measurement, meaning that the removal was not successful (see Figure 26, upper left corner).

Applying these conditions for longer time (72 hours) also did not give the desired results (Figure 26, upper right corner).

However, when MIP NPs extracted under acidic conditions for 24 hours were stored in ethanol for further 6 days, see the bottom left corner of Figure 26, one could observe differences between MIP and NIP channel during QCM measurement.

When storing the MIP particles in ethanol for an even longer period (14 days), the QCM measurement in the bottom right corner of Figure 26 clearly shows that removing the template from the cavities was successful because there is a significantly higher change in frequency for MIP than for NIP when PenV is injected.

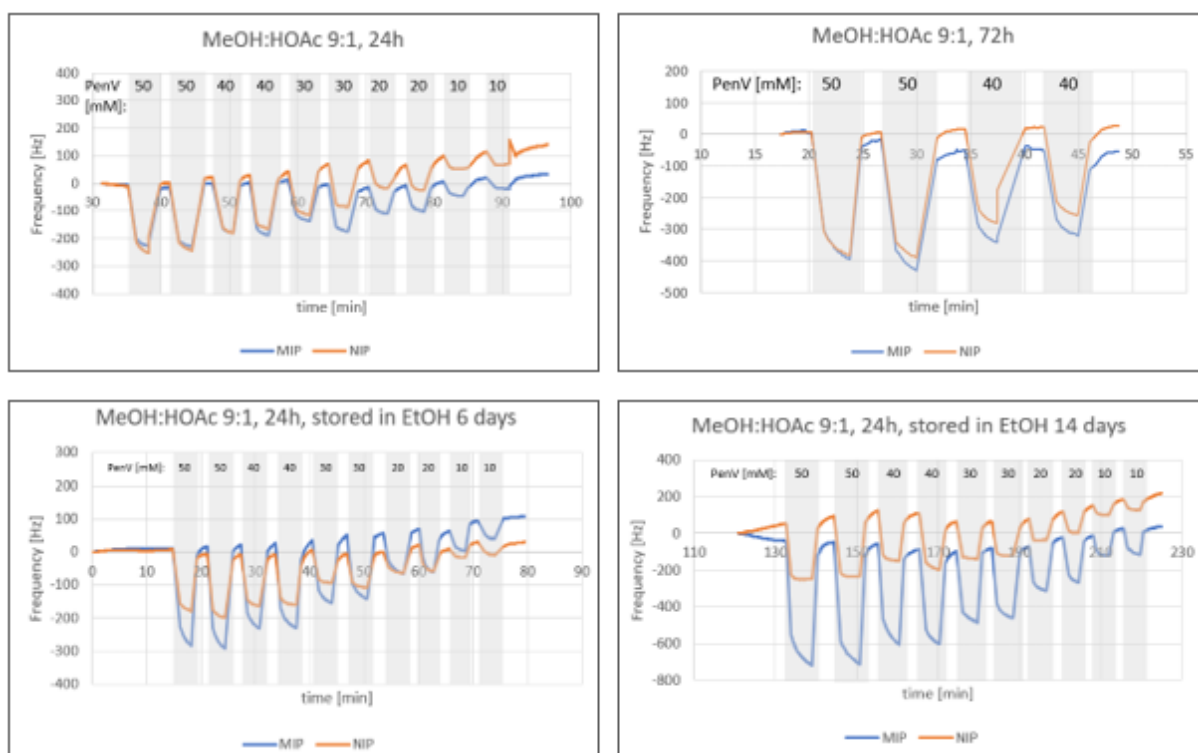


Figure 26: QCM measurements with MIP particles that were extracted under acidic conditions with methanol and acetic acid.

Similar results were obtained for the third template removal technique, where incubation was conducted in alkaline conditions.

Batch mode incubation with alkaline pH (MeOH:Triethylamine 9:1):

When MIP NPs were extracted with methanol and triethylamine for 24 hours, no specific interaction of PenV with the MIP could be observed during QCM measurements (see Figure 27, upper left corner).

When the extraction time was extended to 72 hours, a part of the template seemed to have been removed from the cavities in the MIP, as the MIP showed a higher response to the PenV injection than NIP. (upper right corner of Figure 27)

Again, storing the particles in ethanol after extraction really improved the extraction yield. (see bottom row of Figure 27)

