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DIPLOMARBEIT / DIPLOMA THESIS

Titel der Diplomarbeit / Title of the Diploma Thesis

“*Escherichia Coli* Imprinted Polymer Systems via Emulsion Templating as Sorbents for Preconcentration Methods in Analytical Applications”

verfasst von / submitted by

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angestrebter akademischer Grad / in partial fulfilment of the requirements for the degree of
Magistra der Naturwissenschaften (Mag. rer. nat)

Wien, 2017 / Vienna, 2017

Studienkennzahl lt. Studienblatt /
degree programme code as it appears on
the student record sheet:

A 190 423 344

Studienrichtung lt. Studienblatt /
degree programme as it appears on
the student record sheet:

Lehramtstudium Chemie Englisch

Betreut von / Supervisor:

Univ.-Prof. Mag. Dr. Peter Lieberzeit

Acknowledgements

I would like to express my gratitude to everyone who has contributed to this diploma thesis in one way or the other.

First of all, I would like to thank Peter Lieberzeit and his group for the great support that I have received at all times. A special thanks also goes to Alexander Bismarck and the materials sciences group, whose input has contributed greatly to this thesis. I am especially grateful to Annette Schnettelker and Jiang 'John' Qixiang, who have been very resourceful and have helped me do the best I could do in the course of the project.

Besonderer Dank gilt außerdem meiner Familie, die mich während meines Studiums immer in jeglicher Hinsicht unterstützt hat und es mir ermöglicht hat, meine persönlichen Ziele bestmöglich zu verfolgen. Insbesondere möchte ich mich bei meiner Schwester Lilly für die liebevolle Gestaltung der Grafiken und viele inspirierende Gespräche bedanken.

Für die wertvolle Zeit abseits der Universität, welche immer Quelle für neue Energie und Motivation für mich war, möchte ich mich bei meinem Freund und meinem gesamten Freundeskreis bedanken.

Declaration of originality

I hereby declare that this diploma thesis and the work reported herein was originated and composed entirely from me.

Furthermore, I confirm that the information derived from published and unpublished work of others has been acknowledged in the text and references are given in the list of references.

This work has not been submitted previously or concurrently, insofar as I am aware of, to any examining board.

Vera-Maria Prinz

Vienna, June 2017

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1 Introduction

In the field of biosensors, one of the main issues is the selective detection of one target analyte in the presence of several other, maybe also structurally similar, compounds. Another issue, especially in microsensor technology, is the limited sensibility at low analyte concentrations due to varying limits of detections (LODs) of the sensors. A reliable way to circumvent these problems is via proper sample preparation methods such as solid-phase extraction.

A field of interest in the context of biosensors is the detection of bacteria, which can for example be biological markers for water or food contamination. The bacteria species *Escherichia Coli* (*E. coli*), which is a well studied model organism in microbiology, is often used as an indicator for water contamination. These cells make an interesting and useful template for the development of a target-selective sample pre-treatment system.

Molecular imprinting of polymer surfaces with biomolecules and cells, which has reached a higher level of reliability within the last years, could thus be a feasible way to develop a sorbent material for preconcentration purposes. A special type of polymer systems are so called polyHIPEs, which are produced via emulsion templating. These materials are characterised by a high porosity and thus a large surface area, which is a desirable property for a sorbent material, especially if it were possible to create imprinted sites on the pore walls of such a monolith.

Based on the need for a preconcentration method to detect *E. coli* cells more reliably and in a fitting concentration range, the aim of this diploma project was to develop an *Escherichia Coli* (*E. coli*) imprinted polymer system as sorbent material for sample pre-treatment and filtering applications using the experimental approach of emulsion templating. The developed polymer system should then be characterised, especially concerning bacteria uptake and cross-selectivity, and if possible, the developed SPE sorbent should be coupled to a molecularly imprinted quartz-crystal-microbalance measuring cell developed for the detection of *E. coli* bacteria.

The experimental research for this thesis has been conducted at the Department of Physical Chemistry of the University of Vienna in the group of Univ.-Prof. Mag. Dr. Peter Lieberzeit in collaboration with the group of Prof. Dr. Alexander Bismarck.

2 Chemical Sensing and Sample Pre-Treatment Methods

The acquisition of information about biological systems in real-time has become a field of ongoing research and remains an ongoing challenge due to the complexity of such systems. In recent years, the use of molecularly imprinted polymers (MIPs) in biochemical sensing has become well-established and numerous papers have been published on the matter. The following section will give a brief overview of the state of the art in the field and introduce some important concepts related to biochemical sensing which are especially relevant to this thesis.

2.1 Principles of Chemical Sensing

Chemical sensors allow us to acquire information by an interaction between the sensor and the species of interest. In general, the target species is first recognised and the signal is then amplified, a process also known as transduction.

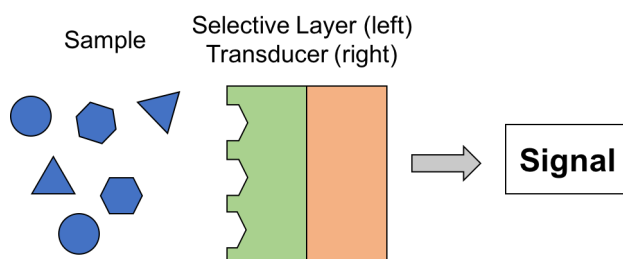


Figure 2.1: Principle of a chemical sensor

Figure 2.1 shows the basic principle of a chemical sensor. A recognition layer interacts with the target analyte or analyte group and undergoes some physical change. This change is then detected by the transducer, which converts this variation into a sensor-specific physical parameter into an electrical output signal. Together, the recognition layer and the transducer form a chemical sensor.

Chemical sensors have to possess a few important characteristics in order to be of analytical use. A good sensor is rugged, i.e. behaves the same at varying environmental conditions, which is key to interpreting any data obtained at several

points in time or at different places. Quality control of any process or product, the understanding of chemical reactions and process optimisation depend heavily on reliable and reproducible data. Furthermore, the higher the sensitivity of a sensor, i.e. the lower the limit of detection (LOD) towards a target analyte or analyte group, the better. If a sensor does not have high sensitivities, a large amount of analyte is needed to gain data, which in turn means that a lot of product needs to be sacrificed for characterisation or, in the case of the analysis of biological matrices, it might not even be possible to carry out any analysis because the sensor is not sensitive enough to detect the often minimal target analyte concentrations [1].

Sensors which can detect smallest amounts of target analyte are referred to as microsensors. They can detect even trace amounts of target analyte abundant in a sample, but are often prone to cross-selectivities and work best in a very specific concentration range. Such microsensors are most relevant to sectors such as clinical analysis, where the amount of sample accessible, e.g. blood, is restricted. To avoid false positive results, which could have a great impact on patients, measures such as sample pre-treatment are employed. Sample enrichment or the separation of a certain analyte or analyte group from a biological matrix such as blood or urine, where several structurally similar compounds are abundant, play an important role in these sectors. A common method to achieve higher concentrations of target analyte is solid-phase extraction, which will be discussed in section 3.3.

A sensor which uses biochemical reactions via a biological selective layer containing specific binding sites to detect chemical compounds is called biosensor. This kind of sensor usually has excellent selectivities and can be used to detect biological target analytes like enzymes, cells, tissues, or antibodies. A binding event causes a biological change in the selective layer and the transducer changes this parameter into an electrical signal. A common problem with biosensors is the strong affinity of the selective layer for the target analyte, which results in very strong and often irreversible binding events about 100 kJ mol^{-1} in energy or more. This irreversibility is the reason why many biosensor systems are made for single-use only [1]. Furthermore, the selective layers are very fragile towards temperature or pH changes as well as towards enzyme digestion [2]. An approach

to mimic and replace real biological antibodies or receptor binding sites is molecular imprinting of polymers, which will be more thoroughly discussed in section 3.2.

Probably the best known example for a microbiosensor is the glucose sensor, which can determine the glucose concentration from a single drop of blood via an amperometric oxygen electrode, and which has been commercially available since the early 1970s. Another sensor that can be used as both a micro- and a biosensor for a great number of target analytes is the quartz crystal microbalance (QCM), which will be briefly presented in the following.

2.1.1 Quartz Crystal Microbalance (QCM)

Mass change detection in very small ranges via quartz crystal microbalances has the potential to evolve into an important tool in clinical diagnostics. These mass-sensitive sensors can give a signal based on their piezoelectric properties. A mass change on the sensor will result in a frequency shift of the oscillation of the quartz material proportional to the additional mass of the system. QCM balances are the most prominent example of so called Thickness-Shear Mode (TSM) Resonators.

The Sauerbrey-equation establishes the relationship between the frequency change and mass change on the quartz surface:

$$\Delta f = -\frac{2f_0^2}{A\sqrt{\rho_q\mu_q}}\Delta m$$

As can be seen, the frequency shift Δf depends on the resonance frequency (f_0) of the material, the area between the electrodes (A), density (ρ_q) and shear modulus (μ) of the quartz and finally on the mass change (Δm).

The fact that quartz is the most widely commercially used material for such sensors is due to its mechanical, electrical, chemical and thermal properties [3]. What determines the frequency and thus the sensitivity of a QCM-balance is the quartz thickness, typical frequencies used range from 5 to 10 MHz. Although higher frequencies could be obtained using thinner quartzes, which would increase sensitivity, however, thinner quartz wafers are more fragile and crack more easily. Figure 2.2 shows the basic principle of a QCM coated with a gold surface.

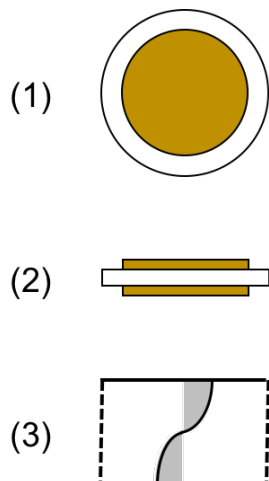


Figure 2.2: Principle of a QCM. (1) Top view of quartz wafer with gold coating. (2) Side view. (3) Mode of vibration side view. After Reference [1].

What makes QCM so applicable as biosensors is the fact that they can be used in gas phase, but also in liquid environments using flow-through circuits. This allows an application of these sensors not only in mass sensing but also in interfacial studies. The addition of a selective layer on top of the gold electrode surface transforms the QCM balance into a powerful biological sensor. However, using QCM in liquids also adds several parameters which might influence the measurements, especially when an additional selective layer is added on the surface of the quartz wafer [1]. Especially with regards to the roughness and wettability of the additional layer, a few key principles have to be kept in mind: High roughness can lead to hydrophobic cavities which might entrap air or produce a vacuum and can thus lead to false sensor responses. A similar observation can be made about hydrophilic surfaces, which can entrap liquids and thus increase the mass load on the quartz surface [3]. Since the dynamics of solvent and sample interaction with any selective (polymer) layer on an electrode surface is very complex and has not been completely and thoroughly described yet, new protocols need to be developed for every target analyte, which is an ongoing field of study in sensor development.

Since effects from the solvent interacting with the selective layer such as shear effects or entrapment of water molecules can never be completely ruled out, it is good practice to use a two-electrode system (Figure 2.3) connected to two separate oscillating circuits. One electrode has a selective layer and the other



Figure 2.3: QCM wafer with two-electrode system for use in microsensing

one serves as a reference electrode. This way, it is possible to distinguish solvent effects from actual binding events and relevant interaction between target analyte and sensor surface.

Although QCM-measurements are very sensitive, some drawbacks to this method remain. Factors such as solvent effects or experimental issues like air bubbles in the measuring chamber might influence the measurement. Also, it is important that the electronics which are used are reliable in order to obtain reliable results. Although QCM quartzes can normally be used several times without the need for regeneration, it is still not possible to obtain completely reproducible results from different quartzes which have the same selective layer. This is probably due to several complex interactions between solvent, analyte and surface and also a great factor of randomness in surface structure, especially when polymer systems are used.

Further problems especially relevant to the detection of cells such as *E. coli* include the fact that cells on a quartz surface behave like viscoelastic bodies rather than like an ideal rigid mass in flow-through circuits. This can lead to considerable damping effect and false results of actual mass load [3]. Furthermore, sensitive QCM layers which are not rigid, or which do not interact strongly with the analyte and result in analyte particles moving freely in the solvent medium can contribute to a so-called anti-Sauerbrey effect which results in a positive frequency shift although additional mass might be added [4].

Although several constraints are still present when using QCM measurements for the detection of bioanalytes, the possibility to measure quickly, sensitively

and label-free makes this technology an exceptionally useful tool in fields such as clinical analytics or in the food industry. The method has been successfully used to detect numerous bioanalytes and remains one of the most important tools in biosensor development, especially in context with molecularly imprinted polymer layers, which will be discussed in the next section.

2.2 Molecularly Imprinted Polymers (MIPs)

The field of molecular imprinting of polymers is growing rapidly and it has become a well-established technique in several areas, especially in analytical chemistry, due to its manifold applicability and its flexibility in terms of target molecules and polymer matrices. Molecular imprinting has amongst other areas been applied in catalysis, biosensors, for purification and isolation purposes as well as for chiral separation [5].

Molecular imprinting is a process to create imprints of a specific molecule on the surface of a polymer in order to imitate biological receptors. As is shown in Figure 2.4, these imprints resemble the target analyte in size and shape and represent preferential binding sites for the respective target analyte.

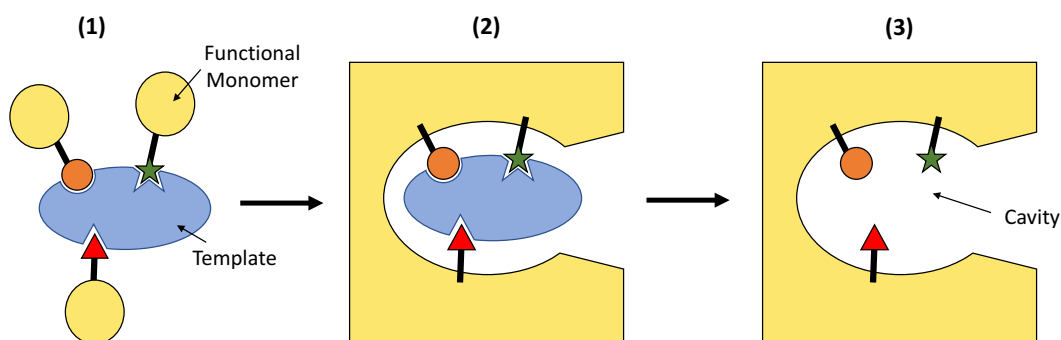


Figure 2.4: Principle of Molecular Imprinting of Polymers: (1) Self-Assembly of Functional Monomer around the Template Molecule. (2) Polymer Matrix after Polymerisation. (3) Molecularly Imprinted Polymer after Removal of the Template.

There are several methods of imprinting, which all include the formation of a polymer network around a target analyte molecule often called template molecule. Through this process, sites of molecule-specific binding are created in an otherwise homogeneous polymer surface. There are three main methods to create molecularly imprinted polymers, namely bulk imprinting, epitope imprinting and surface imprinting. The latter is also often the method of choice for imprinting macromolecules such as proteins, as efficient template removal is otherwise difficult for those molecules [5].

Molecular imprinting is also an interesting technique when it comes to creating selective layers for (bio)sensors due to the special characteristics of the material. Especially attractive qualities compared to their natural equivalents are robustness and high stability under varying operating conditions such as pH and temperature variations as well as the relatively easy preparation and cheap starting materials to produce MIPs [6]. Furthermore, the production of natural receptors often includes animals [2] and could thus also raise ethical concerns.

2.2.1 Microorganism Imprinting for Biosensor Applications

The technique of molecular imprinting has become successfully established especially when it comes to smaller target molecules. For macromolecule or microorganism imprinting, there are still several issues at hand which need further development. Since in protein binding, the current state-of-the art is still mostly dependent on expensive antibodies which are often usable only once, the demand for well-developed imprinting techniques for these larger molecules and cells is large [7].

Several proteins and macroorganisms have already been successfully imprinted in a variety of polymer materials. Examples of imprinted proteins include haemoglobin, lysozyme, ribonuclease A or myoglobin [8]. Bacteria species which have been successfully imprinted are amongst others species such as *B. subtilis*, *E. coli*, *M. luteus* or *S. aureus*. The polymer systems chosen for imprinting are as manifold as the template molecules and range from polyurethane, pyrrole and a variety of silanes to methacrylic acid and several acrylate based polymers [2].

Some of the problems only faced when imprinting macromolecules and thus also microorganisms are related to the analyte size, solubility, complexity and flexibility of conformation. Due to the size of the template molecules, entrapment of template in the polymer network is an issue which is often reported in literature. Another difficulty is the fact that most biomolecules are mainly soluble in water, which is not a good solvent for molecular imprinting procedures. Due to the complexity and existence of several possible binding sites in these molecules, cross-selectivity is often an issue when dealing with large template molecules. Furthermore, proteins and microorganisms are often flexible in conformation and might, upon thermopolymerisation, adapt conformations which are not abundant at standard conditions, leaving unnatural binding sites in the material [7], [9].

To remove template molecules from the molecularly imprinted polymer, several procedures have become established. The most commonly employed techniques to remove biomolecules include washing with highly concentrated salt solutions (0.5 M salt concentration), washing with 10% SDS/10% Acetic Acid or Tween-20 (as detergent) or with NaOH/SDS mixtures [9]. In several biomolecule-imprinting papers published, washing procedures are not well explained or not mentioned at all and the question of potential template leakage is not addressed, which can be a factor severely influencing any sensitivity measurements. Another severe drawback to using SDS as a washing agent reported by Verheyen et al. is the finding that any residual SDS molecules which might have entered the polymer network can significantly increase nonspecific binding or even lead to protein precipitation [9].

2.3 Solid-Phase Extraction (SPE)

For analytical purposes, sample preparation is one of the most crucial steps to obtain highly reliable results. The right choice of sample preparation methods is thus an important factor to consider in experimental design. Especially in bio-analytical methods, extraction of some kind will often be performed. There are several extraction methods, which are mostly based on the principles of volatilization, hydrophobicity, acid-base equilibria or the distribution of hydrophobic ionogenic organic compounds. The most common extraction methods include liquid-liquid extraction (LLE), where an analyte is separated via two immiscible liquid

phases [10] and solid-phase extraction (SPE), where the liquid sample is partitioned via a solid phase. Advantages such as effective pre-concentration, high recovery, a greater possibility for automation and the need for less organic solvent make SPE a very popular approach for sample preparation [11].

