Preparation and self-assembly behavior of anisotropic polymer patchy particles

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To my parents
"There is a place that you are to fill and no one else can fill, something you are to do, which no one else can do."

— Florence Scovel Shinn —
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Publications

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  Fatemeh Naderi Mehr, Dmitry Grigoriev, Nikolay Puretskiy and Alexander Böker,

- "Self-assembly behavior of oppositely charged inverse bi-patchy micro-colloids"
  Fatemeh Naderi Mehr, Dmitry Grigoriev, Nikolay Puretskiy and Alexander Böker
  To be submitted, 2019.

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  Fatemeh Naderi Mehr, Dmitry Grigoriev, Nikolay Puretskiy and Alexander Böker
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  Fatemeh Naderi Mehr, Dmitry Grigoriev, Nikolay Puretskiy and Alexander Böker
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- "Multifunctional Patchy Particles via Microcontact Printing"
  Marc Zimmermann, Fatemeh Naderi Mehr and Alexander Böker
Table of content

Table of content .................................................................................................................. I
List of abbreviations ............................................................................................................ V
Abstract ................................................................................................................................. VII
Zusammenfassung .................................................................................................................. IX
1 Background and motivation ............................................................................................. 1
2 Introduction and literature review ................................................................................... 5
3 Theory and methods ......................................................................................................... 11
   3.1 Patchy particles ........................................................................................................... 13
   3.2 Microcontact printing ................................................................................................ 13
   3.3 Analytical techniques ................................................................................................. 15
      3.3.1 Fluorescence microscopy ..................................................................................... 15
      3.3.2 Scanning electron microscopy ............................................................................. 17
      3.3.3 Transmission electron microscopy ...................................................................... 18
      3.3.4 Dynamic light scattering .................................................................................... 19
      3.3.5 Brunauer-Emmett-Teller theory ........................................................................ 20
      3.3.6 Mercury intrusion porosimetry ......................................................................... 21
      3.3.7 Zeta potential measurement .............................................................................. 22
      3.3.8 Scanning force microscopy ............................................................................... 23
4 Epoxy-functionalized particles for covalently bonded patches ..................................... 25
   4.1 Synthesis and characterization .................................................................................... 27
      4.1.1 PGMA-co-DVB particles .................................................................................... 27
      4.1.2 PVP seeded PGMA particles ............................................................................. 31
      4.1.3 PGMA modified PS particles ............................................................................. 36
   4.2 Fabrication of patchy particles .................................................................................... 38
5 MF particles with electrostatically bonded patches ........................................ 43
  5.1 Mono-patches made of PMVEMA or PEI ................................................. 45
     5.1.1 Patch thickness and the yield of µCP .............................................. 45
     5.1.2 Effect of PDMS treatment with ethanol .......................................... 49
  5.2 Electrostatic interaction and force between mono-patchy particles ............... 51
     5.2.1 Self-aggregation behaviour of patchy particles in a solution with an increased
          ionic strength ................................................................................. 56
  5.3 Oppositely charged bi-patches on particle poles ......................................... 57
     5.3.1 Characteristic and the yield of patchy particles ................................. 57