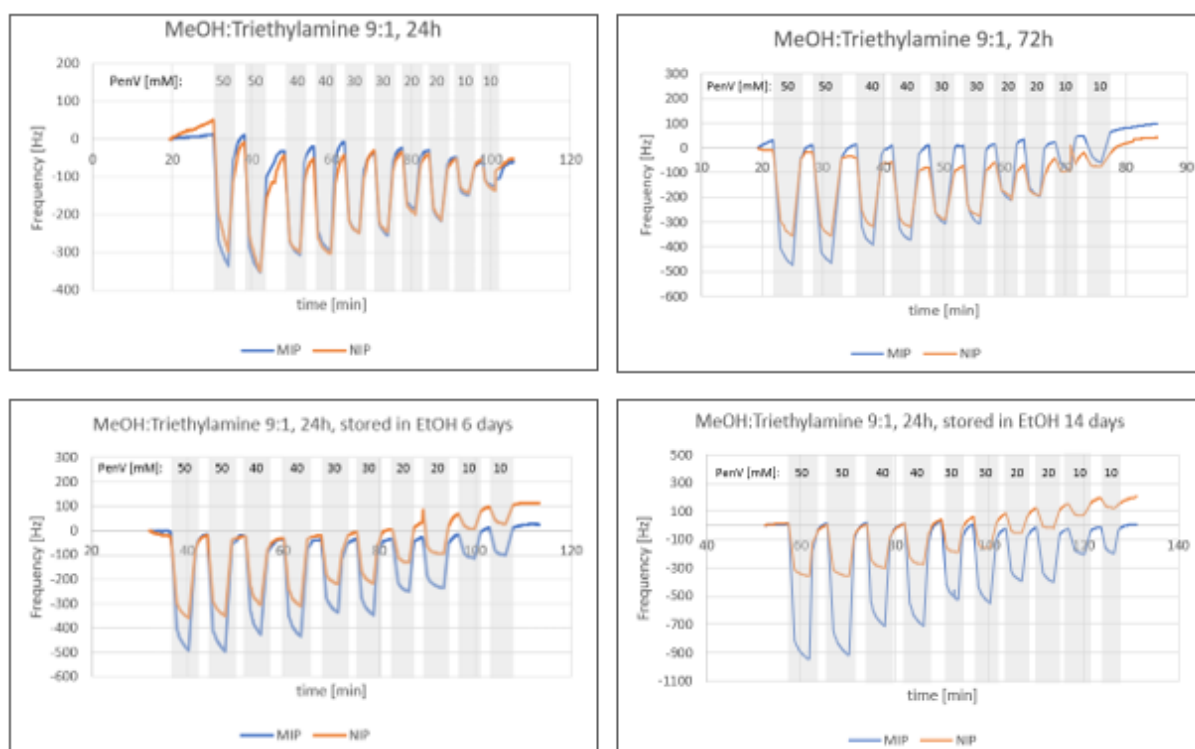


Figure 27: QCM measurements with MIP particles that were extracted under alkaline conditions with methanol and triethylamine.

In conclusion, the different template removal experiments showed that time played a seminal role in extraction. Appreciable extraction yield of the template from the MIP NPs was only observed after storing the particles in ethanol for 14 days.

Furthermore, when comparing the first method to the other two, one can see that incubating only in ethanol did not work and that a first extraction step had to be carried out under harsher conditions (acidic or alkaline pH) so that the template could then be slowly extracted over time with ethanol as a solvent.

One explanation could be that under the harsh acidic or alkaline conditions the lactam ring of PenV is split and then the molecule can be released from the cavity. Or the acidic/alkaline pH changes the protonation/deprotonation of groups within the PenV and is therefore decreasing its affinity to the cavity.

4.1.3. QCM Sensor characteristics

When evaluating the performance of a chemical sensor, there are four important sensor properties that need to be investigated:

Sensitivity – it corresponds to the slope of the calibration curve. The steeper the calibration curve, the higher sensitivity. Sensitivity is assessed by injecting solutions with different concentrations of the analyte and setting up the calibration curve.

Selectivity – A chemical sensor is considered selective when it can detect the analyte alongside other or similar molecules in the matrix. Selectivity of a sensor is examined by injecting solutions of the analyte and structurally similar compounds and calculating the selectivity factors.

Reversibility – In QCM measurements, the signal is considered reversible if the analyte is removed from the binding sites completely and therefore the frequency returns to the baseline.

Reproducibility – Reproducibility means that injecting a certain analyte concentration several times always leads to the same sensor response. [45]

Sensitivity, Reversibility and Reproducibility:

In the following, one can see the sensitivity measurements for two batches, containing three QCM each.

Batch 1:

Figure 28 depicts the MIP channel in blue and the NIP channel in orange color. The graph on the right-hand side shows the corresponding sensor characteristics.

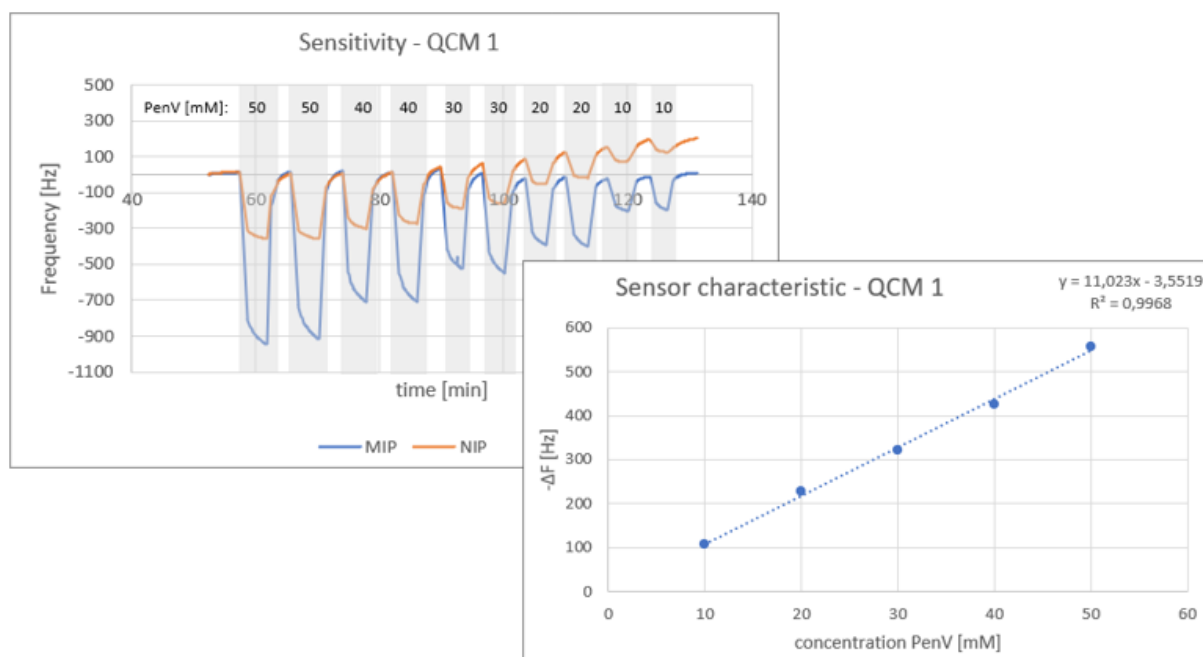


Figure 28: Sensitivity measurements of QCM 1-3 (batch 1).