The retention mechanism in solid-phase extraction is based on sorption of the analyte onto the solid phase. To obtain a sorption effect strong enough to be interesting for analytical purposes, the solid phase must exhibit a strong affinity for the target analyte. This affinity of the material can be expressed mathematically through the Nernst distribution law:

$$K_D = \frac{C_A}{C_B} \quad (1)$$

In this equation, K_D is the distribution coefficient, and C_A and C_B are the concentrations of the analyte in the liquid sample phase and in the sorbent extracting phase respectively. Thus, if a sorbent is effective in separating the analyte, i.e. has a strong affinity to the target analyte, a large K_D value will be observed. It is important to note that solid-phase extraction is not a filtration process, as the analyte molecules are in a homogeneous solution in the sample and adsorption are the reasons for retention of the analyte, rather than size exclusion (see Figure 2.5).

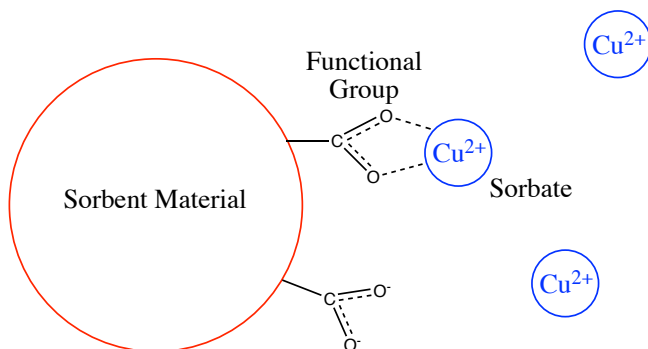


Figure 2.5: Example of interaction between sorbate and sorbent material.

The most important consideration when doing SPE is the choice of sorbent, which determines selectivity, affinity and capacity and depends strongly on the physical and chemical properties of the analyte [11]. A good sorbent material should take up a high, reproducible percentage of the analyte and allow for the analyte to be eluted again after the sorption process. Additionally, factors such as a large surface area, the absence of impurities and stability towards the sample matrix and elution solvents are important. Nowadays, a great variety of sorbent materials is available for a wide range of analytes. SPE sorbent materials include polar polymeric bonded silica, graphitized carbon sorbents, ion-exchange sorbents, immunoaffinity sorbents and molecularly imprinted polymer sorbents [12].

2.3.1 Molecularly Imprinted Polymer Solid-Phase Extraction (MISPE)

Molecularly imprinted polymer solid-phase extraction (MISPE) is based on materials designed for highly specific analyte extraction via molecular imprinting. The great potential of molecularly imprinted materials for SPE purposes has first been mentioned in 1994 by Sellergren [13]. Especially in bioanalytical purposes, it can occur that several analytes exhibit similar chemical and physical properties and will thus not be sufficiently separated through conventional SPE sorbents. Molecular imprinting offers a promising alternative through its ability to selectively extract one target analyte or a defined group of compounds and reduce co-extraction issues, thus avoiding further clean-up steps in the sample preparation [14]. The principles of molecular imprinting have already been discussed in a previous section. What makes these MIP materials suitable sorbents for SPE is not only their high affinity for the target molecule(s), but also their robustness and stability under conditions such as high temperature or varying pH environments. Drawbacks are the general difficulties of removing the template molecules from MIP materials, which might lead to leaking of these template molecules during the extraction and give false results. Furthermore, since the affinity of the MIP sorbents for the target analyte is very high, it is often difficult to quantitatively and quickly desorb the analyte molecules or particles [15].

Several studies have already implemented MISPE successfully, however, there are still some areas which need further investigation. Chianella et al. reported

effective pre-concentration up to 1000-fold for their MISPE material for extracting the toxin microcystin-LR and the use of molecular imprinted polymers for both the extraction and the corresponding sensor to increase sensitivity and specificity. They used a bulk imprinting technique to prepare the MIPs and could effectively recover the analyte for concentrations from 1-50 nM. The MIPs performed slightly better than the controls with non-imprinted polymers (NIPs), however, especially in low concentration range, the errors increased significantly. Cross-reactivity was tested against analogues of the target analyte and a good affinity for microcystin-LR was found. Chianella et al did not mention any measures to confirm that there is no leakage of template molecule from the MISPE material [16].

Watabe et al (2004) showed effective use of a molecularly imprinted polymer for preconcentration to detect trace amounts of the endocrine disruptor bisphenol A (BPA), which is often abundant in environmental water up to a detection limit of 0.36 ng/L. They developed a fitting MIP material via a two-step swelling and polymerisation method and successfully used it in a column-switching auto pre-treatment system for HPLC detection. Their results showed good repeatability for higher concentrations of BPA (100 ng/L), with a relative standard deviation (R.S.D.) of only 0.5%, but an increasing error of up to 9.3% R.S.D. for concentrations at 1 ng/L. Tests showed that the set-up worked well for the purpose, could avoid contamination from manual pre-treatment and gave reproducible results also with actual river water samples, with a R.S.D. of 2.4% for approximately 20 ng/L BPA content. The group also reported issues with interference due to small retention capacities and poor selectivity of the pre-treatment column and suggest a surface modification of the MIP as a possible solution, but did not report of testing this hypothesis. They did not mention running tests for cross-reactivity of the sorbent material, but they reported an effort to avoid leakage of template molecule by using p-tert.-butylphenol as a pseudo-template which can be separated from the real target compound BPA [17].

In an effort to develop a fast and sensitive way to detect the broad-spectrum antibiotic Florfenicol (FF) in food, Sadeghi and Jahani (2013) produced a MIP-SPE material via bulk imprinting and precipitation imprinting of FF. They described good site accessibility, high adsorption capacity and selectivity for FF, especially for the MIP produced via precipitation imprinting. They also reported the successful implementation of the sorbent material as means of preconcentration for

detection in HPLC, with recovery-rates ranging from 96.2% for honey samples to 88.9% for fish samples. Cross-selectivity with coexisting substances was tested and it was found that the MISPE sorbent showed high cross-selectivity against the non-fluorinated original compound chloramphenicol, but also against the common amino acid histidine, with a tolerable concentration ratio of less than 1 for both substances. The authors also emphasised the importance of the washing step and the elution step, since these parts of the analysis are more difficult for MISPE than for standard SPE sorbents [18].

As can be seen from these examples, MISPE is an interesting alternative for sample pre-treatment purposes, especially when it comes to more complex sample matrices where several structurally similar compounds might coexist. The application of this type of sorbent has successfully been implemented for relatively small molecules like BPA, but also for larger compounds such as microcystin, although there are still some issues about cross-reactivity and the potential leakage of template molecule from the imprinted polymer. That the technology is promising is shown by the fact that some MISPE systems are already commercially available [19], including systems selective for BPA and aminoglycosides. Bacteria-imprinted MIPs have been developed but not tested specifically for SPE purposes, and therefore it seems to be an interesting area of investigation.

2.4 *Escherichia Coli* Bacteria

The bacteria species *Escherichia Coli* (*E. Coli*) has been selected as template cell for this thesis for several reasons.

The gram-negative, rod-shaped *E. Coli* cells (see Figure 2.6) have long been used as a model organism in microbiology and thus, their surface structure is well studied. As gram-negative bacteria, these cells possess a bacterial outer membrane, in contrast to gram-positive cells. While most *E. Coli* strains are harmless, some can cause illness in humans. What is more, this type of bacterium is always of fecal origin and is thus often used as an indicator organism for water contamination.

Their cell structure is characterised by an outer membrane made of polysaccharides, a peptidoglycan layer and an cytoplasmic inner membrane. The outer

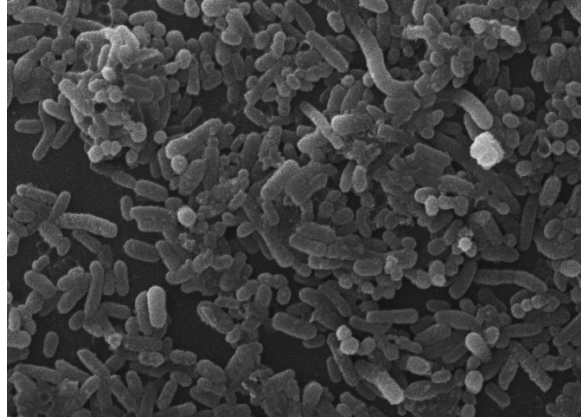


Figure 2.6: SEM image of *E. coli* cells on a polymer surface

polysaccharide membrane distinguishes this kind of bacterium from so called gram-positive bacteria, which do not possess this outer membrane. The outer membrane works as an additional layer, which makes it also harder to induce cell lysis and destroy the cells.

Several methods to induce cell lysis in microbial cells, i.e. the breakdown of the cell wall, exist, such as mechanical lysis, liquid homogenization, sonication or manual grinding. Additionally, physical disruption can be facilitated by adding reagents such as lysozyme, which can digest the polysaccharide layer. A commonly employed lysis method is alkaline lysis, which is often used for plasmid DNA extraction and consists of suspending bacteria cells in a lysis buffer made from sodium hydroxide and sodium dodecyl sulfate (SDS). The cell wall is solubilised by SDS and NaOH disrupts the hydrogen bonding between the DNA bases leading to protein denaturation.

To work with bacteria cells under meaningful conditions, it is often important to work at a stable pH and temperature. Such conditions can be created when working in a buffered environment with a pH around 6-8, since most biological reactions take place in that pH range.

3 Polymer materials via emulsion templating

It has now been established that polymer materials can be very useful due to their applications in biosensors. The following section will introduce the materials relevant to this thesis, such as PolyHIPEs and structures made from Pickering Emulsions.

3.1 Emulsions

An emulsion is a disperse colloidal system which normally consists of two immiscible liquid phases. Most emulsions consist of an aqueous and a hydrocarbon (oil) phase as well as an emulsifying agent (stabilizer), which enhances kinetic stability of the system.

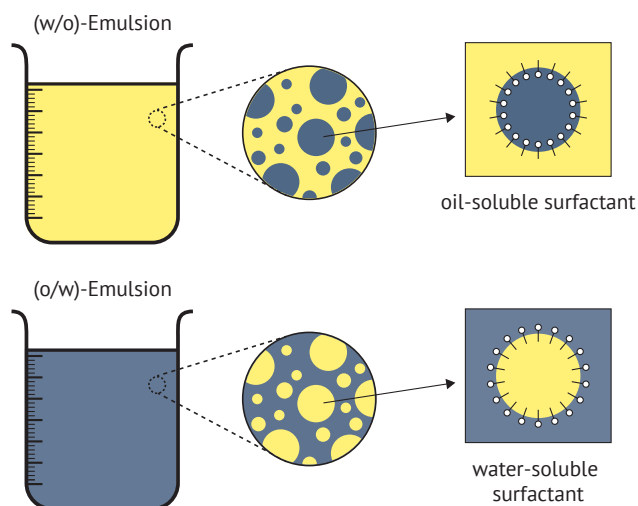


Figure 3.1: Principle of emulsion stabilisation.

Depending on which phase is the dispersed (internal) phase, the emulsion is referred to as water-in-oil (w/o) or oil-in-water (o/w) emulsion (Figure 3.1). The continuous phase is also referred to as external phase. The stabilizer is normally a surfactant, macromolecule or finely spread solid particles. Surfactants contain a hydrophilic and a lipophilic part. The relationship between hydrophilic and lipophilic portion of a nonionic surface active agent can be expressed via the so-called hydrophile lipophile balance (HLB). If a surfactant has a high HLB-value,

it has good water-solubility, i.e. the water-loving part of the surfactant makes up a large part of the total functionality. To prepare (w/o)-emulsions, the HLB-value of the used surfactant has to be low, whereas for (o/w)-emulsions, a high HLB-value is required.

Emulsions are not thermodynamically stable systems, but can be (kinetically) stable for hours up to years depending on storage conditions and stabilising agents. Several breakdown processes, such as coalescence, phase inversion or Ostwald ripening, can occur [20]. If one or both phases of an emulsion contain monomers, they can be polymerised. The application of this principle as a technique in material science is often referred to as emulsion templating.

Emulsion templating is a well-established and flexible method to produce (macro)porous polymer materials. The technique allows for a great variety of starting materials and can also be adapted to specific needs in terms of form, structure or surface chemistry of the product. Emulsion templating can be used to create either porous materials or beads, if it is a reversed emulsion system. In the following, two types of emulsions and their cured products will be discussed, namely high internal phase emulsions (HIPEs) and Pickering Emulsions.

3.2 HIPEs and PolyHIPEs

3.2.1 Introduction

Poly-(High Internal Phase Emulsions) (PolyHIPEs) are a special kind of porous material which is prepared via emulsion templating from a high internal phase emulsion (HIPE). These materials are often characterised by the internal phase ratio of the HIPE, which typically makes up 74% or more of the total volume of the emulsion. Although 74% is the maximum volume ratio that non-deformable, uniform spheres can take up, much higher internal phase ratios can be achieved through choice of the right stabilising agent. It is clear, however, that the droplets in a HIPE will not be uniform in size and can also take on polyhedral shape [21]. Upon polymerisation, the internal phase is removed through drying, creating a 3D-scaffold made up from the polymer in the continuous phase. The droplets of the dispersed phase leave pores resembling their shape and size upon drying as well as pore throats connecting the pores, sometimes also referred to as pore windows, which is shown in Figure 3.2.

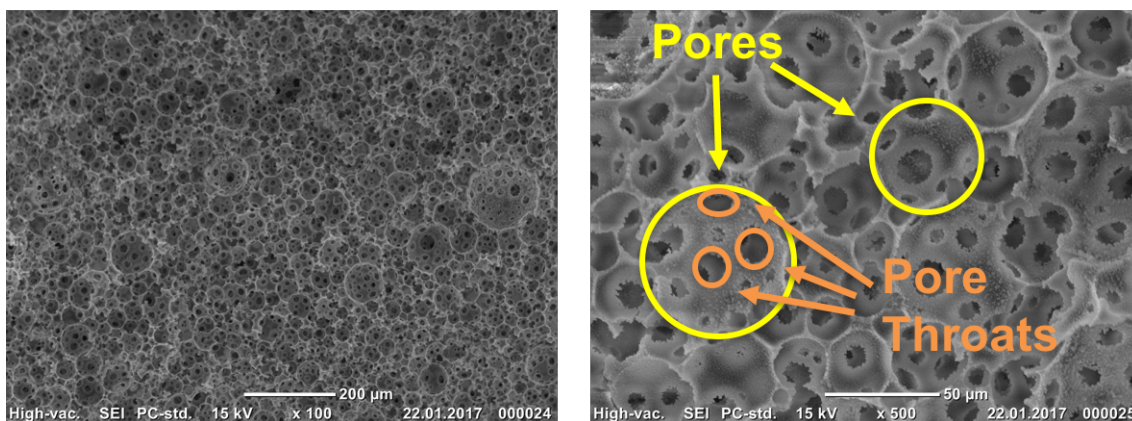


Figure 3.2: Typical PolyHIPE structure obtained from an acrylate-based HIPE; left: 100x magnification, right: 500x magnification

3.2.2 PolyHIPE Morphology

One advantage of polyHIPEs is that their structural and chemical surface properties can be specifically designed towards a certain outcome through emulsion templating. Emulsion templating is a common technique to produce macroporous systems and it offers many advantages through the flexibility of the experimental design and the quite simple process of preparing emulsions. Apart from being used as a means to produce macroporous systems, emulsions have manifold applications in everyday life, especially in the cosmetics and food industries, but also in industrial separation techniques such as flotation.

There are various factors which can be experimentally altered in order to achieve certain properties in the resulting polyHIPE. This section will give a brief overview of some of the most important features which can be tuned via experimental design. A schematic protocol to produce the precursor high internal phase emulsion for a (w/o)-emulsion and the resulting porous polymer (polyHIPE) is shown in Figure 3.3. The continuous phase, consisting of oil, surfactant and initiator, is stirred at a continuous speed, usually using an overhead stirrer. The disperse phase is dripped in constantly to give a homogenous emulsion while stirring is continued.

Emulsion Templating Process

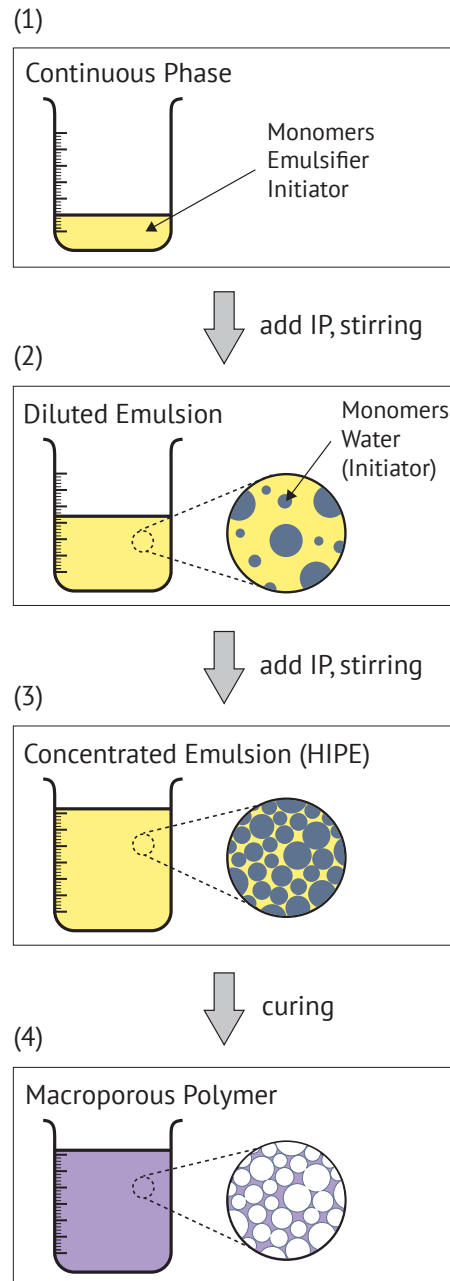


Figure 3.3: Emulsion templating process resulting in a macroporous polymer (PolyHIPE) material

First of all, the nature of the chosen monomer influences the surface qualities of the polyHIPE. A well-established monomer mixture to produce a polyHIPE is for example based on an (w/o)-emulsion of the hydrophobic monomer styrene and the crosslinker divinylbenzene (DVB). The resulting polymer will thus be hydrophobic as well. Choice of a hydrophilic monomer such as an acrylate and a fitting crosslinking monomer to give an (o/w)- emulsion will result in a hydrophilic material [22]. Although the available options seem endless, there are limits as to the stability of the structure of the resulting materials. However, it is also possible to create a compound material by using monomer in both the internal and continuous phase, resulting in a layer of internal phase monomer copolymerised onto the continuous phase scaffold [23]. This approach allows creating a stable support structure together with the desired surface properties for the application of the polyHIPE.