     5.3.2 Electrostatic interactions between bi-patchy particles ....................... 59
     5.3.3 Effect of ionic strength on self-aggregation behavior of bi-patchy particles .. 62
6 Summary and conclusion .............................................................................. 65
7 Experimental and materials ......................................................................... 69
  7.1 Materials .................................................................................................. 71
  7.2 Preparation of epoxy-functionalized particles .......................................... 71
     7.2.1 PGMA-co-DVB ................................................................................ 71
     7.2.2 PVP seeded PGMA .......................................................................... 71
     7.2.3 Determination of epoxy content on the surface of PGMA particles by titration ... 72
  7.3 Microcontact printing .............................................................................. 72
     7.3.1 Preparation of inked PDMS and monolayer of particles .................... 72
     7.3.2 PDMS treatment with ethanol ......................................................... 73
  7.4 Preparation of dispersion medium at different pH ...................................... 73
  7.5 Characterization ...................................................................................... 73
     7.5.1 Fluorescence microscopy ................................................................... 73
     7.5.2 Scanning force microscopy .............................................................. 74
     7.5.3 Scanning electron microscopy ......................................................... 74
     7.5.4 Zeta potential measurement ............................................................ 74
Bibliography ................................................................................................... XII
| Appendix | XIX |
### List of abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
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<tbody>
<tr>
<td>μCP</td>
<td>Microcontact printing</td>
</tr>
<tr>
<td>μm</td>
<td>Micrometer</td>
</tr>
<tr>
<td>ACN</td>
<td>Acetonitril</td>
</tr>
<tr>
<td>AIBN</td>
<td>Azobisisobutyronitrile</td>
</tr>
<tr>
<td>BET</td>
<td>Brunauer–Emmett–Teller</td>
</tr>
<tr>
<td>BSE</td>
<td>Backscattered electrons</td>
</tr>
<tr>
<td>DLS</td>
<td>Dynamic light scattering</td>
</tr>
<tr>
<td>DVB</td>
<td>Divinylbenzene</td>
</tr>
<tr>
<td>ERO</td>
<td>Epoxy ring opening</td>
</tr>
<tr>
<td>FITC</td>
<td>Fluorescein isothiocyanate</td>
</tr>
<tr>
<td>Geom</td>
<td>Geometric</td>
</tr>
<tr>
<td>GMA</td>
<td>Glycidylmethacrylate</td>
</tr>
<tr>
<td>IEP</td>
<td>Isoelectric point</td>
</tr>
<tr>
<td>kDa</td>
<td>Kilodalton</td>
</tr>
<tr>
<td>KPS</td>
<td>Kalium persulfate</td>
</tr>
<tr>
<td>LCC</td>
<td>Liquid core clusters</td>
</tr>
<tr>
<td>LV</td>
<td>Low voltage</td>
</tr>
<tr>
<td>MF</td>
<td>Melamine formaldehyde</td>
</tr>
<tr>
<td>MIP</td>
<td>Mercury intrusion porosimetry</td>
</tr>
<tr>
<td>M&lt;sub&gt;w&lt;/sub&gt;</td>
<td>Molecular weight</td>
</tr>
<tr>
<td>nm</td>
<td>Nanometer</td>
</tr>
<tr>
<td>ODTAB</td>
<td>Octadecyltrimethylammonium bromide</td>
</tr>
<tr>
<td>PDI</td>
<td>Polydispersity index</td>
</tr>
<tr>
<td>PDMS</td>
<td>Polydimethylsiloxane</td>
</tr>
<tr>
<td>PEI</td>
<td>Polyethyleneimine</td>
</tr>
<tr>
<td>PGMA</td>
<td>Polyglycidylmethacrylate</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Full Form</td>
</tr>
<tr>
<td>--------------</td>
<td>-----------</td>
</tr>
<tr>
<td>PMVEMA</td>
<td>Poly(methyl vinyl ether-alt-maleic acid)</td>
</tr>
<tr>
<td>PS</td>
<td>Polystyrene</td>
</tr>
<tr>
<td>PVP</td>
<td>Polyvinylpyrrolidone</td>
</tr>
<tr>
<td>RT</td>
<td>Room temperature</td>
</tr>
<tr>
<td>S</td>
<td>Styrene</td>
</tr>
<tr>
<td>SDS</td>
<td>Sodium dodecyl sulfate</td>
</tr>
<tr>
<td>SE</td>
<td>Secondary electrons</td>
</tr>
<tr>
<td>SEM</td>
<td>Scanning electron microscopy</td>
</tr>
<tr>
<td>SFM</td>
<td>Scanning force microscopy</td>
</tr>
<tr>
<td>SPM</td>
<td>Scanning probe microscopy</td>
</tr>
<tr>
<td>TEM</td>
<td>Transmission electron microscopy</td>
</tr>
<tr>
<td>Tol</td>
<td>Toluene</td>
</tr>
<tr>
<td>wt %</td>
<td>Weight percent</td>
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Abstract

In framework of this thesis, microcontact printing was used as a mild and quick method to prepare anisotropic particles with pre-defined sites on their surface “Patches”. In this technique, a monolayer of particles on a substrate is pressed against a stamp, which is made of Polydimethylsiloxane (PDMS) and coated with the desired ink. The covalent bonds and electrostatic interactions serve as two possible tools for irreversible and reversible attachment of the patches to the surface of particles. According to this, the corresponding work is also divided into two main sections.