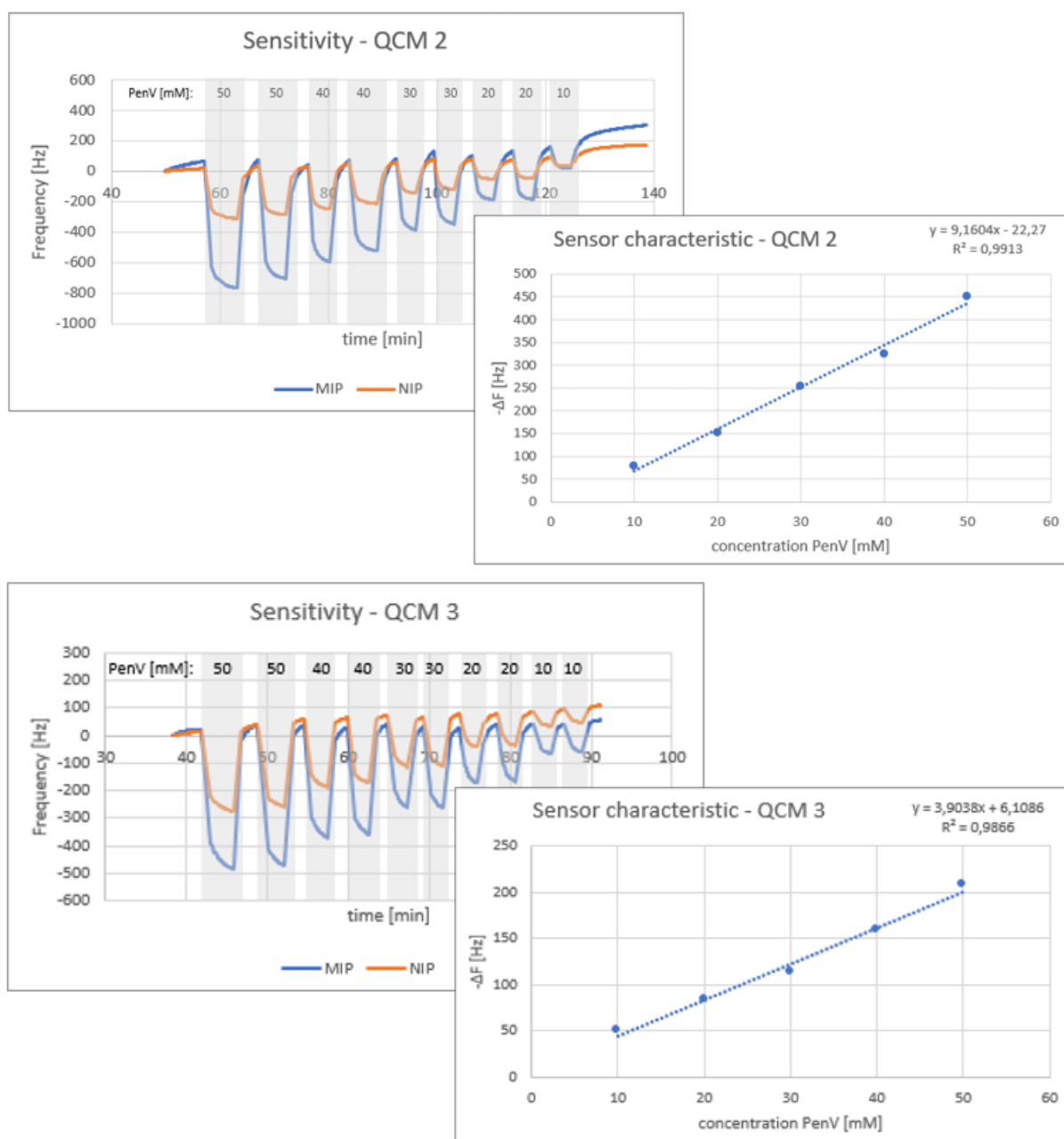


Figure 28: continued.

All three QCM from batch 1 showed concentration-dependent signals when different concentrations of PenV were injected. Clearly, there was a higher response on the MIP side than on the NIP side, indicating that there are specific binding sites present in the MIP NPs. As can be seen in Figure 28, a linear model fitted the calibration curves in a concentration range from 10 mM to 50 mM PenV.

In addition, the signals were fully reversible. When the measuring cell was washed with distilled water, the frequency went back to the baseline for MIP and NIP as well.

In most cases, each PenV concentration was injected twice to see if the response was reproducible. For QCM 1-3 similar sensor responses were obtained for the injection of the

same concentrations. Reproducibility within one QCM measurement was good. However, one can see that the performance within one batch of three QCM was different, even though QCM 1, 2 and 3 were prepared in the same way. QCM 1 and 2 showed about two times higher sensitivity to PenV than QCM 3. The differences could be due to differences in the occupation of the electrodes with particles. Table 13 shows the thickness of the layer on the quartz, which was determined with a network analyzer. The thickness is very similar for MIP and NIP of QCM 1 and 2, however, somehow the thickness of the layer on QCM 3 is much higher. One can therefore assume that there were too many particles present on both MIP and NIP sides of QCM 3. Presumably, multiple particle layers were present on the electrodes. Therefore, the performance of this quartz was lower than that of QCM 1 and 2. Presumably, it is the washing step after spin coating, that removes some of the loosely bound particles and is probably the least reproducible step in the sensor preparation.

Table 13: Thickness of the layer on the electrodes of QCM 1-3, determined with a network analyzer.

QCM	Thickness [nm]	
	MIP	NIP
1	420	360
2	420	300
3	600	480

The limit of detection (LOD) and limit of quantification (LOQ) were calculated for QCM 1-3 using the following equations and are listed in Table 14.

$$LOD = \frac{3 * s}{k} \quad (3)$$

$$LOQ = \frac{10 * s}{k} \quad (4)$$

s standard deviation (baseline)

k slope of the calibration curve

Table 14: Limit of detection and limit of quantification for QCM 1-3.

QCM	LOD	LOQ
1	1.5 mM	4.9 mM
2	1.8 mM	5.9 mM
3	3.0 mM	10.1 mM

Batch 2:

As can be seen in figure 29, in the second batch the signals in the QCM measurements were also all reversible and similar responses were obtained for injections of the same concentration.

The sensor characteristic was linear, but for QCM 5 the measured frequency for 10 mM PenV had to be excluded from the calibration curve to achieve appreciable fit.