The size of the resulting pores can also be influenced up to a certain point. For example, a low internal phase ratio will result in small pores. Apart from that, it mostly depends on the kinetic stability of the HIPE, which is influenced by factors such as surfactant concentration and energy input from stirring [24]. Also the amount of surfactant plays a certain role as to how big the droplets and their resulting pores can become in the emulsion. Furthermore, the chemical composition of the continuous phase has been shown to influence the pore size as well [21].

Whether a polyHIPE has a closed or open cell structure (i.e. possesses pore throats) also depends on several factors. The origins of pore throats, which interconnect the pores, are not yet completely studied. Cameron et al. suggest that the pore throats arise from shrinking after the gel point during the polymerisation phase. Menner and Bismarck [25], however, theorise that the pore throats are a result of the purification process of the polyHIPE, explaining why there is no observable overall shrinkage of the resulting monoliths, a theory which has been met with scepticism [24]. Although the mechanism is not clear yet, some factors can be mentioned which favour an open cell system. A higher internal phase ratio, which reduces the layer thickness of polymer between the droplets, leads to a more open-porous system. Type and amount of surfactant also play an important role, with a general rule that an increase in surfactant will also lead to an increase in pore throat size [24]. Lastly, there is also evidence suggesting the

type of initiator which is used influences the openness of the system [26]. For a further review on the morphology of polyHIPEs, see [21], [24] or [22].

3.2.3 PolyHIPE Surface-Modification

Apart from the correct choice of monomer system, surfaces of the polyHIPE can be modified by various means, such as grafting, copolymerisation or molecular imprinting. Especially in organic synthesis, polymer supported chemistry has become widespread, for example as support for catalysts or reagents. Chemin et al. produced tin hydride and thiol grafted polyHIPEs and tested the use of these materials as reducing agent and catalyst respectively. The DVB-based polyHIPE surface was modified by adding a thioacetic acid, which reacts with the vinyl groups of the polymer surface and results in supported mercapto-SH groups on the polyHIPE after aminolysis. Tin hydride grafted polyHIPEs were produced via polymerisation of a SnCl-functionalised styrene monomer together with styrene and DVB as crosslinker. The SnCl-containing surface was then treated with NaBH₄ to give the desired SnH-functionality. The authors mentioned the great potential for polyHIPE based supports in organic chemistry due to permanent porosity, good accessibility of active sites and the great stability against solvents of different kinds as well as less contamination from the reactants [27].

Another way to modify a polyHIPE surface is by grafting macromolecular chains on the surface. Desforges et al (2002) applied this principle by grafting TEMPO, a widely used catalyst in organic synthesis, onto modified macroporous poly(styrene-*co*-DVB) beads. The beads were produced by replacing a part of the styrene starting material with a functional styrene monomer in the precursor HIPE. Such beaded supports filled by a non-crosslinked functional polymeric chain could be a potential alternative to gel-type or macroporous beads in solid-phase organic chemistry due to their large pore size and high loading. The group did not report testing the obtained material for their intended purpose [28].

To investigate the influence of the surface chemistry of a polyHIPE on their applicability as supports for cell growth, Akay, Birch and Bokhari (2003) produced a poly(styrene-*co*-DVB) HIPE with hydroxyapatite on the surface of the porous material. Hydroxyapatite is a bone-like mineral and thus supports the growth of

bone forming osteoblast cells. They introduced the functionality by adding 0.5% hydroxyapatite and 15% phosphoric acid to the aqueous phase prior to HIPE formulation and confirmed the presence of the intended groups via X-ray diffraction analysis. Their investigation showed that the osteoblast cells they wanted to grow could penetrate the material better when functionalised by this surface modification approach [29].

Similar to introducing functional groups at the surface of a polyHIPE, it is also possible to graft another polymer-system onto the macroporous material. In an effort to combine the advantages of a stable polymer system such as poly(styrene-*co*-DVB-*co*-EHA) with the chemical properties of a more hydrophilic material such as the hydrogel poly(MAA-*co*-MBA), Jiang, Menner and Bismarck (2017) developed a one-pot-synthesis to obtain such a grafted polyHIPE. By incorporating EHA into the HIPE system, the resulting polyHIPE was not brittle like a typical poly(styrene-*co*-DVB) material would be. This approach together with the hydrogel grafting made the material an interesting candidate for filtration purposes [23].

As can be seen from these examples, surfaces of polyHIPE materials can be designed and altered in various ways, such as introducing a modified monomer into the HIPE system or grafting another polymer onto the polyHIPE. This adaptability is one of the characteristics which makes emulsion templating such an interesting and widely applied field and it can be assumed that the full potential of possibilities has not yet been realised and there is still a lot of room for investigation.

3.2.4 Applications of PolyHIPEs

Due to their versatility, polyHIPE materials have a wide range of possible applications in various fields. Their large surface area makes them extremely useful in fields such as tissue engineering [29], [30], for separation purposes [31], [32] and for materials science, recently for example as a means to recover spilled oils [33].

In tissue engineering, polyHIPEs have been investigated not only as cell growth medium [29] but also as an injectable bone graft [34]. The first application has already been shortly discussed in the previous section. For the latter purpose, a

research group has developed a PDMA-based, biodegradable, compressive poly-HIPE material curable in situ and successfully added calcium phosphate nanoparticles as well as demineralized bone matrix without sacrificing emulsion template stability. The material showed similar compressive properties to the trabecular bone. Tests showed that the oxygen inhibition might affect the properties of the material in clinical settings and thus in another study, a thiol-based crosslinking agent was applied to prevent oxygen inhibition [35].

PolyHIPE materials have also been investigated for their potential applications in electrochemical sensing. Zhao et al (2007) assessed functionalised polyHIPE membranes for their use in the development of sensors with integrated separation and detection. They produced thin membranes (100-200 μm thickness) from styrene, EHA and DVB and used it as a potentiometric, ion-selective electrode material to detect potassium ions. They achieved positive primary results and mentioned the potential of macroporous polymer materials in the field of electrochemical sensing.

Further applications of polyHIPEs include ammonium production or the clean-up of oil spills. For example, an alternative ammonium production method using a sulphonated polyHIPE acting as an acid in a neutralisation reaction to recover the product was recently been patented by Akay [36]. Another application with great potential is a superhydrophobic poly(styrene-*co*-DVB)HIPE containing Fe_3O_4 particles, which reaches oil contact angles close to 0° and can adsorb up to 57.6 gram oil per gram polymer material. It was proven to be reusable and 90% of the adsorbed oil could be recovered.

In general, the possible applications of macroporous materials made via emulsion templating seem endless in number and field due to their versatile nature, but also the facile processing and relatively low cost of the material.

3.3 Pickering Emulsions

3.3.1 Introduction

Emulsions which are stabilised by particles instead of a surfactant are called Pickering emulsions, named after Spencer Pickering. Sometimes, these emulsions are also referred to as Pickering-Ramsden emulsions after Walter Ramsden, who was among the first to describe the phenomenon at the beginning of the twentieth century. Solid particles, which have a strong tendency to assemble at fluid-fluid interfaces, lower the interfacial tension between the two phases of the emulsion and thus contribute to a greater kinetic stability, reducing coalescence and flocculation [37]. Figure 3.4 shows the principle of Pickering emulsion stabilisation by particles, for example by *E.Coli* cells.

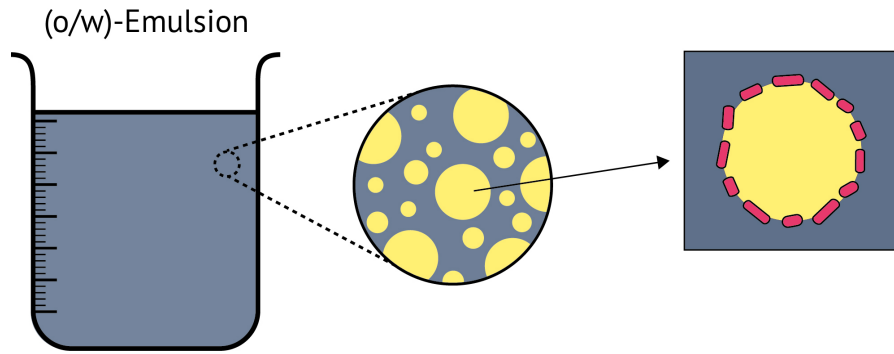


Figure 3.4: Principle of Pickering Emulsion Stabilisation

In contrast to surfactant-stabilised emulsions, which are metastable, and where coalescence of the droplet occurs after some time, particle-stabilised emulsions are more stable due to the formation of a dense interfacial barrier at the phase boundary [38]. Factors particularly influencing the degree of stabilisation are particle size and the particles hydrophobicity or hydrophilicity [39].

Pickering Emulsions have various applications, especially in the food industry, but also in heavy oil processing and as templates for microcapsules and hollow spheres.

3.3.2 Pickering Emulsion Templating

Like HIEs, Pickering emulsions which contain monomer can be used as a template to produce poly-Pickering-HIEs or beads. These materials are interesting in materials science due to the flexibility in the choice of stabilising particles and thus the possible tuning of material properties. Another advantage in comparison with surfactant-stabilised emulsions is that relatively little material has to be used (mostly less than 1 wt%). Furthermore, the stabilising particles are often less hazardous and do not leak from the product material, which makes them more environmentally friendly [40].

The factor determining which phase of the emulsion will be stabilised, i.e. whether an (o/w)- or a (w/o)-emulsion will be produced, is wettability of the particle, which can be determined via contact angle measurements. A contact angle smaller than 90° means that the particle is more water-soluble, resulting mostly in (o/w)-emulsions, and vice-versa [41]. Knowledge about this principle makes it possible to choose the right stabilising particle for the intended purpose. Furthermore, the surface of the solid particles can also be modified via grafting or adsorption of different molecule-types, and thus the contact angle can be influenced accordingly [42].

Droplet size of the emulsion can be partly controlled by the formulation and process parameters. It has been shown that there is a link between the droplet size and the mass ratio of the solid particles to the internal phase of the emulsion. The resulting droplets should be bigger if less solid particles are used, since the particles can only cover small interfacial areas. Furthermore, depending on the energy input to produce the Pickering emulsion, the droplets will be bigger or smaller with low or high energy input respectively. Any excess particles in the aqueous phase will lead to thickening of the emulsion, which is often advantageous for storage purposes since emulsions with higher viscosities experience slower breakdown processes [42]. This means that by choosing the right experimental parameters, further adjustments to the resulting polymer materials can be made.

3.3.3 Pickering PolyHIPEs and Particles

Several reports have been published on the production of monoliths and particle spheres from particle stabilised emulsions using a wide range of particles, such as titania, silica, carbon nanotubes, or copolymer particles.

Poor mechanical properties often restrict the application of PolyHIPE materials. One way to improve these properties is by using particles to strengthen the polymer material. Ikem, Menner and Bismarck [43] described successful improvement of the properties of surfactant stabilised Poly(medium internal phase emulsions) (PolyMIPEs) by adding silica particles with a diameter of 20 nm to the precursor emulsion. They reported a crush strength of the resulting material of 5.6 ± 0.1 MPa as well as a gas permeability of 0.92 ± 0.09 D, which are good mechanical properties for a polyMIPE, since it has a naturally lower gas permeability due to the higher density of the material compared to polyHIPEs.

Sun et al [40] developed spherical dummy-imprinted polymers via Pickering emulsion templating selective for different types of bisphenol A (BPA). 4-vinylpyridine and ethylene glycol dimethacrylate were chosen as monomer and crosslinker and SiO₂ particles were chosen as stabilising agents as well as 0.2% Triton X-100 as a water-soluble surfactant. Shaking by hand was enough to form a Pickering emulsion stable enough to endure 16h polymerisation at 70°C in a water bath. Dummy imprinting is often used to avoid template leakage if a structurally closely related analogue to the template is available. The group successfully applied the material as a solid-phase extraction sorbent by packing it into an SPE cartridge and could couple the material to an HPLC system.

Most relevant to this thesis is the work by Shen et al [44], who developed *E. coli* and *M. luteus* imprinted beads via Pickering emulsion templating by using N-acrylchitosan (NAC) to form a NAC-bacteria network which would stabilise the oil-in-water emulsion. The self-assembly tendency of bacteria at oil-water-interfaces was exploited to produce bacteria-imprinted beads. Apart from the NAC-pre-polymer, the oil phase consisted of trimethylolpropane trimethylacetate (TRIM) and divinylbenzene (DVB). The group studied bacteria binding and displacement of bound *E. coli* with competing bacteria for this material and found that it can lead to effective bacteria-specific binding. They further suggested that the surface-bound *E. coli* bacteria might attract self-assembly of even

more cells. It was also found that the nature of the pre-polymer and the target bacteria strongly influences bacterial recognition of the MIP. They did not report any problems removing the template from the polymer surface.

In general, Pickering emulsion templating is another versatile method to create polymer materials with specific structures and chemical and physical properties. Increased stability of the emulsion system, environmental friendliness and reduced costs are further advantages of this method over emulsion stabilisation with traditional surfactants. Especially with regard to imprinting larger structures such as bacterial cells, the Pickering emulsion templating seems a promising alternative to often more difficult bulk imprinting techniques.

4 Experimental Results and Discussion

4.1 Approaches towards an *E. coli* imprinted PolyHIPE

The goal of this work was to create bacteria-imprinted polyHIPEs for use as a sorbent for preconcentration in analytical methods. PolyHIPEs have already been used for separation purposes and through emulsion templating, the structure of the resulting material can be influenced to relatively great extent. As *E. coli* bacteria are hydrophilic, a feasible monomer had to be chosen for imprinting.

A first approach was to use HEMA, a monomer which has already been used for producing classical bacteria-imprinted polymers (BIPs) [6], together with the crosslinking agent methylene bisacrylamide (MBA) as continuous phase. Protocols, which had already been developed to produce polyHIPEs from this monomer mixture with cyclohexane as disperse phase [45], were repeated with the alteration that *E. coli* bacteria were added to the aqueous monomer mixture. The survival rate of *E. coli* in contact with cyclohexane was tested before for 24 hours. No significant reduction in cells was observed. Control polyHIPEs produced without bacteria showed good structural features, however, addition of even a small amount of bacteria (10^9 cells in a total volume of 32 mL HIPE) to the emulsion mixture led to phase separation before a polyHIPE structure could be formed, or to a non-stable 3D-structure. To produce a monolith with a surface area containing a great number of bacteria imprints, the amount of bacteria added to the HIPE mixture would have to be at least 10-fold. Thus, this procedure was discontinued

In a next screening step, the production of an acrylate based MIP-polyHIPE was tested for its suitability. Pre-testing with bacteria has shown that ethylhexyl acrylate (EHA) most likely kills the bacteria cells, and UV-light, which was used to start the polymerisation reaction, can destroy bacteria as well. As suspected, no bacteria cells could be found on the surface of the resulting polyHIPE when examined with scanning electron microscopy (SEM). Therefore, this approach did not seem promising either.

4.2 Emulsion-templated Poly(MAA-*co*-MBA) grafted Poly(Styrene-*co*-DVB)HIPE with bacteria embedded in hydrogel surface

Finally, a more promising method was found in the production of supported hydrogel membranes via emulsion templating. Methacrylic acid (MAA) and MBA were grafted onto a scaffold made from a styrene and DVB copolymer in a one-pot synthesis approach, adapting a protocol developed by Jiang, Menner and Bismarck [23]. Via one-pot synthesis, a grafted polyHIPE with bacteria embedded in the surface of the hydrogel was created. The bacteria were added to the aqueous phase prior to preparing the (w/o)-emulsion. This method yielded the most promising results, as a polyHIPE structure with bacteria on the surface of the hydrogel could be observed via SEM imaging. The material will in the following sections be referred to as *E. coli*-PolyHIPE. The principle of how to obtain an *E. coli*-imprinted PolyHIPE via this method is presented in Figure 4.1.

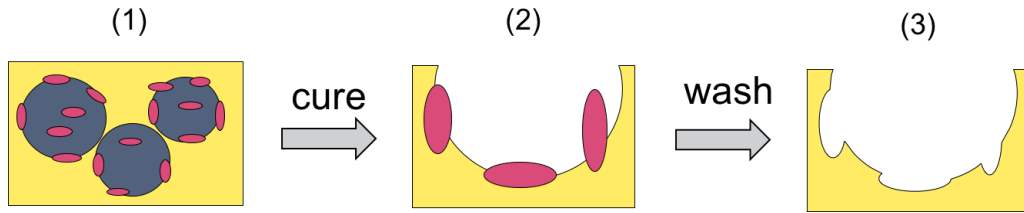


Figure 4.1: Schematic representation of producing a bacteria-imprinted PolyHIPE. (1) w/o-Emulsion containing *E. coli* cells. (2) Material after polymerisation. (3) Material after template removal via washing.

The materials used for synthesis were chosen according to the desired final properties of the material. The highly crosslinked copolymer of styrene and DVB results in a stable 3D-structure that acts as a solid scaffold for the grafted hydrogel, since the latter is not form-stable. Polymer materials based on methacrylic acid and methylene bisacrylamide have already been used for imprinting macromolecules [46] and are thus considered feasible for this work. The oil-soluble surfactant Hypermer B246 used to stabilise the emulsion is based on polyhydroxystearic acid, which is also used in the cosmetics industry, and polyethylene glycol. Calcium chloride acts as an electrolyte, which is added in order to decrease coalescence of the emulsion droplets.

Since producing a bacteria-imprinted polyHIPE was a novel procedure, several issues were encountered during process development. The main problems to report in this respect were phase separation, the absence of pore throats in the material, as well as pore and pore throat size in general. It can be assumed that phase separation was induced by the presence of *Escherichia Coli*, because control HIPEs always gave stable polyHIPEs without any observable phase separation. Pore size and the absence of pore throats were similar in both the control and the bacteria-containing polyHIPEs, so this issue most likely is due to the properties of the materials used.

In order to create a stable HIPE, i.e. to avoid phase separation, and to make the material an open-porous system, several parameters in the experimental design were altered and the results were confirmed via scanning electron microscopy (SEM). Table 4.1 summarises the results.

Parameter	Range Tested	Results
Internal phase ratio	60% - 85%	80% yielded best results in terms of stability of the emulsion and pore size.
Addition of CaCl ₂	0.01 g/mL aqueous phase	Additional CaCl ₂ was used to reduce Ostwald Ripening effect and give more homogeneous pore size distribution.
Surfactant amount	4:4:2, 4:4:1.5, 4:4:1 (Volume ratio of Styrene:DVB: Hypermer B246)	4:4:2 gave the best results for emulsion stability, but did not result in a porous structure due to copolymerisation of the internal and continuous phase. 4:4:1 did not result in optimal stability and increased coalescence and air bubbles in the resulting PolyHIPE, but gave better structural results.
Crosslinking degree (XL) of oil phase	4:4:1, 4:2:1, 4:2:0.75, 4:1:1	The 4:2:1 mixture gave the best results in terms of stability of the emulsion. 4:2:0.75 and 4:2:1 were also the only mixtures to result in an open-porous polyHIPE.

Stirring speed	750 rpm - 1500 rpm	750 rpm gave bigger pores, but sometimes was not enough stirring speed to result in a stable emulsion. If that was the case, stirring speed was increased to 1000 rpm.
Monomer concentration in internal phase	1.5, 3 & 6 ($\frac{V}{V_{ges}}$)% MAA	No obvious difference was observed in the stability of the emulsion or in its pore and pore throat size.
Bacteria Concentration	E^7 - E^9 cells/mL	More bacteria lead to phase separation. A smaller amount of bacteria would mean reducing the number of imprinted sites.
Bacteria suspension in Glucose	10 mL 200 mM Glucose in H ₂ O	Interestingly, suspension of the cells in 200 mM Glucose prior to HIPE formation seemed to increase emulsion stability. A similar effect has been described in [47].
Use of initiator	KPS+AIBN, AIBN only	The use of KPS seems to promote formation of closed-cell systems. Pure AIBN seems more practical for this application.

Table 4.1: Influence of experimental parameter variation on emulsion stability and resulting polyHIPE structure

From analysis of the resulting polyHIPEs, the following recipe has turned out to be the most promising in terms of both stability of the precursor HIPE and the structure of the final material:

Continuous phase	Disperse phase
4:2:1 volume ratio	6 ($\frac{V}{V_{ges}}$)% MAA
Styrene: DVB: HypermerB246	21.9 g/L MBA
	(20% crosslinking degree (X_L))
	0.01 g/mL CaCl ₂
	5xE ⁸ cells <i>E. coli</i> bacteria treated with 200 mM Glucose
Conditions	
Internal phase ratio:	80%,
Total HIPE volume:	4 mL
Initiator:	1 mol% (double bond equivalents) AIBN in oil phase,
Stirring speed:	1000 rpm on magnetic stirrer,
Polymerisation	70°C for 4h

Table 4.2: Final Recipe for *E. coli* PolyHIPE

The material obtained had a stable 3D-structure and was not brittle or chalky, which is often the case with Styrene/DVB-based PolyHIPEs, but very rigid. The only difference between the bacteria containing PolyHIPEs and the control PolyHIPEs observable with the eye was a slight colour change from white (control) to bright grey (with bacteria).

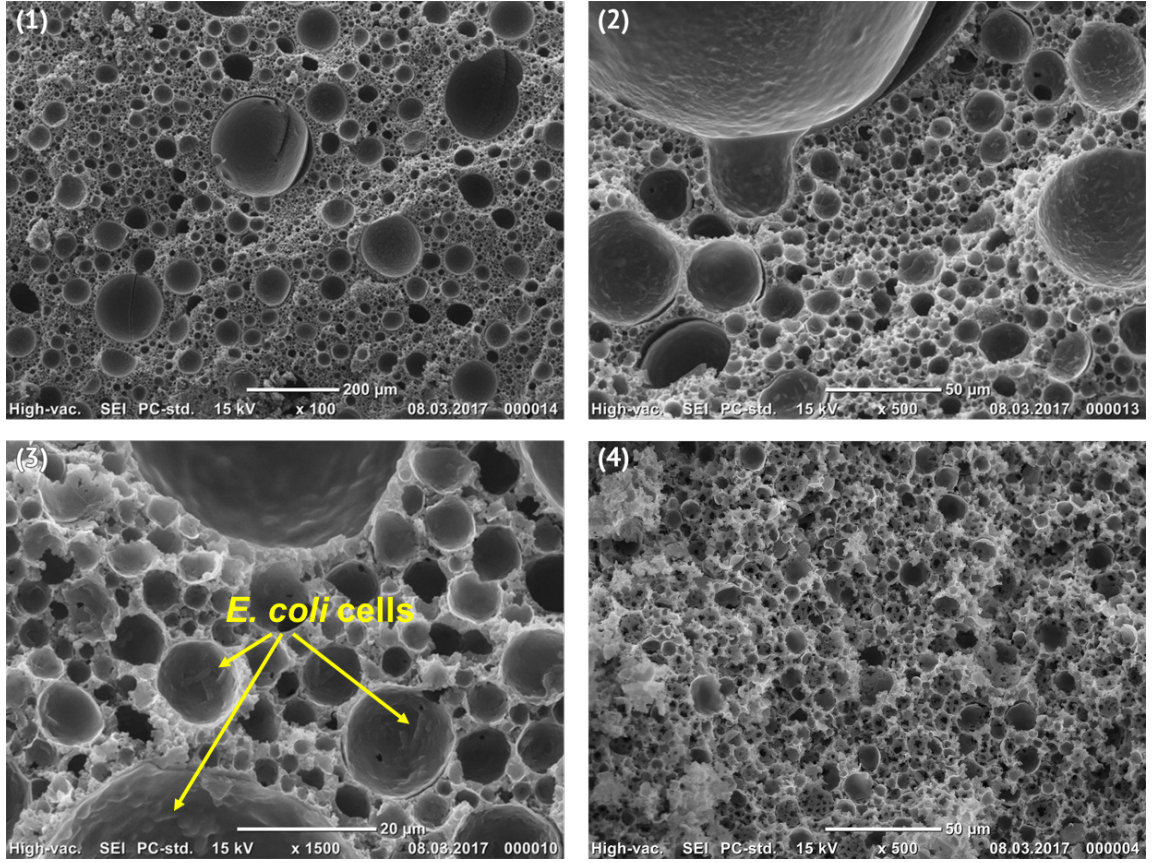


Figure 4.2: SEM images of *E. coli*-PolyHIPE. (1) 100x magnification. (2) 500x magnification. (3) 1500x magnification. (4) Control-PolyHIPE, 500x magnification.

As can be seen from the SEM images (Figure 4.2), the material has a typical macroporous polyHIPE structure including small pore throats, and bacteria cells are embedded in the surface of the material, which can be best seen in (3). The grafted Poly(MAA-*co*-MBA) hydrogel, which seems disconnected from the surface in the large pore visible in (1) and (2), is also clearly distinguishable. When comparing the SEM images of the control PolyHIPE (4) with the bacteria-containing material, it can clearly be seen from the resulting structure how the bacteria cells lead to destabilisation of the emulsion.

While the control PolyHIPE has regular pore-size distribution, the *E. coli*-PolyHIPE shows several pores which are much larger than the other ones. This is a result of a destabilisation process called Ostwald ripening, which leads to coalescence of small droplets due droplet phase molecule migration in the continuous phase and is a sign of kinetic instability of an emulsion [21].

From these images, it can be seen that the pores and especially the pore throats might be too small for the material to be useful as a sorbent material for *E. coli* bacteria. A typical bacterium has a diameter of 0.25-1 μm and a length of 2 μm . The pore throats visible in the polyHIPE are much smaller; however, since the surface layer is a hydrogel, potentially bigger pore throats in the Poly(Styrene-*co*-DVB) scaffold might be present after washing. Furthermore, bacteria removal with the usual washing procedures could not be observed during SEM measurements, and there might be issues with the original bacteria used for imprinting being embedded too deeply in the structure of the polyHIPE to be removed. If such a material is to be used as a sorbent, it needs to be completely free of any remaining template bacteria to avoid any leakage.

Although imprinting at this stage could not be confirmed after washing of the material, it has been shown in principle that it is possible to embed large structures such as whole cells in a hydrogel supported by a Poly(Styrene-*co*-DVB) scaffold via one-pot synthesis. It can be assumed that it is in principle possible to remove cells from the hydrogel surface, because this was shown in other successful cases [48]. However, the question whether this particular polymer is sufficiently rigid to yield stable, clearly defined imprints that correspond to template size and shape, could not be answered within this study.

The produced *E. coli*-PolyHIPEs did not seem to work for the purpose of this thesis, and the material seemed difficult to be characterised with regards to bacteria uptake. Therefore, another method has been tested for this work, which is Pickering emulsion templating.

4.3 Poly-Pickering-HIPE from *E.coli*-stabilised Pickering Emulsions

Since bacteria cells show the same tendency to assemble irreversibly at phase boundaries as solid particles do, they can be expected to be useful in forming and stabilising emulsions.

Following the principles discussed in Section 3.3, and as a result from the hydrophilicity of the gram-negative *E. coli*, (o/w)-emulsions are expected to be formed preferentially. However, using a suitable strategy, such as adding positively charged surfactant, it should be possible to modify the hydrophilic bacteria particles in order to make them more hydrophobic and produce (w/o)-emulsions. These (w/o)-emulsions, which would be closed-cell in nature, should, after treatment with additional surfactant, produce a final, open-cell polymer product.

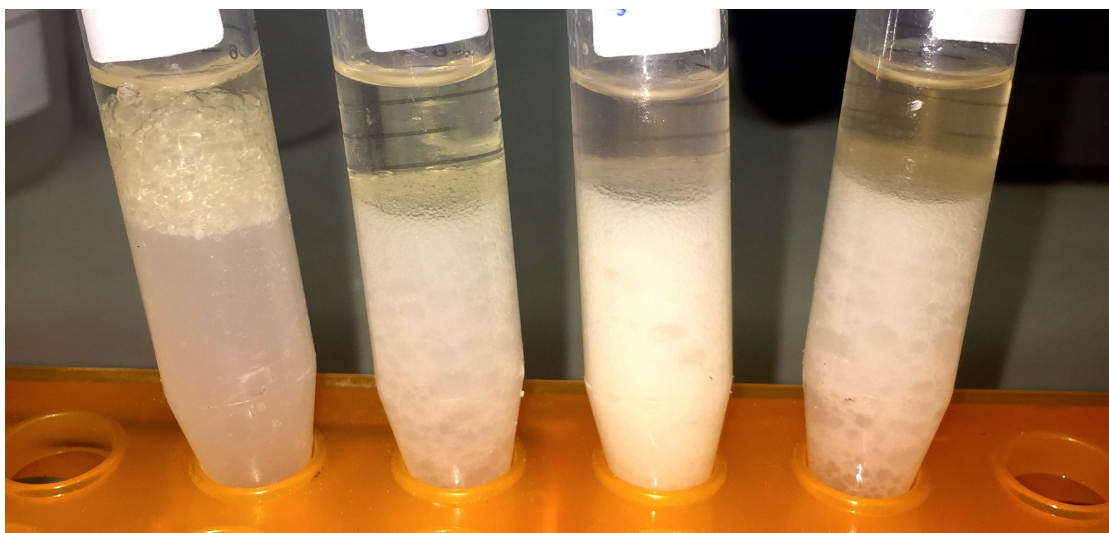


Figure 4.3: *E. coli*-stabilised Pickering emulsions. (o/w)-emulsion (1st emulsion on the left side) and (w/o)-emulsions.

Several approaches towards such a stable *E. coli*-stabilised (w/o)-emulsion were tested. Using positively-charged surfactant to sufficiently compensate for negative surface charge of the cell turned out to be the key to modify the contact angle of the bacteria just enough to encourage the *E. coli* to form a (w/o)-emulsion. After finding the right procedure for emulsification, this approach led to the formation of stable emulsions (Figure 4.3). It can be assumed that the emulsions were

indeed stabilised by the *E. coli* bacteria, since the same system without *E. coli* did not form emulsions stable for a longer period of time. The *E. coli*-containing Pickering emulsions were stored for several days without any visible changes in their structure.

The three emulsions on the right hand side shown in Figure 4.3 are (w/o)-emulsions. Whether an emulsion is a (w/o)- or an (o/w)-emulsion can be easily determined experimentally. If transferred to water, (o/w)-emulsions will disperse, whereas (w/o)-emulsions will remain stable for a short time, since the aqueous droplets are dispersed in a protecting continuous phase which prevents the droplets from falling apart immediately. Furthermore, in Figure 4.3, it can be seen that the droplets are either in the top or bottom phase. Since the disperse phase makes up most of the volume, the droplets are in the top phase if a (o/w)-emulsion is formed and vice versa.

Table 4.3 shows which parameters were changed to obtain a stable *E. coli*-stabilised Pickering emulsion.

Parameter	Range Tested	Results
Bacteria / Surfactant ratio	E^6 - E^9 cells/mL H_2O , 0.5- 2 mM DODAB in oil phase (or 1-10 mM CTAB in water phase)	Bacteria concentration was hard to control, thus, no reproducible results were obtained. Varying this param- eter resulted in different bead sizes (approx. 100 μ m - 1 mm diameter).
Addition of $CaCl_2$	0.01 g/mL aqueous phase	No observable effect on emul- sion stability
Initiation type	Thermoinitiation (AIBN), Redox initiation (TEMED/KPS)	Neither worked, but with ther- moinitiation, small fragments of monolithic, poly-Pickering- HIPE form were obtained twice.

Table 4.3: Overview of parameters tested and results for the production of a Poly-Pickering-HIPE

Although several (w/o)-emulsions could be formed with this approach, the Pickering emulsions seemed very sensitive to temperature changes and phase separation was observed when thermo-polymerisation was initiated at 70°C. In consequence, the procedure was changed towards redox-polymerisation, which can be done at room temperature. Since this project was limited in time, no positive results were obtained from this process. Since the bacteria-stabilised (w/o)-Pickering emulsion was not stable at higher temperatures, most emulsions phase separated completely. In two occasions, however, small pieces of a porous material were obtained, leading to the conclusion that it would indeed be possible to make porous *E. coli*-stabilised monoliths if the right polymerisation method was found. SEM imaging was used to characterise the surface structure of the polymer pieces obtained.

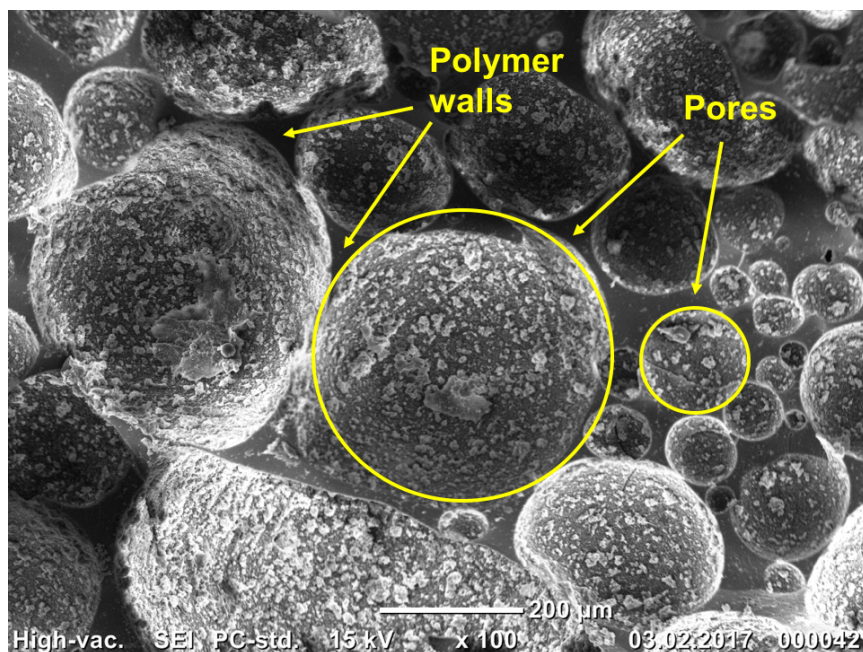


Figure 4.4: SEM image of a Poly-Pickering-HIPE, 100x magnification.

Figures 4.4 and 4.5 show the resulting structures from the Pickering emulsion polymerisation. The resulting polymer material is Poly(Styrene-*co*-DVB) with a crosslinking degree of 50%. As can be seen from the SEM images, the pores are an order of magnitude bigger in size than the pores of the traditional surfactant-stabilised polyHIPE, but, typical for a poly-Pickering-HIPE, there are no pore throats. The small bright structures on the surface of the pores are remaining

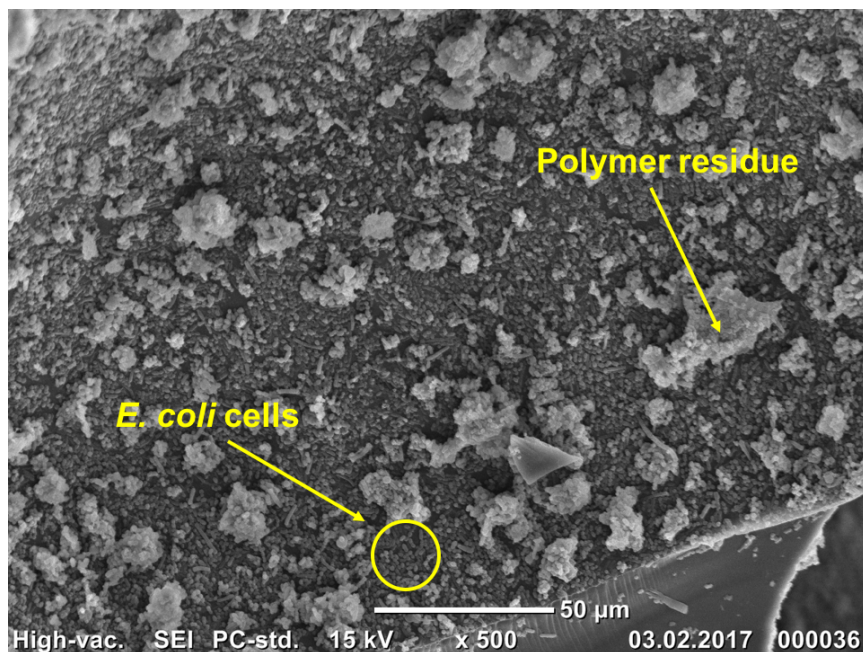


Figure 4.5: Poly-Pickering-HIPE 500x magnification.

polymer, the pore walls are covered in *E. coli* cells (Figure 4.5).