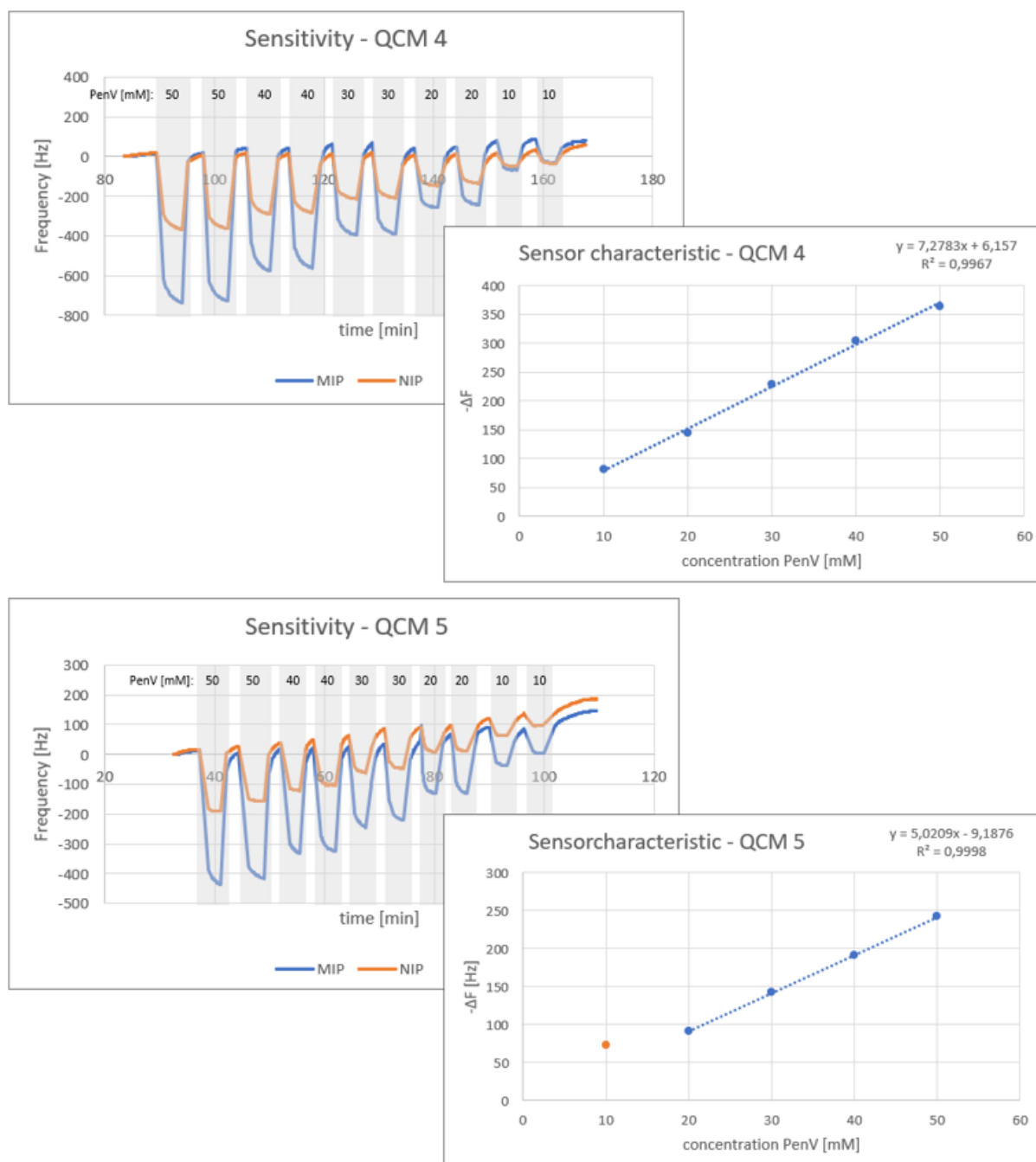


Figure 29: Sensitivity measurements for QCM 4-6 (batch 2).

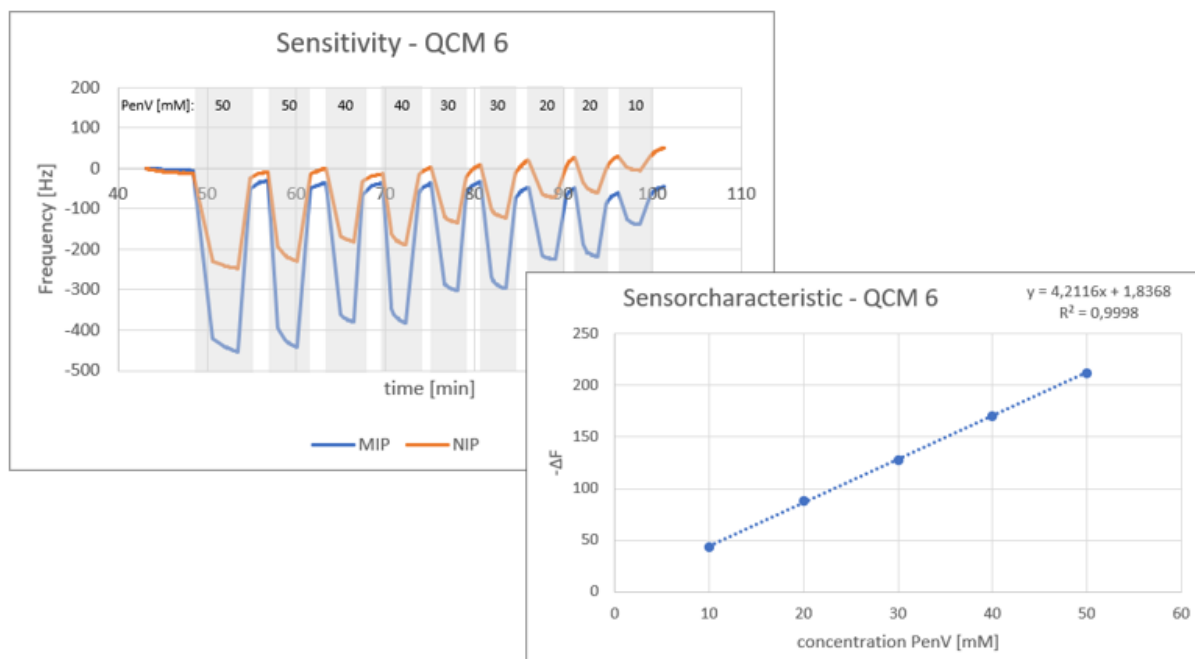


Figure 29: continued.

QCM 4 worked similarly well as QCM 1 and 2 from the first batch. From Table 15 one can see that QCM 4 had similar layer thickness on MIP and NIP like QCM 1 and 2. QCM 5 and 6 were less sensitive to PenV than QCM 4, but one can see that the layer thickness for these two quartzes was lower.

Table 15: Thickness of the layer on the electrodes of QCM 1-3, determined with a network analyzer.