A more thorough study of this approach would be useful to establish a route towards macroporous bacteria-imprinted Poly-Pickering-HIPEs. Especially promising in this respect is the systematic measurement of the change of the contact angle of the *E. coli* cells with varying surfactant concentrations and an investigation in how to obtain open-cell polymer systems from Pickering emulsions. Due to the large pore sizes, such a system could be very useful for filtering and sorption applications.

4.4 Molecularly Imprinted Poly(Styrene-*co*-DVB)-Particles from Pickering Emulsions

Apart from macroporous materials, emulsion templating can also lead to the production of polymer beads if the dispersed phase of the emulsion is polymerisable. This approach was used to produce Poly(Styrene-*co*-DVB) beads with *E. coli* cells on the surface of the material. Since it was not clear whether the developed PolyHIPE material was of use as a sorbent material for *E. coli* due to the aforementioned small pore and pore-throat sizes, another type of material was developed from phase-inversed Pickering emulsions.

Some of the Pickering emulsions obtained were phase inversed (o/w)-emulsions, and could thus be polymerised in order to produce polymer beads containing bacteria cells on the surface. Several recipes have been tested to produce beads via Pickering emulsion polymerisation, which are presented in Table 4.4.

	Aqueous:Oil Phase (mL/mL)	<i>c(E. coli)</i> in aqueous phase (cells/mL)	<i>c</i> (DODAB) in S/DVB (mM)	Comments
1	3:3	E ⁸	0.5	Sometimes successful, sometimes (w/o)-Emulsion
2	2:4	E ⁸	0.5	(w/o)-Emulsion
3	1:5	E ⁸	0.5	(w/o)-Emulsion
4	4:2	E ⁸	0.5	(o/w)-Emulsion, medium sized beads
5	5:1	E ⁸	0.5	(o/w)-Emulsion, very large droplets, disintegrated during curing
6	3:3	E ⁷	0.5	Sometimes (w/o)-Emulsion, large sized beads from (o/w)-Emulsion
7	2:4	E ⁷	0.5	(w/o)-Emulsion

8	4:2	E ⁷	0.5	(o/w)-Emulsion, large sized beads after curing
9	4:2	E ⁹	0.5	(o/w)-Emulsion, small sized beads after curing

Table 4.4: Tested compositions for the production of *E.Coli*-stabilised particles.

Table 4.4 summarises the parameters and outcomes of the different Pickering emulsion approaches tested. *E. coli* concentrations were not determined more exactly for these first trials. From these trials, it was found that a ratio of 4:2 mL *E. coli* suspension to S/DVB gave the best results in terms of both emulsion stability and amount of droplets and thus resulting particles. The droplet size was tuneable by varying the *E. coli*-concentration in the aqueous phase, with higher concentrations resulting in smaller droplets. The beads obtained from recipes 4, 7 and 9 were then used for further tests to confirm their potential as sorbent material for *E.Coli* cells.

It was found experimentally that the principle of better stabilisation from more particles also applies to bacteria as stabilising particles. The higher the bacteria concentration, the smaller the droplets and thus also the resulting beads. From the SEM images, it can also be seen that the larger beads contain more cells on the same area than the smaller ones do. Figure 4.6 shows the SEM images of typical beads obtained from this approach. As can be seen, the particles were not always spherical, especially if they were larger. The brighter structures visible on the polymer surface are *E. coli* cells.

It can be assumed that during emulsion formation, storage or polymerisation, some coalescence occurs, since the beads obtained from the Pickering emulsions were not uniform in form and size. This effect is consistent with the principle of droplet deformation in emulsions with a high internal phase ratio.

In general, the experimental design for the production of these Poly(Styrene-*co*-DVB) particles is very straightforward and the recipes were found to be very reproducible. The *E. coli*-imprinted beads could upon template removal potentially also be used as sorbent material for preconcentration purposes. Spherical

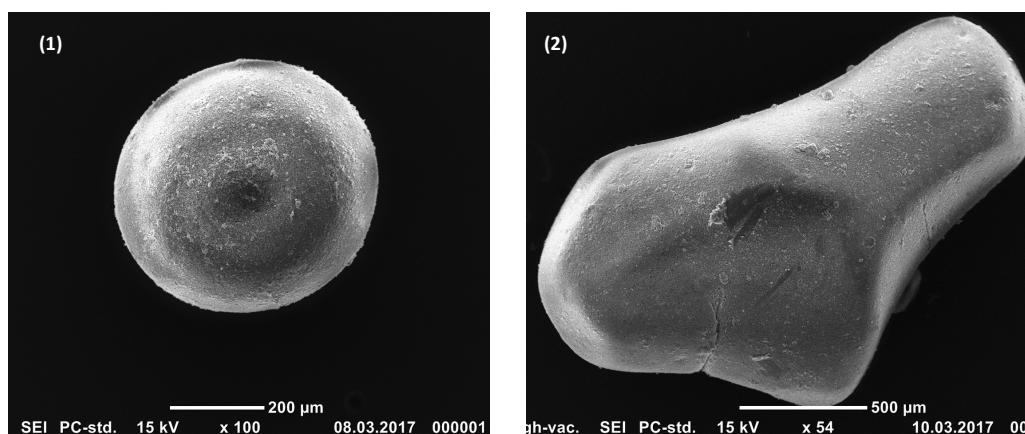


Figure 4.6: SEM image of (1) small spherical bead, 100x magnification and (2) large non-spherical bead 54x magnification

particles have a large surface area and solvent or air could pass through the holes between the individual beads if they were packed in a column or cartridge. Also the fact that the particle ensembles show size distribution might be beneficial for SPE purposes, since a cartridge or column could be more closely packed if the smaller beads occupy the space between the larger beads. This could then lead to a higher density of imprinted sites per volume unit and thus to a more efficient extraction of target analyte.

Compared to other *E. coli*-imprinting protocols [44], the procedure found from this work seems more straightforward, since all chemicals can easily be ordered and time and money can be saved by not having to functionalise any chemicals. Pickering emulsion templating is not only very practical, it also takes away one of the main drawbacks of molecular imprinting techniques for biomolecules: The analyte can be suspended in the aqueous phase, but an apolar monomer can still be used, since the template will adsorb to the phase boundary of the emulsion given it is large enough in size to act as a stabilising particle.

4.5 Template Removal

MISPE approaches frequently suffer from leakage of template molecule during the washing or elution step. If additional analyte molecules enter the solution, the data obtained becomes unreliable and cannot be accurate. In order to avoid leakage of any template molecule left in the material, intensive washing of the sorbent material prior to use is of great importance.

For this work, several washing procedures were tested and the degree of removal of the template bacteria were confirmed via SEM. Unfortunately, the removal of template bacteria from bead surfaces turned out very challenging as some of the *E. coli* cells seem to be deeply embedded in the surface or might be covered with a thin polymer film.

Table 4.5 lists the washing procedures tested. It turned out that combining several steps often lead to better results.

	Washing procedure	Success	Comments
1	70% EtOH, 12 h	No	No template removal at all visible
2	10mM NaOH, 0.1 ($\frac{w}{V}$)% SDS, then dist. water, 3x	Partly	Still large areas covered in template bacteria
3	40mM NaOH, 1 ($\frac{w}{V}$)% SDS, then dist. water, 3x	Partly	Still some areas covered in template bacteria
4	2M NaOH, 1($\frac{w}{V}$)% SDS, then dist. water, 6x	Partly	No significant change from lower NaOH concentrations
5	Ultrasonic bath, 1 min	Yes	Beads in washing solution, seems to have positive effect on removal
6	Ultrasonic bath, 10 min	No	Longer period of time in ultrasonic bath seems to remove imprints by smoothing of the surface

7	10% Acetic Acid, 1 ($\frac{w}{V}$)% SDS, then dist. water, 6x	Partly	As described in [44]. Similar to NaOH/SDS results
8	Lysozyme treatment, 38° C, 30 minutes before washing with NaOH/SDS	Partly	Seems to improve results of further washing, still some bacteria visible
9	Conc. sulfuric acid, H_2O_2 , 3:1 ratio	No	No improvement in template removal, but colour change of particles from cloudy white to brown

Table 4.5: Washing procedures tested for template removal

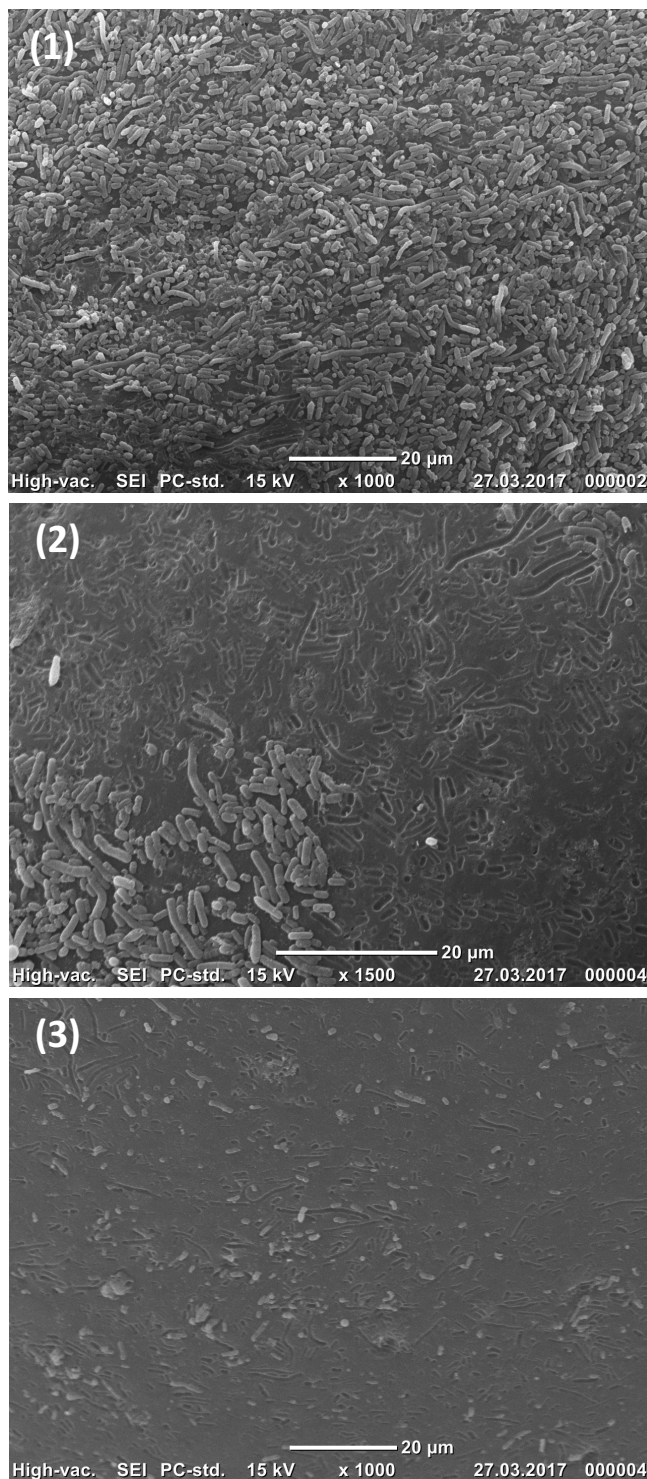


Figure 4.7: Varying success in removal of template from the polymer surface. (1) Almost no removal, 1000x magnification. (2) Template partly removed, 1500x magnification. (3) Good removal of template, 1000x magnification.

SEM-images taken after washing with 40 mM NaOH / 1 ($\frac{w}{V}$)% SDS (Figure 4.7) reveal that some parts of the surface do not contain any bacteria which indicates complete removal. However, the removal was not evenly distributed and not reproducible. Different parts of the same bead are still covered with bacteria cells and on other beads from the same washing batch, almost no template was removed. Such inconsistent removal might be explained by shear effects which help removing template and do not affect all parts of the bead surface equally.

Since all washing procedures failed to completely remove all template *E. coli* cells, the final protocol comprises first washing with 40 mM NaOH solution containing 1 (w/V)% SDS followed by water, which led to reasonable template removal in the trials. All beads and polyHIPEs were washed with this procedure for at least five times.

It should be mentioned that removal of the template is reported in many papers as one of the most challenging steps in the development of MIP materials, especially if any technique other than stamp-imprinting is used. Even on the SEM-images of the *E. coli*-imprinted beads Shen et al [44] present, some unremoved template can be seen on the surface, so it can be assumed that although the group does not mention it in the paper, they may have encountered difficulties in that respect too.

4.6 Bacteria Uptake

The uptake of the developed *E. coli*-imprinted beads was tested after extensive washing of the material. Adsorption of large amounts of analyte, rapid binding kinetics and appreciable selectivity for the intended species are the most important characteristics of a sorbent material.

Figure 4.8 sketches the interaction of molecularly imprinted polymer particles with *E. coli* bacteria during a solid-phase extraction step. Of course, bacteria assembly on the surface has to be reversible, e.g. by washing the respective beads.

To assess binding efficiency, all bacteria suspensions were analysed via fluorescence spectrometry, which gives more accurate results than cell counting. Furthermore, since the template bacteria were not treated with any fluorescent labelling agent, it can be assumed that any leakage of template *E. coli* cells will

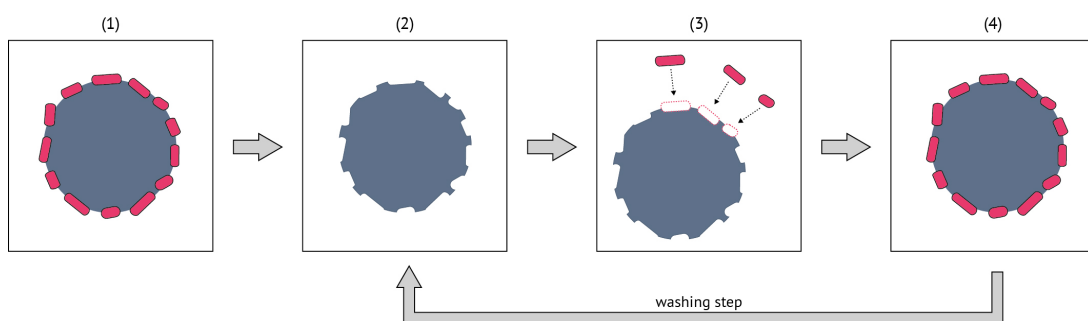


Figure 4.8: Interaction of MIP particles with *E. coli* bacteria. (1) Particle containing template *E. coli* cells after polymerisation. (2) bacteria-imprinted particle with *E. coli* imprints. (3) Affinity of material for target cells. (4) Bacteria uptake.

not influence the results. However, via this method it is not possible to distinguish colony forming units from dead cells, which has to be kept in mind when interpreting the data obtained.

A calibration curve was produced from a series of dilutions of TRITC-labelled *E. coli* bacteria in phosphate buffer solution. The usefulness of this method was tested by measuring TRITC-labelled *E. coli* suspensions of known concentrations and after obtaining data peaks corresponding to their concentrations (e.g. Figure 4.9), the method was found feasible for the purpose of this work.

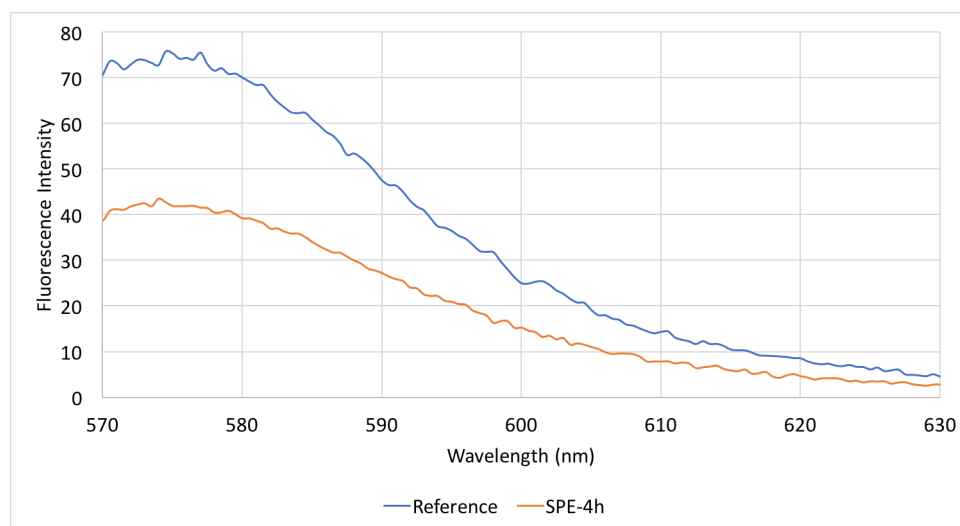


Figure 4.9: Fluorescence spectra of reference *E. coli*-suspension (blue) and *E. coli*-suspension after 4h SPE step.

4.6.1 PolyHIPE *E. coli* Uptake

The *E. coli*-PolyHIPEs bacteria uptake was measured. Emission wavelength maxima measured at 576 nm were 76.86 ± 1.33 nm for the reference and 45.93 ± 0.93 nm for the suspension after the SPE step. From these values, the bacteria uptake of the *E. coli*-PolyHIPE were calculated. The difference in fluorescence intensity corresponds to a change of 1.55×10^6 *E. coli* cells/mL, which equals an uptake of 1.26×10^4 cells per mg material (for a sample uptake calculation, see section 5.8).

These results confirmed that SPE indeed considerably reduces cell concentrations in solution. However, when interpreting these results, one has to keep in mind that it has not been confirmed that any *E. coli* imprints were actually formed after washing of the material, since even after extensive washing of the material, no imprints were discovered on the polyHIPE surface via SEM imaging. Rather than that, *E. coli* cells were still visible on the surface. This could either mean that the bacteria cells are bound too tightly to the hydrogel and cannot be removed by means of washing, or be due to the fact that via SEM imaging, only a very small part of the surface is visible so there might still be some imprints left on the surface of some pore walls. Furthermore, if any uptake of bacteria was detected, it could also be due to entrapment of the cells in the porous material rather than through adsorption at any imprinted sites.

Due to these circumstances, it is not possible to draw any conclusions about the cell uptake and imprinting effect, if there is any, of the *E. coli*-PolyHIPE. Further investigation of the material and a way to confirm template removal are needed for more reliable results.

4.6.2 MIP-Particles *E. coli*-Uptake

To determine whether the developed MIP-beads showed affinity for the target cells, first tests of *E. coli* uptake of the material were performed via a similar procedure to the one reported by Shen et. al to characterise their *E. coli*-imprinted material [44]. The results from these measurements are presented in Figure 4.10.

	Sample	Material used (mg)	Max (576 nm)	Std. Dev.	c(<i>E. coli</i>) (cells/mL)	Error c(<i>E. coli</i>)	Uptake (%)	Error (Uptake (%))	Uptake (cells/mg)	Error (Uptake)
1	Reference		74.330	0.368	3.06E+06	±1.51E+04				
	Beads P53 (~1mm ø)	88.8	41.910	1.533	1.44E+06	±5.26E+04	53.012	±1.9	5.48E+04	±2.00E+03
2	Reference		76.868	0.350	3.18E+06	±1.45E+04				
	Beads P25 (~150 µm ø)	124.1	35.294	0.688	1.11E+06	±2.15E+04	65.272	±1.3	5.03E+04	±9.79E+02
	Beads P59 (~600 µm ø)	68.1	29.106	0.255	7.97E+05	±6.99E+03	27.975	±0.5	1.36E+04	±1.20E+02
3	Reference		300.479	5.538	1.44E+07	±2.65E+05				
	Beads P60 (~600 µm ø)	99.1	151.232	1.810	6.90E+06	±8.26E+04	51.947	±1.0	2.26E+05	±4.16E+03
	Beads P60 (~600 µm ø)	409.5	74.618	1.705	3.07E+06	±7.02E+04	78.614	±1.8	8.27E+04	±1.89E+03

Figure 4.10: Results from three different bacteria uptake experiments

Figure 4.10 shows that indeed, after every measurement, considerable reduction in cell concentration after a 4h SPE-step can be observed. For example, based on the amount of beads used in measurement 1 (88.8 mg), one can calculate a bacteria uptake of $5.48 \cdot 10^4$ cells per mg material, which corresponds to an uptake of 53 ± 1.9 % of the suspended cells.

In the course of the material synthesis process, bacteria-imprinted beads of different sizes were produced. Target cell uptake results of the different sized MIP particles range from $1.36 \cdot 10^4 \pm 1.2 \cdot 10^2$ to $2.26 \cdot 10^5 \pm 4.16 \cdot 10^3$ cells/mg.

Figure 4.11 presents the different values obtained from the uptake measurements of different bead sizes. It can be seen that the medium sized beads (~600 µm diameter) could extract nearly twice the amount of cells than the small (~150 µm) and large (~1 mm) sized beads. In order to make any valid observations with respect to the uptake of the different bead sizes, complete removal of template cells would be a prerequisite. Since this was not possible, no general observation can be made regarding a correlation between bead size and cell uptake.

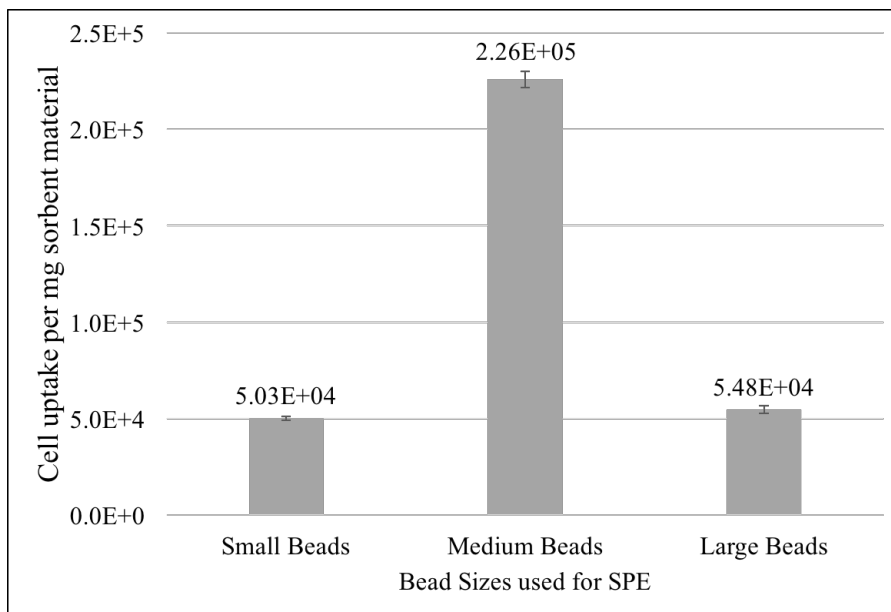


Figure 4.11: Uptake efficiency of various bead sizes after 4h SPE step

Several uptake measurements were performed, and bacteria uptakes of up to 2.26×10^5 cells/mg or up to 52 ± 1 % of the original cell concentration were measured with beads of medium size. The fact that different bead batches gave different uptake results can be attributed to incomplete removal of template, so that different beads contain different amounts of imprinted sites. Furthermore, bead size distribution can also influence the number of imprints per mg. Uptake results for *E. coli*-imprinted beads described in literature are 1.6 cfu/mg or 23% [44]. However, direct comparison to these values is not possible since fluorescence spectroscopy does not distinguish between dead cells and colony forming units.

4.6.3 MIP-Particles Uptake Kinetics

Another important material characteristic is its ability to bind quickly to the target analyte. To investigate binding properties, *E. coli*-concentrations of a suspension in contact with the sorbent material was measured regularly over a period of 24h.

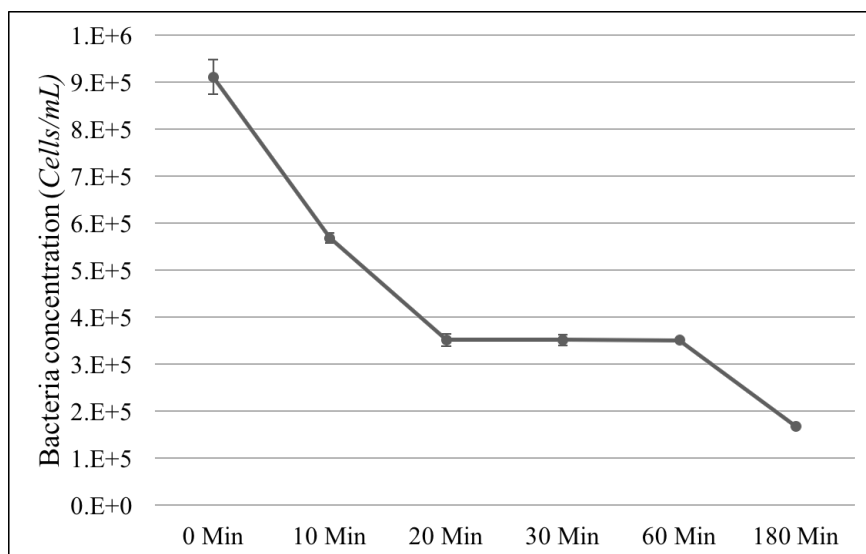


Figure 4.12: Change of *E. coli* concentration over time during Solid-Phase Extraction.

Time	Max. Values (576 nm)	Std. Dev.	c (<i>E. coli</i>) (cells/mL)	Error (c(<i>E. coli</i>))
0 Min	31.377	1.258	9.10E+05	±3.65E+04
10 Min	24.522	0.441	5.67E+05	±1.02E+04
20 Min	20.195	0.778	3.51E+05	±1.35E+04
30 Min	20.191	0.673	3.51E+05	±1.17E+04
60 Min	20.178	0.164	3.50E+05	±2.84E+03
180 Min	16.506	0.515	1.67E+05	±5.20E+03
24h	9.818	0.364		

Figure 4.13: Data obtained from Uptake Kinetics experiment

The results presented in figure 4.12 and 4.13 show that already after 20 minutes, a significant decrease in unbound bacteria cells by more than 60% could be observed via fluorescence spectroscopy. Another decline in *E. coli* concentration could be seen after 3h and 24h, respectively, although it has to be said that the biggest effect was observed in the first 20 minutes. Further reduction in fluorescence intensity was observed after 24h, however, the signal was too weak to determine the final cell concentration.

These results show that the material can be useful as an SPE sorbent also with respect to quick binding, which is an important criterium for such a material.

A first reduction of the cell concentration can already be observed after a few minutes. It seems that even more *E. coli* cells are targeted by the material after some time, but that the biggest effects can be observed within the first 20 minutes.

4.6.4 Control-NIP-Particles Uptake

To evaluate the effect the imprinting has on the uptake of target analyte, it is good practice to produce a non-imprinted polymer (NIP) for comparison purposes.

	Sample	Material used (mg)	Max (576 nm)	Std. Dev.	c(<i>E. coli</i>) (cells/mL)	Error c(<i>E. coli</i>)	Uptake (cells/mg)	Error (Uptake)
1	Reference 1		143.282	2.840	6.51E+06	±1.29E+05		
	MIP Beads P25	200	82.250	0.638	3.45E+06	±2.68E+04	4.58E+04	±9.07E+02
	NIP-Particles unwashed	267	91.219	1.125	3.90E+06	±4.81E+04	2.92E+04	±5.80E+02
2	Reference 2		256.774	3.540	1.22E+07	±1.68E+05		
	NIP-Particles washed	105.2	164.010	5.025	7.54E+06	±2.31E+05	1.32E+05	±4.05E+03

Figure 4.14: Results from NIP-uptake experiments.

To evaluate the effect of the washing agent SDS on the bacteria uptake, MIP vs. NIP uptake studies were performed twice, once with SDS washed and once with unwashed NIP material. Figure 4.14 presents the *E. coli*-uptake of the NIP material. It can be seen that for both washed and unwashed material, the uptake of *E. coli* was higher than the MIP uptake. Both NIP bead batches showed better uptake than the MIP material, which might be due to high non-specific binding between material and *E. coli* cells. Since the non-washed NIP also showed high *E. coli* affinity, the main source of these non-specific interactions did not seem to stem from SDS molecules embedded in the polymer network but rather from a general affinity of the material for the negatively charged cells. Due to the complex surface structure of *E. coli* cells, which possesses numerous different

functional groups, the number of possible interactions with the material is high and thus, such an effect is not surprising.

The fact that the material shows high affinity for the target cells does not necessarily mean that there is no imprinting effect at all. Cross-selectivity measurements can give further insights in the affinity of the material.

4.6.5 Cross-Selectivity

One of the most important characteristics of a sorbent material is selectivity. The material needs to be selective for the specific analyte and interact with other molecules or cells that might be present in the sample. To test the cross-selectivity of the material, uptake measurements with *B. Cereus* were performed and differences in bacteria concentration were investigated. This bacterium was chosen due to its similarity in size and shape to the template *E. coli* bacterium.

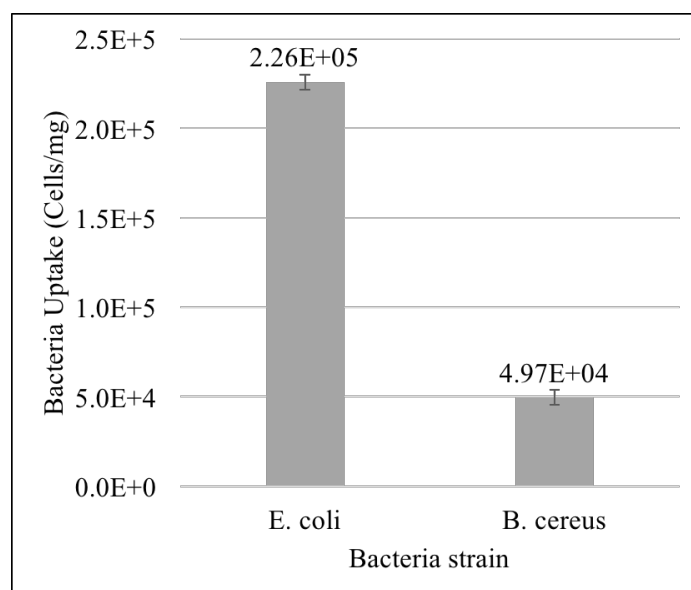


Figure 4.15: Cross-selectivity of medium-sized MIP beads after 4h SPE

The results presented in figure 4.15 show that the material, although selective for *E. coli*, could also take up a considerable amount of *B. Cereus* (4.97×10^4 cells/mg). Reasons for this might be the size and shape of *B. cereus*, which is slightly smaller and can thus easily fit into the imprints created from *E. coli*. Furthermore, non-specific interactions could also lead to affinity of the material for one or both

species of bacteria. Nevertheless, a difference in uptake between the strains can be observed, which is a sign of material affinity for the imprinted target bacteria strain over *B. cereus* cells.

Bacteria Strain	Max (576.5 nm)	Std. Dev.	Max (656.5 nm)	Std. Dev.	Concentration (cells/mL)	Error c(cells/mL)	Uptake (%)	Error (Uptake (%))	Uptake (cells/mg)	Error (Uptake)
<i>E. coli</i> reference	226.195	3.492			1.07E+07	±1.64E+05				
<i>E. coli</i> after SPE	62.258	0.751			2.45E+06	±2.96E+04	76.96	±0.93	9.36E+04	±1.13E+03
<i>B. cereus</i> reference			50.124	0.823	9.37E+06	±1.54E+05				
<i>B. cereus</i> after SPE			25.193	0.621	4.38E+06	±1.08E+05	53.21	±1.31	5.69E+04	±1.40E+03

Figure 4.16: Results from competitive binding experiment.

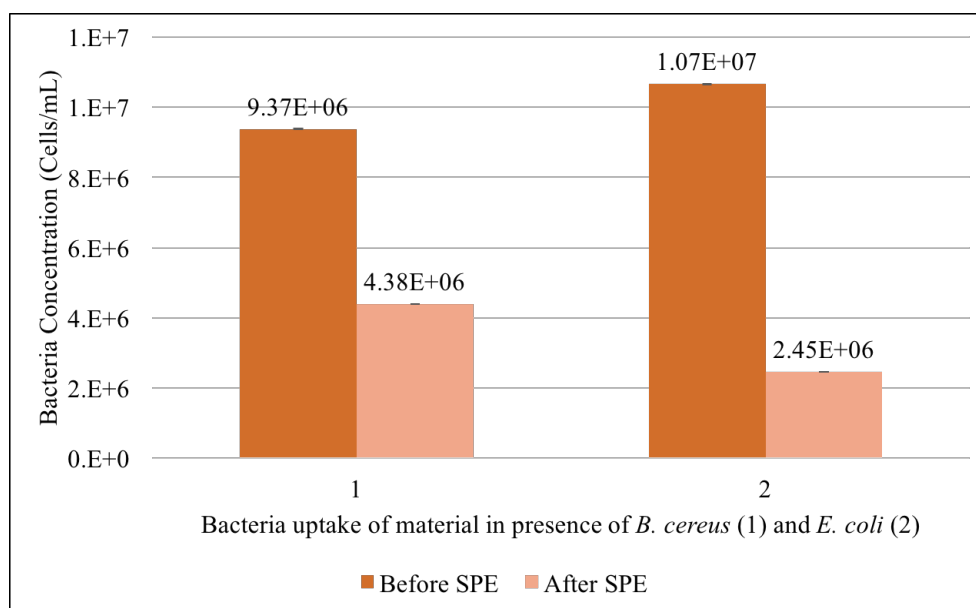


Figure 4.17: Uptake of different bacteria strains of 262.7 mg material after 4h Solid-Phase Extraction.

In a competitive binding experiment, direct competition between the two bacteria strains was examined and the results (Figures 4.16 and 4.17) show that the material is selective for *E. coli* when both species are present. Although the initial

concentration of both bacteria strains in the experiment were similar, only 53.2% of *B.Cereus* cells were taken up by the material compared to 77% of *E. coli* cells. These results confirm that although non-specific binding seems to take place, bacterial imprinting can lead to a selective uptake of target analyte even if structurally similar competing analytes are present.

4.6.6 QCM-Measurements

Although fluorescence spectroscopy yields good results for the detection of labelled bacteria cells in a reasonable concentration range, a label-free detection method would yield several advantages, such as immediate detection without the need for any sample preparation. Furthermore, effects such as photobleaching or unbound labelling reagents can influence the intensity measured via fluorescence spectroscopy. Therefore, QCM-measurements were performed.