QCM	Thickness [nm]	
	MIP	NIP
4	420	360
5	360	240
6	240	120

The limit of detection (LOD) and limit of quantification (LOQ) for QCM 4-6 are summarized in Table 16.

Table 16: Limit of detection and limit of quantification for QCM 4-6.

QCM	LOD	LOQ
4	0.9 mM	2.8 mM
5	1.3 mM	4.3 mM
6	0.7 mM	2.3 mM

For batch 1 and 2 the lowest obtained LODs were around 1 mM PenV, which corresponds to about 0.4 mg/mL.

Selectivity:

The presence of imprinted binding sites was verified during sensitivity tests, as shown in the previous section, by comparing the sensor responses for MIP and NIP particles, respectively. However, in order to demonstrate that the imprinted binding sites are selective, the sensor response of the MIP for the analyte must be compared with that for a structurally similar molecule. [39] In this case penicillin G was chosen because, like PenV, it is a beta-lactam antibiotic and has a similar chemical structure, as can be seen in Figure 30.

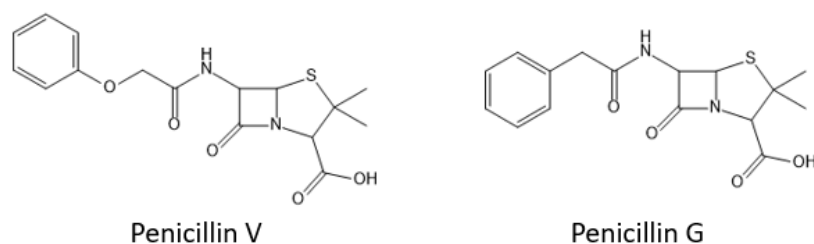


Figure 30: Comparison of the chemical structure of PenV (left) and PenG (right).

For the three QCM sensors that performed best during sensitivity measurements (QCM 1, QCM 2 and QCM 4), selectivity measurements were performed using aqueous solutions of PenV and PenG with concentrations of 50 mM, 25 mM and 12.5 mM.

Figure 31-33 show the results of selectivity measurements of these three QCM. On the left-hand side it shows how the frequency changed for MIP (blue line) and NIP (orange line) side of the sensor when different concentrations of aqueous PenV and PenG solutions were injected. For each injection, the frequency difference between MIP and NIP was calculated and the bar chart on the right shows the frequency difference of the injections normalized to the 50 mM PenV injection. The blue bars represent injections with PenV and the green ones stand for injections with PenG. The selectivity factor (SF) for each concentration is also given in the bar chart.

In the selectivity measurement of QCM 1 (Figure 31) one can see that the selectivity factor between PenV and PenG is 2 for the highest concentration (50 mM). For the two lower concentrations (25 mM, 12.5 mM) the selectivity factor is about 3.

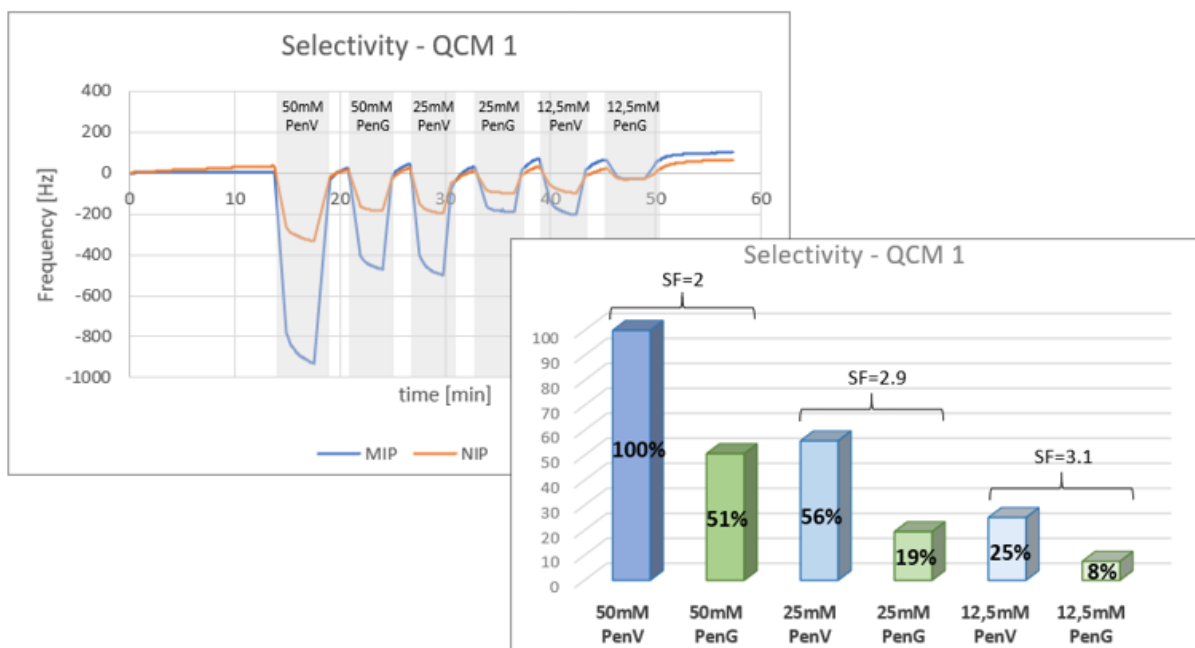


Figure 31: Selectivity measurement for QCM 1.

The difference in the selectivity coefficient for lower and higher concentrations presumably comes from the heterogeneity of the binding sites.

Binding sites in MIPs do not all have the same affinity. In fact, they are very heterogeneous concerning the arrangement of functional monomers and the properties of the environment in the cavities. Particularly in non-covalent imprinting, not all functional monomers are assembled around the template, which leads to the formation of non-specific binding sites. Damaging and collapse of binding sites also increases heterogeneity and non-specific sites. In reality, not only specific and non-specific binding sites are present in MIPs, there rather is a continuous spectrum of binding sites with more or less selective binding properties. [39]

One can assume that injecting aqueous PenV solutions at lower concentrations, the analyte mostly binds to specific binding sites. Higher specificity and affinity of binding sites towards the analyte also correlates with higher selectivity. Therefore, the selectivity factor for injections of lower concentrations of PenV is higher. While the injection of very high concentrations of PenV leads to the occupation of specific and non-specific binding sites as well. Since the non-specific binding sites function less selectively, the selectivity factor is lower for higher concentrations.