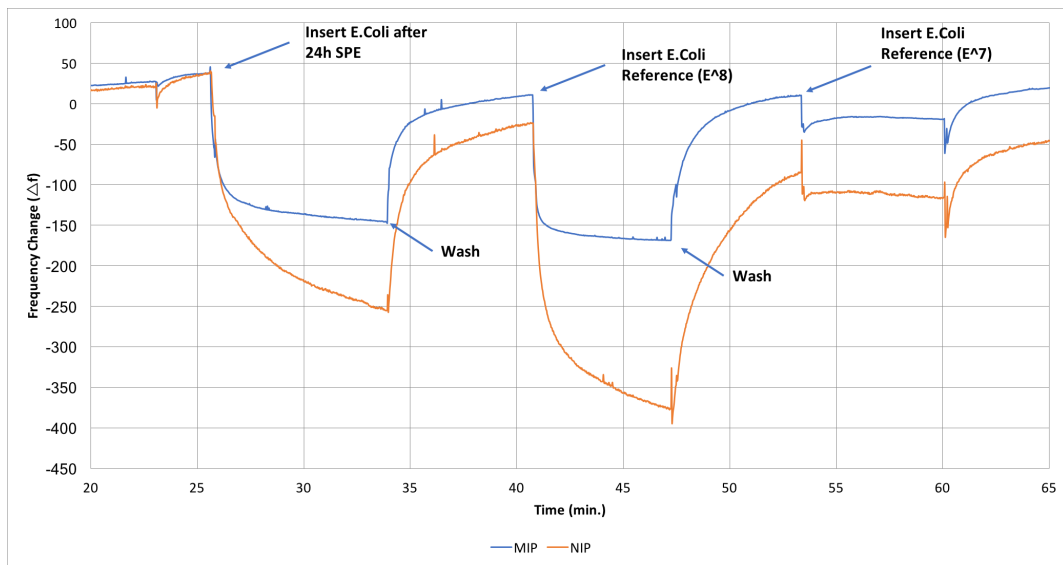


Figure 4.18: Resulting frequency shift over time with different *E. coli* concentrations

Figure 4.16 shows a plot of data obtained from a QCM measurement. It can be seen that the frequency decreases when target analyte is added and that upon washing the frequency goes back to the quartz' resonance baseline. However, based on previous experience in the group , a frequency shift of $\Delta f = -150$ Hz is comparably low for such a high concentration of target analyte added. It can also

be seen that the sample which had been exposed to the MIP sorbent beads for 24h and should contain less cells per mL than the reference sample does not give a significantly different signal. To test the general sensitivity of the QCM, the reference suspension was diluted by a factor of 10 and inserted to the measuring chamber. Here, a much smaller frequency shift is detected, so it can be said that the system is indeed mass sensitive up to a certain sensitivity.

For several measurements, rather than obtaining a negative frequency shift upon addition of analyte cells, a positive frequency shift was observed, a phenomenon called anti-Sauerbrey effect, which has been briefly described in Section 2.1.1. A more detailed account of the problems encountered and the countermeasures taken can be found in Section 5.10.

Possible reasons for these results are that the MIP material cannot take up such a high number of analyte as to make a significant difference on a scale of 10^8 cells/mL. Indeed, from the fluorescence measurements it can be seen that the sorbent takes up around 10^5 cells per mg. If this value is transferred to 3mL *E. coli* suspension with a concentration of 10^8 cells/mL and 500 mg sorbent beads are used, only a change of 16% in bacteria concentration can be induced, resulting in a concentration change probably not detectable via QCM measurement. Another explanation might be that the selective layer does not possess sufficient affinity for the *E. coli* cells, which would explain the anti-Sauerbrey-effect which was sometimes observed.

Further investigation in why the change in *E. coli* concentration was not measurable via this method would be needed to re-adjust the measuring conditions and eventually replace the polyurethane layer on the gold surface of the QCM quartz wafer with another material.

5 Experimental Methods

5.1 Bacteria Cultivation and Handling

Bacteria Cultivation

The bacteria strains used were *Escherichia coli* (*E. coli*) ATCC®31303™ and *Bacillus cereus* (*B. cereus*) ATCC®11778™, obtained from LGC Standards.

The *Escherichia coli* or *Bacillus cereus* bacteria were incubated at 37° C in lysogeny broth (LB), which is a common nutrition medium for bacteria.

The lysogeny broth was prepared from the following recipe:

Chemical	Amount
Yeast extract	10 g
Peptone	10 g
NaCl	0.5 g
Glucose	0.1 g
d. H ₂ O	1 L

Table 5.1: Recipe for Lysogeny Broth

The mixture was boiled for 30 minutes and upon cooling filtered into 45 mL Falcon tubes via 120 μ m syringe filters to be stored at -20° C.

Washing and Storage

To remove any byproducts of bacteria cultivation, the bacteria cultures were washed regularly. The bacteria containing broth was centrifuged and the supernatant liquid layer was removed. The resulting bacteria pellet was suspended in distilled water and shaken vigorously to spread the bacteria in the tube for washing. Upon washing, a part of the cells were resuspended in new lysogeny broth and the rest was kept for storage. The bacteria were washed and suspended into new nutrition medium three times a week for the time of the study.

If the cells were to be used within the same day, the bacteria were kept in the fridge suspended in water at 6°C. For longer periods, the bacteria suspension was centrifuged, the supernatant water layer was removed and the bacteria pellet was stored at -20° C.

Fluorescence Labelling

The *E. coli* cells were suspended in 10 mL d.H₂O or 10 mL PBS-puffer and 20 µL fluorescein isothiocyanate (FITC) solution (10 $\frac{mg}{mL}$ in DMSO) were added. 5 µL TRITC (1mg/mL in DMF) or 15 µL Fluorescent Red 630 (1 mg/mL in DMF) were added to a 10 mL bacteria suspension for TRITC- or Fluorescent Red labelling, respectively. The mixture was placed on a rocker shaker for continuous, gentle mixing and was excluded from light for one hour to avoid photobleaching and then washed twice with d.H₂O or PBS-puffer. To determine the amount of labelling agent necessary, a range of concentrations were tested with the same cell-suspension and observed via fluorescence spectroscopy.

For fluorescence spectroscopy measurements, labelling the bacteria with fluorescein isothiocyanate (FITC) did not give valid results from fluorescence spectroscopy because the data obtained would indicate that after an SPE step, much more *E.Coli* cells would be present than in the reference suspension. Therefore, Rhodamine (TRITC) was chosen as more useful labelling agent.

5.2 Hemocytometry

For the polyHIPE and MIP-particle synthesis part of this work, the *E. coli* cells were counted using a counting chamber. For the synthesis part, it was sufficient to know roughly how many cells were used, and therefore counting with a counting chamber was a quick and facile way to determine the bacteria concentration.

For bacteria counts, a Neubauer-improved counting chamber with double net ruling was used as hemocytometer, which was obtained from Paul Marienfeld GmbH & Co. KG.

Standard procedures were followed for preparing the samples, filling the counting chamber and counting the particles. The following formula was used to calculate the bacteria concentration from the cell count:

$$Cells/mL = \frac{Number\ of\ cells \times 1000}{Counted\ area\ (mm^2) \times Chamber\ depth\ (mm) \times Dilution\ factor} \quad (2)$$

Example calculation cell count:

Let the number of cells counted in one count be 12 and in the other count be 10. Before counting, 10 μ L of the suspension had been diluted with 990 μ L d.H₂O.

(1) The average of the two counts is formed, which is 11. The results of the two cell counts did not differ by more than 10 cells.

(2) The values are transferred into the formula, where the counted area and the chamber depth are taken from the counting chamber manual:

$$Cells/mL = \frac{11}{0.8mm^2 \times 0.1mm \times 0.01} \times 1000 = 1.37E^9 cells/mL \quad (3)$$

5.3 Fluorescence Spectroscopy

Fluorescence is a form of photoluminescence resulting from a singlet-singlet electronic relaxation. It occurs when an orbital electron of a molecule or atom which has been excited by energy input relaxes to its ground electronic state and emits a photon, i.e. light, at a characteristic fluorescent emission frequency. The ability of a molecule to absorb light is called molar absorbtivity ε_λ , also referred to as extinction coefficient.

This emission of light can be detected and its intensity depends among other factors also on the concentration C_F of the emitting molecule:

$$I_F = \gamma\zeta\varepsilon C_F L$$

In this equation, ζ is the quantum efficiency, which is the ratio of emitted to absorbed photons, ε is the molar absorptivity and L is the pathlength. If all factors apart from the concentration of the analyte are kept constant, the concentration of the fluorescent analyte can be determined after producing a calibration curve from a reference sample with a known concentration.

The functioning principle of a fluorescence spectrometer relies on the detection of emitted light by the sample. A light beam from a light source, for example a xenon discharge lamp, passes through the sample at a wavelength selected by a monochromator. The sample molecules absorb some of the incident light and emit fluorescent light in all directions, which is then detected by a photomultiplier. The detector is placed at an 90° angle to the light source in order to detect no stray light from the original light beam and thus increase sensitivity and lower the signal-to-noise ratio (see Figure 5.1).

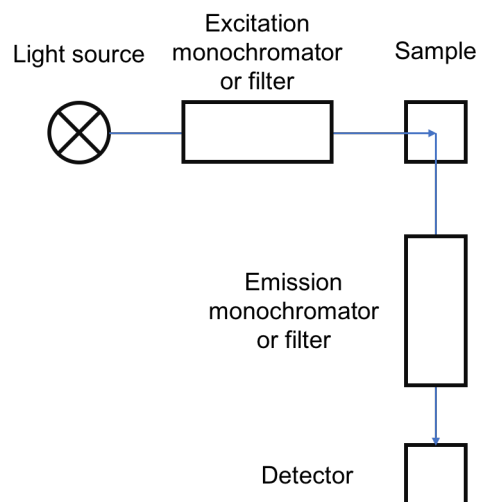


Figure 5.1: Principle of a Fluorescence Spectrometer

Since it is possible to label biomolecules and cells such as bacteria with fluorescent dyes, their concentration in a sample can indirectly be measured via fluorometry. Important aspects to consider are photobleaching, which is the loss of fluorescence intensity over time when the sample is exposed to light, and the removal of any excess labelling agent which would otherwise lead to false results. Furthermore, a calibration curve of a series of dilutions has to be generated. General advantages of this method compared to counting cells with a counting chamber are reproducibility, higher reliability of the results and less likelihood for human

errors. A potential drawbacks are cross-interferences with any additional fluorescing molecules in the samples. Another restriction lies in the concentration of sample analyte, since at low concentrations, the signal can hardly be distinguished from background noise.

Fluorescence spectroscopy measurements were performed with a Perkin Elmer LS50B Luminescence Spectrometer. The quartz cuvette had a path length of 10 mm. For all TRITC-labelled bacteria measurements, the excitation (λ_{ex}) and emission (λ_{em}) wavelength were 557 nm and 576 nm, respectively. The scan rate was 100 $\frac{nm}{min}$ and the slit widths were 5 nm each. For Fluorescence Red 630-labelled bacteria measurements, the excitation wavelength was 620 nm and the emission wavelength was 656.5 nm. Slit widths and scan rate were not changed.

A reference series of dilutions of 2.72×10^8 Cells/mL TRITC-labelled *E. coli* bacteria (original concentration counted via counting chamber) was measured. Each concentration was measured three times to account for inconsistencies of the instrument and derive a standard deviation. *E. coli* concentrations of 10^5 cells/mL or lower did not give any representative signal. Due to the large differences in proportions of the values on the x-axis of the graph, the linear data points might appear out of proportion. The value at a concentration of zero corresponds to a background measurement of PBS-buffer solution.

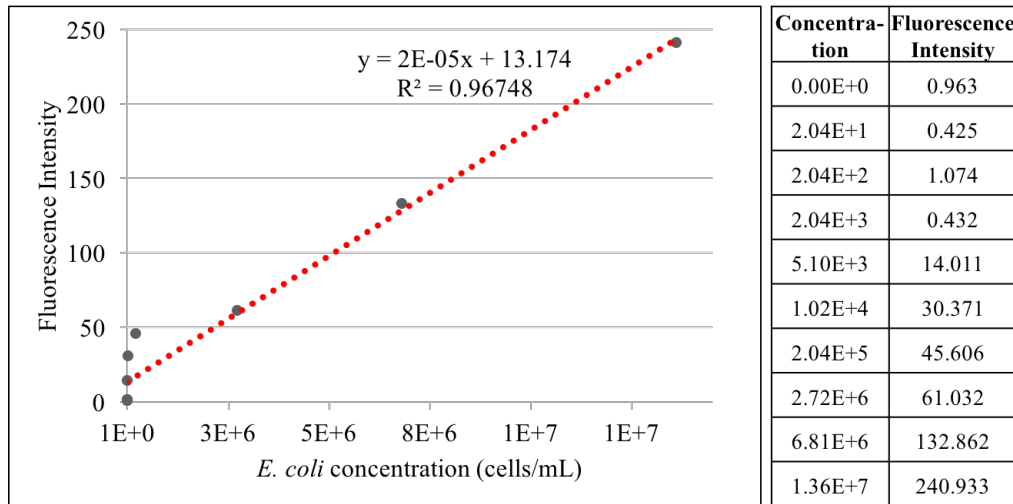


Figure 5.2: Calibration curve obtained from TRITC-labelled *E. coli* dilution series.

From this calibration graph (Figure 5.2), the bacteria concentration before and after SPE was determined. Since the bacteria used as template were not TRITC-labelled, it can be assumed that any potential leakage of template molecules will not be detected by fluorescence spectroscopy measurements.

For fluorescence measurements of bacteria concentration after solid-phase extraction, the glass vial was moved in order to resuspend any bacteria which might have assembled at the bottom of the glass. After the sorbent beads had settled, the supernatant liquid was transferred into a quartz cuvette. To measure uptake kinetics, the suspension was re-transferred into the glass vial containing the sorbent material, in other cases the suspension was either kept for further QCM measurements or thrown away.

5.4 *E. coli*-PolyHIPEs

Styrene, Divinylbenzene (DVB), Methacrylic Acid (MAA), Methylene Bisacrylamide (MBA), Potassium Hydroxide (KOH), Calcium Chloride Dihydrate ($\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$), Potassium Persulfate (KPS) and 2,2'-Azobis(2-methylpropionitrile) (AIBN) were obtained from Sigma-Aldrich. Hypermer B246 was kindly supplied by Croda (UK). All chemicals were used as received.

To produce a stable grafted polyHIPE with *E. coli* bacteria embedded in the surface, the following experimental one-pot synthesis procedure was developed following similar, already existing protocols [23]. Since the availability of *E. coli* cells was restricted, the emulsification process had to be scaled down from the typical overhead-stirring procedure normally used for the preparation of HIPEs. By using a set-up including a magnetic stirrer, an 8 mL glass vial and a syringe attached to a needle to add the disperse phase, emulsification on a smaller scale was realised (Figure 5.3).

The continuous oil phase consisted of styrene, the crosslinking monomer divinylbenzene (DVB) and the oil-soluble surfactant Hypermer B246 in varying ratios (See section 5.1) and was used from a stock solution.

The disperse aqueous phase was produced by adding methacrylic acid (MAA) and the crosslinking monomer methyl bisacrylamide (MBA) with a crosslinking degree of 20% and 0.01 g/mL CaCl_2 to distilled water, the total monomer amount in the aqueous phase was varied (See section 4.1). Since in some cases, potassium persulfate was used as an initiator, the solution was brought to a pH=9 with potassium hydroxide (KOH). Before producing any emulsions, the template *E. coli* cells were suspended in the aqueous phase. Preliminary experiments revealed that *E. coli* subjected to alkaline conditions (up to pH=10) remained viable for up to 24h. To produce control polyHIPEs without template, the last step was omitted.

Immediately before emulsification, 1% initiator with respect to the double-bond equivalents of the total monomers in solution were added. Initiators used were either just AIBN or AIBN and KPS dissolved in the oil or aqueous phase respectively. If KPS was added next to AIBN, the amount of initiator was calculated with respect to the monomer in each phase individually.

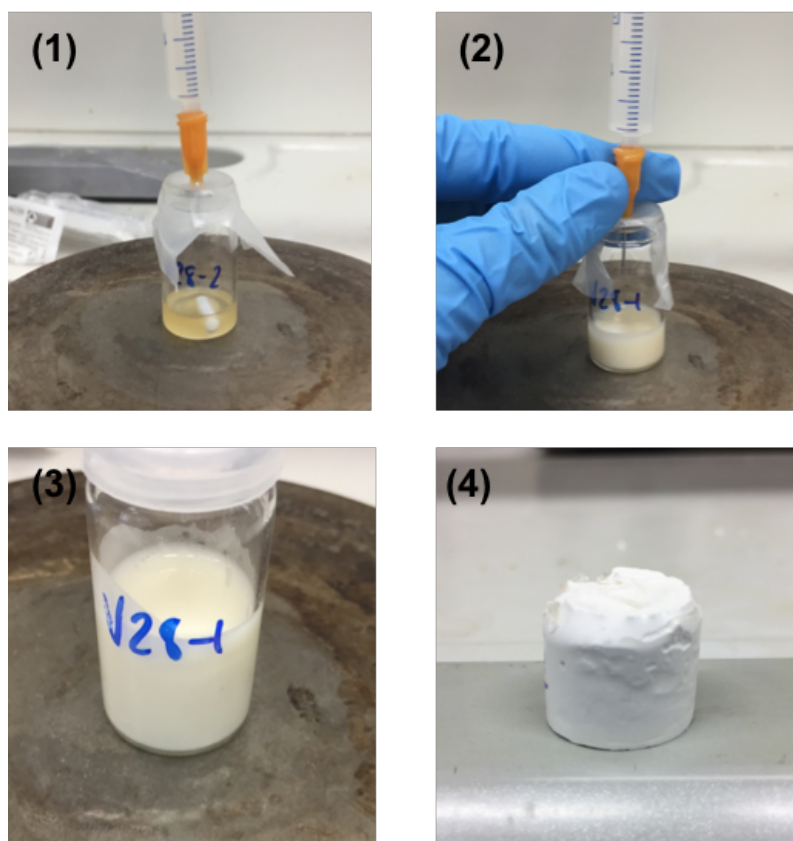


Figure 5.3: Small-scale process to create high internal phase emulsions used for this work. (1) Stirring of continuous phase. (2) Dripping in dispersed phase. (3) Highly viscous high internal phase emulsion prior to curing. (4) Resulting PolyHIPE.

Emulsions with a total volume of 4 mL were produced by adding the disperse phase manually to the oil phase via a plastic syringe piercing through laboratory tape at constant stirring. To account for any irregularities in dripping speed, the emulsion mixture was stirred for an additional 3 minutes at constant stirring speed.

After emulsification, the glass vial was closed with a lid and placed into a 45 mL Falcon tube to prevent any spillage should the glass vial break unexpectedly. The emulsion was cured at 70°C for at least 4 hours. Afterwards, the polymer material was removed from the glass vial, washed with distilled water and left to dry for another 12h at 50°C.