Figure 32 shows the selectivity measurements of QCM 2. Again, one can see that the selectivity factor is slightly higher for lower concentrations of PenV. For injections of 25 mM and 12.5 mM PenV and PenG the selectivity factor is 2.4 and 2.1, respectively. The injection of 50 mM PenV and PenG resulted in a lower selectivity factor of 1.9.

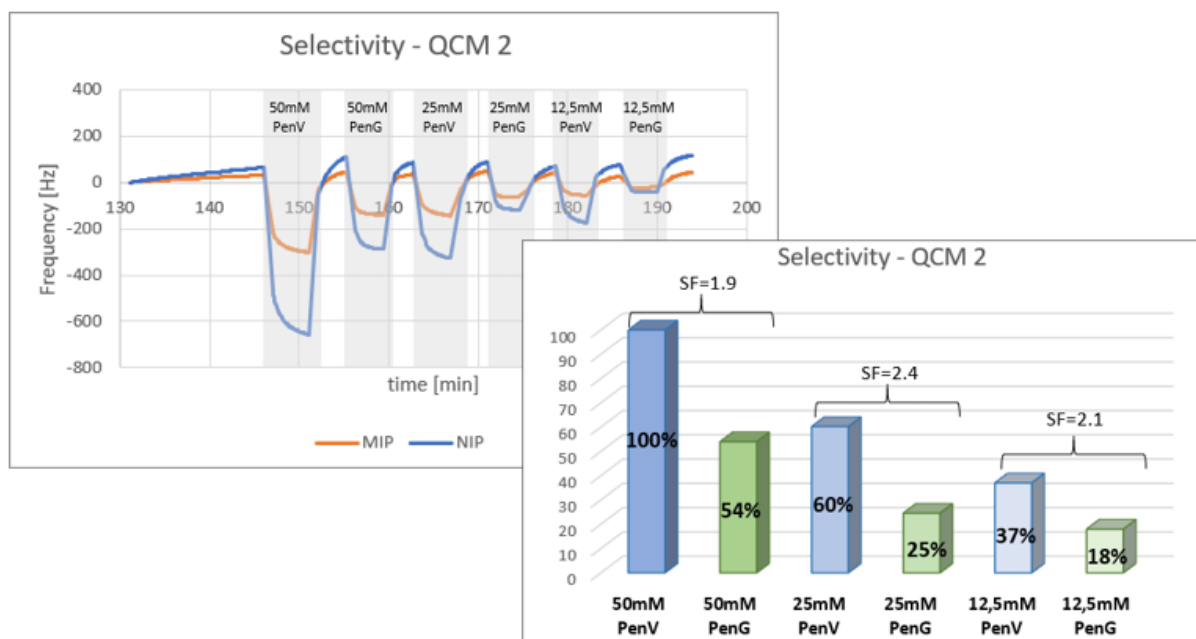


Figure 32: Selectivity measurement for QCM 2.

For QCM 4 (see Figure 33) the selectivity factor was lower than for the other two QCM, namely between 1.5 and 1.9. In this case one does not see the same effect as in the selectivity measurements for QCM 1 and 2. The selectivity factor is the same for the highest and the lowest concentration, namely 1.9.

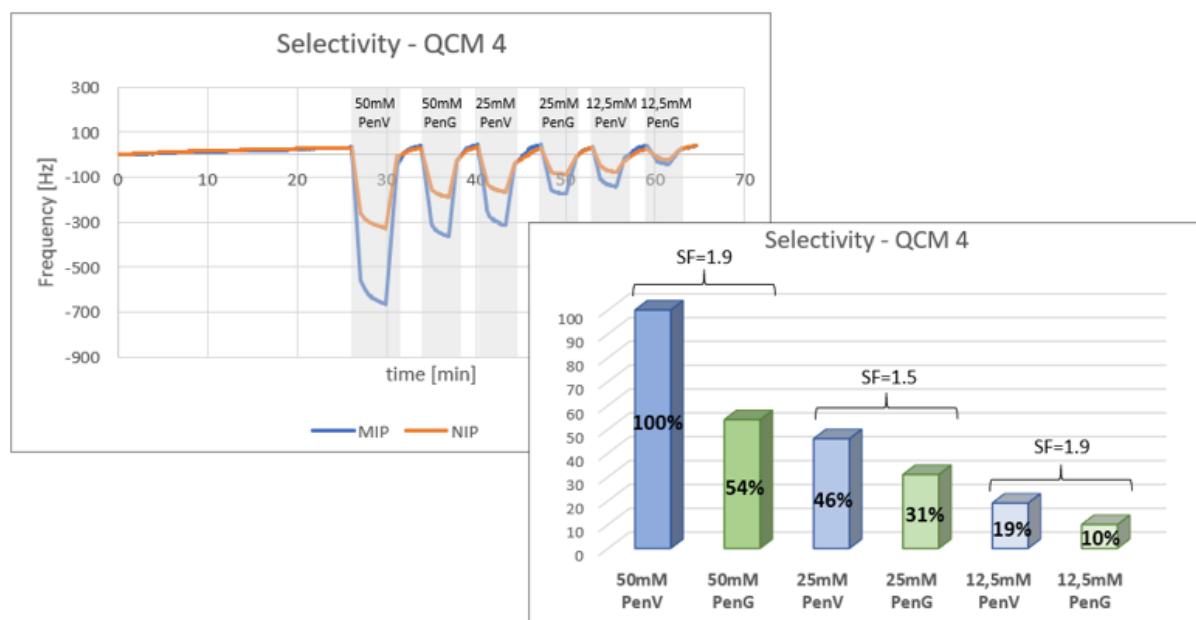


Figure 33: Selectivity measurement for QCM 4.

4.2. Penicillin G imprinted particles

To prepare PenG imprinted MIP NPs, an attempt was made to use the sodium salt of PenG as template. Since the salt type is not soluble in acetonitrile alone, ethanol had to be added to the solvent.

The result can be seen in Figure 34. It shows that the particles are very large and agglomerate rather than having spherical particle shape. Presumably, the agglomeration of the particles results from the ionic interactions because the salt type of the template was used. In addition, the mixture of acetonitrile and ethanol may not have been optimal to perform precipitation polymerization with this polymer system.

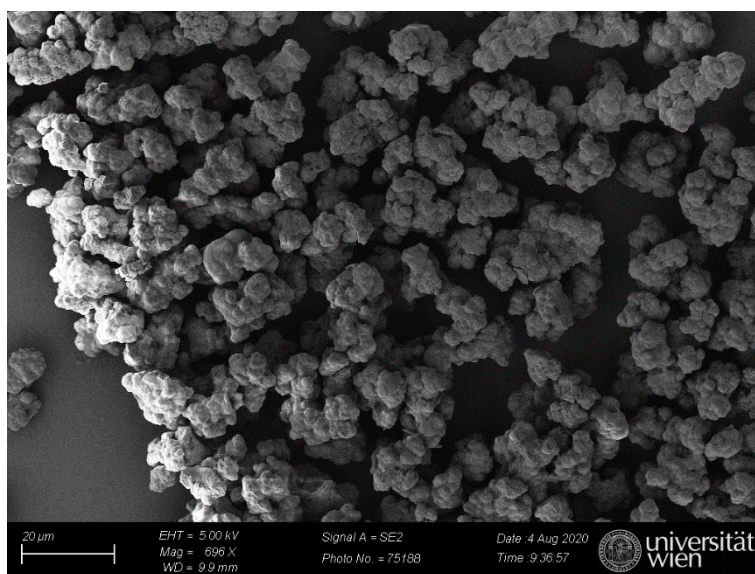


Figure 34: SEM image of PenG imprinted particles.

As already described in chapter 3.3, an attempt was made to prepare the free acid of the template, since then only acetonitrile could have been used as solvent. Since PenG is not stable under acidic conditions, the sodium salt could not be successfully converted to the free acid.

In order to solve this problem more finetuning of the conditions for the conversion to the free acid is needed, but this exceeded the time frame of this work.

4.3. Amoxicillin imprinted particles

As mentioned in chapter 3.4, it was possible to successfully convert the sodium salt of amoxicillin into the free acid. The free acid could therefore be used as template for the MIP NPs. However, another problem occurred because amoxicillin free acid is not soluble in acetonitrile. For this reason, methanol had to be used as the solvent for the precipitation polymerization. As one can see in Figure 35, this also results in very large and agglomerated particles for MIP and NIP. In this case the main problem is to find a suitable solvent that is capable of dissolving all the compounds and at the same time is a poor solvent for the forming polymer and therefore leads to the precipitation of spherical particles.

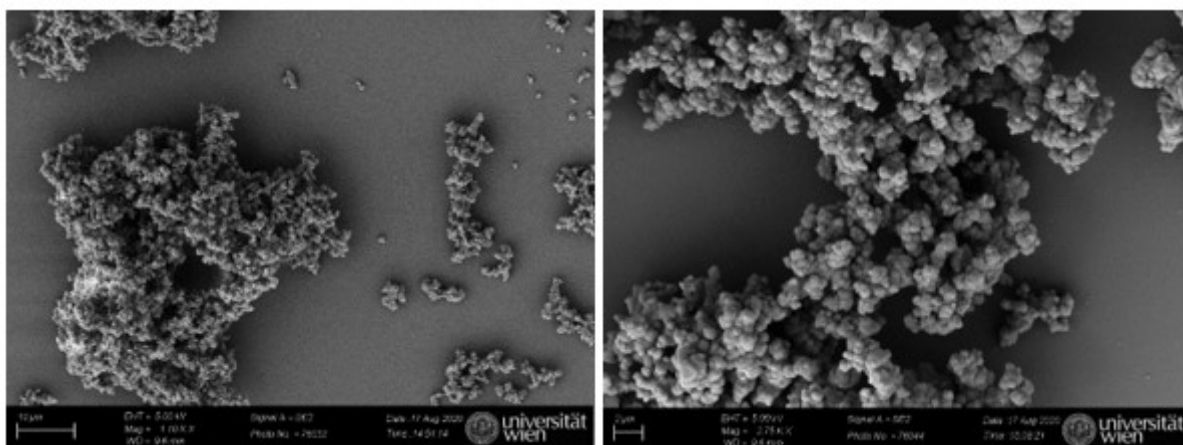


Figure 35: MIP (left) and NIP (right) particles prepared with methanol as solvent in precipitation polymerization.

Therefore, as a next approach a post-dilution polymerization attempt was used by prepolymerizing in a small amount DMSO. Then the prepolymerized solution was added dropwise to an excess of acetonitrile while stirring vigorously.

From Figure 36, one can see that using this approach leads to a small fraction of spherical particles between 100 nm and 300 nm. However, most of the polymer material appears to have precipitated in some sort of sand-like structure.

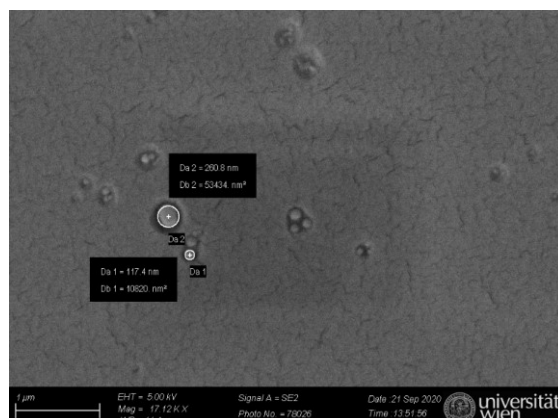


Figure 36: MIP particles for amoxicillin prepared by post-dilution polymerization.

There is further research needed, to improve the particle yield with this technique. It seems to be a promising approach, but the further optimization has exceeded the time frame of this thesis.

5. Conclusion and Outlook

In conclusion, in this work molecularly imprinted polymer nanoparticles were prepared and successfully used as recognition element in a QCM sensor for the determination of penicillin V in aqueous samples.

Studying the influence of polymerization conditions, such as the monomer concentration and the amount of initiator on the size of the particles, the following correlations could be found: A decrease in the used monomer concentration led to smaller particle diameters for MIP and NIP particles. Varying the initiator concentration resulted in different effects in MIP and NIP NPs. While the diameter decreased for NIP NPs with increasing initiator concentration, it was the other way round for MIP NPs. A detailed explanation for possible reasons, including results from relevant literature on this topic, is given in this thesis.