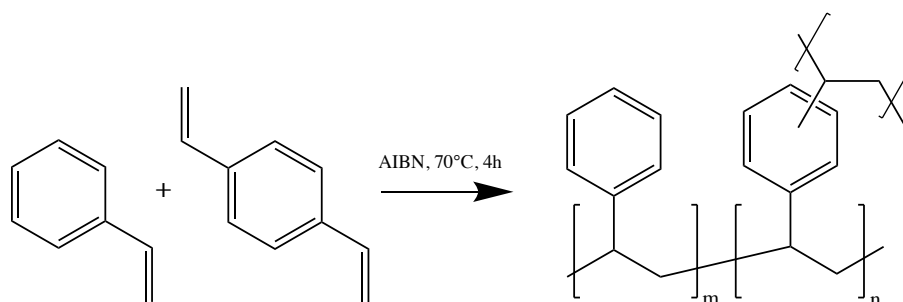


Figure 5.4: Reaction of styrene and DVB to Poly(Styrene-*co*-DVB). Crosslinking degree 50% ($m=n$).

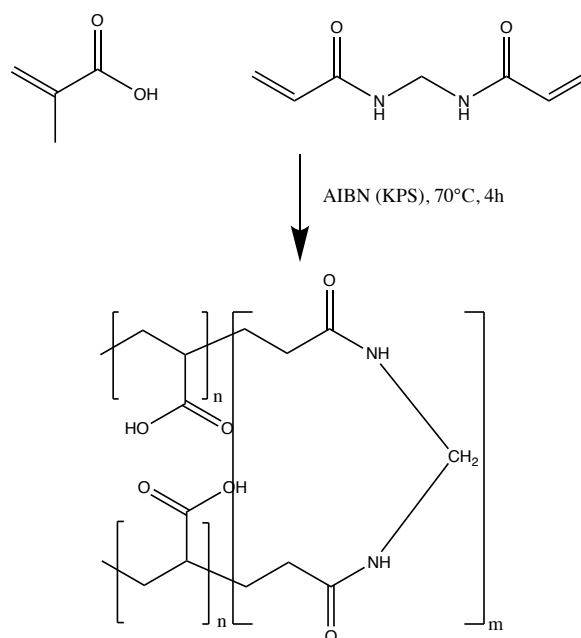


Figure 5.5: Reaction of Methacrylic Acid (MAA) and Methylene bisacrylamide (MBA) to Poly(MAA-*co*-MBA). Crosslinking degree 20% ($m=0.2 \times n$).

Figures 5.4 and 5.5 show the reaction schemes of the thermally initiated free radical polymerisation reactions. The resulting grafted *E. coli*-PolyHIPEs were characterised via SEM imaging.

5.5 Poly-Pickering-HIPEs

Dimethyldioctadecyl ammonium bromide (DODAB) and Hexadecyltrimethylammonium bromide (CTAB) were obtained from Sigma-Aldrich and used as received. The surfactant Hypermer 2296 was kindly supplied by Croda (UK).

In order to create a bacteria imprinted Poly-Pickering-HIPE, a Pickering emulsion of water in Styrene, DVB and the positively charged surfactant Dimethyldioctadecyl ammonium bromide (DODAB) with *E. coli* cells as stabilising particles was produced.

To find the optimal amount of DODAB to make the bacteria more lipophilic and create a (w/o)-emulsion, DODAB was either dissolved in the oil phase at a fixed concentration of 0.5 mM, or dissolved in the aqueous phase. Various surfactant/bacteria ratios were tested (See Section 4.3).

The *E. coli* suspension in distilled water was transferred into a 15 mL Falcon tube and the oil phase was added on top. Then, the mixture was transferred to a shaker over night to provide the energy for the system to build a stable emulsion. It was not tested if it would be sufficient to shake by hand in this case.

For thermo-initiation of the free-radical polymerisation, 1% equivalent to the double bonds of the total monomer concentration thermo-initiator (AIBN) was added. The emulsion was then placed in the oven at 70°C over night. The free-radical polymerisation reaction scheme is described in Section 5.4 (Figure 5.4). To produce an open-porous system, 5 $\frac{V}{V}$ % of the surfactant Hypermer 2296 were added after addition of AIBN and carefully suspended in the emulsion prior to curing.

5.6 *E. coli* imprinted particles from Pickering Emulsions

To create *E. coli* polymer beads with *E. coli* cells on the surface from Pickering emulsion templating, a similar procedure to the Poly-Pickering-HIPE production (Section 5.4) was followed.

To the bacteria stabilised (o/w)-emulsions obtained from mixing various ratios of *E. coli* suspension with oil phase containing DODAB, AIBN was added as

thermo-initiator and the emulsion was cured at 70°C over night. The obtained beads were washed with water for at least 4h and then dried at room temperature. The abundance of bacteria cells on the surface and the size of the beads was confirmed via SEM imaging.

Various bacteria/surfactant ratios were assessed, which have already been discussed in Section 4.4. Washing procedures are described in Section 4.5.

5.7 NIP particles

It is good practice to compare the imprinted materials to non-imprinted equivalents. Since in this case, the imprinted particles were produced by means of *E. coli* stabilisation, a NIP could not be produced via the same procedure. However, it was possible to synthesise poly(Styrene-*co*-DVB), which was then ground with a steel hammer and gave small particles similar in size to the MIP beads.

5.8 Scanning Electron Microscopy

Scanning Electron Microscopy measurements were conducted with a JCM-6000, JEOL GmbH (Eching, Germany) SEM machine.

PolyHIPE samples in a size of roughly 0.5x0.5 cm were broken off from the obtained monolith, mounted on a conductive sticker and placed on the sample holder. The samples were not cut to avoid destroying the surface structure which was to be examined. Beads obtained from Pickering emulsions were put on a conductive sticker and mounted on the sample holder as obtained.

To provide conductivity, the samples on the sample holder were then sputter-coated with gold with a JEOL JFC-1200 Fine Coater for 25 seconds under argon atmosphere. Then, the gold-coated samples on the sample holder were put in the sample chamber in the SEM-machine and the chamber was evacuated. Then, the samples were observed via this technique and images of the samples were saved. If charging-effects occurred, especially among the PolyHIPE samples which were sometimes not completely gold-coated due to their size, the top of the sample was connected to the conductive sticker with silver lacquer with a small paint brush.

For each PolyHIPE sample, images from three different spots on the sample at two different magnifications (500x and 1500x) as well as one image with a 100-fold magnification were taken in order to show that the observed macroporous structure was present throughout the whole sample.

5.9 Uptake Measurements

For bacteria uptake measurements, a suspension of TRITC-labelled *E. coli* cells was added to a known mass of MIP-beads in a 8 mL snap glass and put on a rocker shaker for 4h. The suspension was shielded from light to avoid any photobleaching. The original suspension was put on the rocker shaker as well to ensure reliable results by reducing environmental influences. For cross-selectivity measurements and for the *E. coli*-PolyHIPE uptake measurements, the same procedure was applied to Fluorescence Red-labelled *B. Cereus*-suspensions and *E. coli* suspensions, respectively.

After 4h, the supernatant suspension was removed and transferred into a quartz cuvette for fluorescence spectroscopy measurements or into a Falcon tube for QCM measurements. For fluorescence spectroscopy measurements, both reference suspension and suspension removed from the SPE sorbent material were measured and related to a calibration curve. Each measurement was performed three times to get more reliable results. From that, the concentration of cells and the uptake of the material per mg was calculated. The standard deviation from the spectra was calculated and errors were given relative to that deviation. For error propagation, the largest deviation value was used.

For the time-dependent binding experiment described in Section 4.6.3, 3 mL *E. coli*-suspension (concentration before SPE: 5.82×10^8 cells/mL) in PBS were added to 200 mg of bacteria-imprinted beads of a medium size.

From a series of dilutions of *E. coli* cells suspended in phosphate buffer solution, the following calibration function was obtained:

$$y = 2E^{-5}x + 13.147 \quad (4)$$

In this formula, y is the measured intensity for a given *E. coli*-concentration (x).

Sample calculation for cell uptake of the MIP material:

For the uptake measurement, 3 mL *E. coli*-suspension had been transferred into 88.8 mg large bead SPE material (diameter around 1 mm). Emission maxima obtained at 576 nm were 74.33 ± 1.89 for the reference suspension and 41.91 ± 1.53 for the suspension after a 4 hour SPE step.

(1) The reference formula was rewritten as

$$x = \frac{\text{Measured Intensity} - 13.147}{2 \times E^{-5}} \quad (5)$$

and the *E. coli* concentration was calculated. Here, the measured values correspond to cell concentrations of $2.15E^6$ and $5.34E^5$ cells/mL for reference and SPE-sample solution, respectively.

(2) The concentration after SPE was subtracted from the reference concentration to obtain the difference in bacteria concentration. This value, multiplied by the volume used for the SPE step and divided by the mass of the sorbent material results in the uptake of the material per mg.

$$2.15 E^6 - 5.34 E^5 = 1.62 E^6$$

$$1.62 E^6 \times 3 \div 88.8 = 5.48 E^4 \text{ cells/mg}$$

5.10 Quartz Crystal Microbalance Measurements

Quartz crystal microbalance measurements were performed with a custom made oscillator setup.

Prior to using any QCM, its individual resonating frequency and damping were analysed via a network analyser regarding the respective resonance frequency and damping after each process step.

For each measurement, the measuring cell containing the quartz crystal microbalance was filled with solvent. Careful attention was paid not to have any air bubbles in the liquid chamber and to effectively isolate the air side of the QCM, which should not be in contact with the liquid in order to avoid a short circuit.

The measuring cell was then connected to two oscillator circuits. The frequency of each channel was adjusted to fit the resonance frequency of the respective QCM. In order to reduce shear effects and water entrapment during the measurement, after starting the measurement, the system was left in contact with solvent for at least 20 minutes to establish equilibrium. If no significant changes were observed and the signal was stable and linear, 200 μL of sample were added carefully and the sensor response was observed via a custom made LabView routine. When the signal was stable again, two washing steps were performed in order to get the quartz wafer back to its resonance frequency. These steps were repeated for as many measurements as needed.

For the first QCM measurements, a two-electrode system was used where one electrode was coated with a polyurethane layer, which should have a general affinity for *E. coli* cells, whereas the other electrode was left blank as a reference. Since the SPE steps were performed with bacteria suspended in PBS, phosphate buffer solution was also used as a solvent for the QCM measurements. For first tests, it was tried to reconfirm the uptake results obtained via fluorescence spectroscopy by measuring the same solutions also via QCM. However, several problems were encountered. First, it seemed that using PBS as a solvent was not the best choice for performing QCM measurements, since no stable signal could be obtained from background measurements and a signal drift was observable several times. This is most probably due to charge effects on the electrode surface resulting from the ions in solution. Furthermore, it might be the case that the polyurethane layer

had some undesired interaction with the solvent or considerable shear effects, since upon addition of analyte, the frequency shift was positive, which would mean that instead of adding mass on the polymer layer, mass was removed. Another issue at hand was the bacteria concentration. For reliable detection via fluorescence spectroscopy, a concentration higher than E^7 cells/mL does not give good results. For QCM however, these concentrations are rather in the range of the lower limit of detection.

To avoid the problems encountered during the first QCM-trials, the experimental procedure was adapted in some points: The solvent was changed to distilled water and the primary bacteria concentration was changed to E^8 cells/mL. Furthermore, quartz wafers containing an *E. coli* imprinted polymer layer produced by a colleague [49] were used to increase the sensitivity for the target analyte.

5.10.1 Quartz Surface Coating for QCM measurements

Gold electrodes

The gold electrodes were created on the quartz wafers via screen printing. Gold paste was transferred on top of the wafer through a screen with the desired structure and the quartz was then put in the oven at 400 °C for 4h. The other side of the quartz was coated with gold as well and again put in the oven under the same conditions.

Afterwards, the electrodes were characterised via a network analyser in order to detect possible short circuits or other damages.

Polyurethane layer

To add a sensitive layer on one of the electrode surfaces, polyurethane was synthesised and spin-coated on top of the gold surface.

For the polyurethane synthesis, the following chemicals were mixed:

Chemical	Amount
Bisphenol A (BPA)	118 mg
Phloroglucinol	24 mg
(DPBI)	240 mg
Acetonitrile	200 μ L

Table 5.2: Polyurethane Recipe

From this reaction mixture, 30 μ L were dissolved in 970 μ L acetonitrile. The solution was transferred into a water bath at 70°C for 30 minutes for pre-polymerization. Then, the reaction mixture was stored on ice.

To coat the polyurethane on the gold surface, 20 μ L of the mixture were spotted on the electrode on the quartz which was to be coated and then spin-coated at 3000 rpm for 10 seconds. To prevent the pre-polymer to spread on the second electrode, half of the quartz wafer had been covered with tape before adding the reaction mixture. The wafer was then left at room temperature over night to complete the polymerisation.

To determine whether the polyurethane had successfully formed a layer on top of the electrode, the quartz was studied via light microscopy. Furthermore, the existence of a polymer layer was confirmed via the network analyser. The resonance frequency of the quartz was compared to the frequency before polymer coating to determine whether any layer had been created.

6 Conclusion

The aim of this work was to develop an *E. coli* imprinted polymer material to be used as a sorbent material for preconcentration methods.

After a screening phase, where several approaches of emulsion templating were exploited and led to the development of a macroporous polymer with *E. coli* cells embedded in the surface of the grafted hydrogel as well as to the production of various sizes of bacteria-imprinted Poly(Styrene-*co*-DVB) beads. Upon optimisation of the synthesis processes and investigation of various washing procedures, the materials were tested for their applicability as a sorbent material with regards to several important characteristics.

It was shown that the material can indeed be used as a sorbent material and be applied for preconcentration or sample clean-up. Even though not all template could be removed from the surface, the developed material can take up up to 2.26×10^5 *E. coli* cells per mg. Furthermore, the material shows quick binding of target molecule and selectivity for *E. coli* in a competitive binding experiment with the structurally similar bacteria species *B. cereus*. The material was found to exhibit a tendency for non-specific binding, which can be explained by the complex surface structure of the target analyte, where the great variety of different functional groups make the cell prone to various binding events.

Results show that Pickering emulsion templating can lead to spherical bacteria-imprinted polymer systems which can be used for SPE-purposes. Further investigation is needed with respect to efficient washing procedures and desorption of the analyte. A further step would also be to pack the SPE beads into a cartridge or column and perform 'actual' solid phase extraction, possibly coupled to an *E. coli* detection system. A label-free detection method, e.g. via QCM measurements, would be a good asset to develop an on-line detection mechanism for *E. coli* bacteria which includes an SPE step. Pickering emulsion templating represents an effective way to combine water-soluble template molecules such as biomolecules and hydrophobic monomers for molecular imprinting.

Regarding molecularly imprinted PolyHIPE materials, it has been demonstrated that it is possible to produce a grafted material onto a stable Poly(Styrene-*co*-DVB) scaffold and embed the template molecule in the hydrogel surface via a

one-pot synthesis approach. Bacteria-imprinted sites have not yet been confirmed via this method. Molecular imprinting of smaller biomolecules such as proteins in such a fashion might be an interesting starting point for further research projects due to the large surface areas of such materials, which could lead to a great number of imprints via a straightforward synthesis protocol.

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8 Appendix

8.1 Abstract

In this diploma thesis, the principles of emulsion templating and molecular imprinting of biomolecules were combined to develop *E. coli*-imprinted polymer systems for use in preconcentration methods in analytical applications. *E. coli* bacteria were used as a template organism due to their well-studied surface structure and their application as a biomarker for water and food contamination. A poly(MAA-*co*-MBA) grafted poly(Styrene-*co*-DVB)HIPE with bacteria cells embedded in the surface was developed, which needs to be further investigated for template removal. Highly crosslinked poly(Styrene-*co*-DVB) particles with *E. coli*-cells on the surface were also synthesised in bead diameters ranging between 150 and 1000 μm . Upon template removal, bacteria uptake, uptake kinetics and cross-selectivity as well as competitive binding of these beads were assessed. The material showed a cell uptake up to $2.26 \text{ E}^5 \pm 4.16 \text{ E}^3$ cells/mg and could bind more than 60 % of the cells suspended within the first 20 minutes of extraction. The bacteria-imprinted beads showed selectivity for *E. coli* cells in the presence of *B. cereus*, which is similar in size and shape to the target analyte. The developed protocol of MIP-particle synthesis via Pickering emulsion templating is straightforward and economic.

8.2 Zusammenfassung

Im Rahmen dieser Diplomarbeit wurde *Emulsion Templating* angewandt, um *Molecularly Imprinted Polymers* (MIPs) zur Voranreicherung von Bakterien in analytischen Anwendungen zu synthetisieren. Der Modellorganismus *E. coli* wurde aufgrund seiner hohen Relevanz als Biomarker für Wasser- und Lebensmittelkontaminationen als Template-Organismus eingesetzt. Ein makroporöser Poly(Styrol-*co*-DVB)HIPE mit dem Hydrogel Poly(MAA-*co*-MBA) an den Oberflächen der Poren wurde hergestellt. In diesem Hydrogel waren *E. coli*-Bakterien eingebettet. Weitere Experimente sind nötig, um die Bakterien aus der Oberfläche zu entfernen und deren Imprints nachzuweisen. Außerdem wurden über Pickering-Emulsionen hoch quervernetzte Poly(Styrol-*co*-DVB)-Partikel mit Bakterien an der Oberfläche synthetisiert. Diese Partikel hatten einen Durchmesser von 150 bis 1000 μm und zeigten nach diversen Waschschritten *E. coli*-Imprints. In Tests konnten die MIP-Kügelchen $2.26 \cdot 10^5 \pm 4.16 \cdot 10^3$ Zellen/mg aufnehmen und waren gegenüber dem strukturell ähnlichen *B. cereus* selektiv für *E. coli*-Zellen. Innerhalb der ersten 20 Minuten wurden mehr als 60% der *E. coli*-Bakterien einer Suspension aufgenommen. Das entwickelte Protokoll zur Synthese der *E. coli-imprinted* Partikel via Pickering Emulsionen ist kostengünstig und unkompliziert.