As mentioned, the desired particle diameter for the AQUA NOSE project was below 200 nm in order to introduce the particles into the sensor production process. In order to optimize the particle diameter, an attempt was made to combine the above-mentioned trends to obtain MIP and NIP NPs with diameters below 200 nm. The NIP NPs could successfully be synthesized with a narrow size distribution of around 60-80 nm. While the smallest particle diameters achieved for MIP NPs were around 300 nm. It was assumed that the nature of the template molecule was responsible for this result by changing the solubility of the growing polymer chains in the solution.

However, several possible improvements are still open to study in order to optimize particle size and performance in the sensing process, for example the temperature during prepolymerization and polymerization, photo-initiation, varying the crosslinking degree and finetuning the chemical properties of the binding sites of the MIP to enhance affinity and selectivity, to name a few.

Various methods to extract the template from the cavities were tested and optimized in terms of solvents, the pH value, the extraction mode and the extraction time. The successful removal of the template was confirmed with QCM measurements. Best results were obtained when the particles were incubated in batch-mode in methanol and triethylamine (9:1) for 24 hours, followed by further extraction in ethanol for 14 days. It was shown that for the successful extraction of the template a step under harsh conditions (acidic or alkaline pH) was required and that the complete removal required long extraction times (up to 14 days) in ethanol as a solvent.

These particles were used in combination with a QCM to create a sensor for PenV. Their performance was evaluated in terms of sensitivity, selectivity, reversibility and reproducibility.

By injecting different concentrations of PenV in the sensitivity measurement, it could be confirmed that the signals obtained were fully reversible and that signals for the same concentrations were reproducibly similar. Within the tested concentration range of 10-50 mM PenV, the sensor response was concentration-dependent and linear. The lowest obtained LOD was around 1 mM (~ 0.4 mg/mL) and the LOQ was around 3 mM.

Selectivity was tested by comparing the sensor response of PenV to that of PenG. PenG is also a beta-lactam antibiotic that has similar structural properties. From that, the selectivity factors were calculated. The selectivity factors were different depending on the concentration of the injected solutions. At lower and medium concentrations, the selectivity factor was higher (around 3) than at higher concentrations of PenV and PenG (around 2). It is assumed that this result came from the heterogeneity of the binding sites, since when lower concentrations are injected, only specific binding sites are occupied which can perform rebinding more selectively than the non-specific ones.

With regard to the preparation of PenG and amoxicillin imprinted MIP NPs first steps were made within this work, but to overcome some challenges, further research is needed until MIP and NIP NPs for PenG and amoxicillin can be used as recognition element in chemical sensing, as it has been shown for PenV.

6. References

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7. Appendix

7.1. Abstract

Antibiotics are considered among of the most commonly used drugs in human medicine, but also in the livestock sector, as they allow the control of bacterial infections. However, their misuse and overuse has led to the development of antibiotic-resistant bacteria strains and poses a threat to public health. Monitoring these substances in water systems and the environment is therefore very important. [1, 2, 3]

Ultra-high-performance liquid chromatography high-resolution mass spectrometry (UHPLC-HRMS) is an established method for the qualitative and quantitative measurement of antibiotics. However, it is expensive and time-consuming. For specific analytical problems, chemical sensors can be a simple and non-expensive alternative. [5, 6]

In this work, molecularly imprinted polymer particles were prepared with the beta-lactam antibiotic penicillin V as template in order to use them as selective recognition layer on a quartz crystal microbalance for the detection of penicillin V in aqueous samples.

The influence of different factors, like the monomer concentration, the initiator concentration and the template, on the size of the obtained particles was investigated.

The performance of the prepared sensors was evaluated in terms of sensitivity, selectivity, reversibility and reproducibility. A limit of detection of around 1 mM PenV (~ 0.4 mg/mL) was achieved and the selectivity coefficient between PenV and PenG was between 2 and 3 depending on the used concentration.

The preparation of molecularly imprinted polymer particles for the templates PenG and amoxicillin was challenging and there is further research needed until they can be used in chemical sensing like the PenV MIP NPs.

7.2. Zusammenfassung

Antibiotika ermöglichen die Kontrolle von bakteriell ausgelösten Infektionen und gehören damit zu den am meisten verwendeten Medikamenten in der menschlichen Medizin, aber auch im Veterinärsektor. Der exzessive oder falsche Einsatz dieser Medikamente führt jedoch zur Bildung resistenter Bakterienstämme und stellt eine Gefahr für die öffentliche Gesundheit dar. Deshalb ist das Monitoring dieser Substanzen in Wassersystemen und der Umwelt von großer Bedeutung. [1, 2, 3]

Zu den etablierten Systemen zur qualitativen und quantitativen Bestimmung von Antibiotika zählt die Ultra-Hochleistungs-Flüssigchromatographie gekoppelt mit hochauflösender Massenspektrometrie (UHPLC-HRMS). Diese Methode ist allerdings kostenintensiv und zeitaufwändig. Eine einfachere und günstigere Alternative stellen chemische Sensoren dar, die für spezifische analytische Problemstellungen eingesetzt werden können. [5, 6]

In dieser Arbeit wurden molekular geprägte Polymer Partikel für das beta Lactam Antibiotikum Penicillin V hergestellt, die als selektive Erkennungsschicht auf Quarzkristallmikrowaagen eingesetzt werden können, um PenV in wässrigen Lösungen zu bestimmen.

Es wurden die Faktoren untersucht, die einen Einfluss auf die Größe der entstehenden Partikel haben, zum Beispiel die Monomerkonzentration, die Radikalstarterkonzentration und das Templat selbst.

Die hergestellten Sensoren wurden auf Sensitivität, Selektivität, Reversibilität und Reproduzierbarkeit geprüft. Es wurde ein Detektionslimit von ungefähr 1 mM PenV (~ 0.4 mg/mL) erreicht. Die Selektivitätskoeffizienten zwischen PenV und PenG lagen zwischen 2 und 3 und waren abhängig von der eingesetzten Konzentration.

Die Herstellung von molekular geprägten Polymer Partikeln für das Templat PenG und Amoxicillin brachte Herausforderungen mit sich, die noch weiterer Forschung bedürfen, um in ähnlicher Weise wie die PenV MIP NPs eingesetzt werden zu können.