The first part deals with the synthesis and characterization of epoxy-functionalized microparticles based on the polymerization of glycidylmethacrylate (GMA) monomers. Depending on the chosen strategy, three different types of particles can be achieved. At first, to increase the solvent and temperature stability of particles a cross-linker, Divinylbenzene (DVB), was used. Furthermore, polymerization was performed in the presence of Polyvinylpyrrolidone (PVP) as stabilizer. The third possible way was the preparation of core-shell particles with a core made of preformed Polystyrene (PS) particles with a PGMA shell. The physical and chemical properties of synthesized particles, such as polydispersity, morphology, porosity and the surface density of epoxy-groups were characterized in order to be able to correlate the microcontact printing behavior with the respective particle characteristics. For surface properties, different microscopies were utilized, e.g. optical microscope and SEM imaging. Other microscopic methods, cryo-SEM and ultrathin section TEM were used for internal structures and pores. In addition, BET nitrogen adsorption-desorption isotherms and mercury intrusion porosimetry were used to define the porosity of particles. Determination of the content of epoxy groups was carried out by epoxy ring opening reaction (ERO) of particles with a titrant. Finally, these three types of particles together with a sample of reference epoxy-functionalized particles prepared by Microparticles GmbH were functionalized with high molecular weight polyethyleneimine (Mw 600-1000 kDa). The resulting patchy particles were labelled with Fluoresceinisothiocyanate (FITC) and visualized
by fluorescence microscopy. To check, if the attachment of the patch to the particle surface was via covalent bond and not because of the electrostatic interactions, patchy particles were dispersed in a basic medium. As expected, despite of non-dissociated state of PEI at very basic pH, patches remain attached to the surface of epoxy-functionalized particles via covalent bond.

The second part of the work describes, how electrostatic interactions lead to generation of one or two oppositely charged patches on the surface of a commercially produced melamine formaldehyde (MF) particle. To prepare the ink solutions for μCP, high molecular weight polyelectrolytes, poly (methyl vinyl ether-alt-maleic acid) (PMVEMA) (Mₗ 1980 kDa) and Polyethyleneimine (PEI) (Mₗ 600-1000 kDa) were used, which are positively and negatively charged, respectively. Since the isoelectric point (IEP) of MF particles is around neutral pH, at the contact interface between MF particles and these multivalent polymers strong local electrostatic attractions is induced, which leads to the attachment of patches to the particle surface. This enables simple, quick preparation of patchy particles by μCP at RT. In case of mono-patches, MF particles were printed on one side of their surface with either PMVEMA or PEI. Whereas, bi-patchy particles with oppositely charged patches on their poles were fabricated by sandwich μCP. The effect of the thickness and molecular weight of polyelectrolytes as well as treatment of PDMS stamps with ethanol on yield of patchy particles and patch parameters were studied. To this end, thickness and diameter of patches were measured using AFM and SEM images of patchy particles. Self-assembly behavior of zwitterionic mono-patchy particles at different pH values was studied. The theoretical electrostatic force between patchy particles as function of pH was quantified using a simplified model based on Coulomb's law. These values were then compared with experimental results. The increase of ionic strength of dispersion medium leads to reduction or even elimination of self-aggregation of patchy particles. Comprehensive quantitative analysis of a diversity of short and long, linear and branched chains of bi-patchy particles, which were spontaneously formed via electrostatic interactions between oppositely charged patches, was investigated. The reliability of statistics was checked using a Hemocytometer for counting of particles by fluorescence microscopy.
Zusammenfassung


Zusammenfassung


reduziert oder sogar eliminiert werden kann. Durch die elektrostatische Wechselwirkung zwischen den entgegengeladenen Patches bildeten sich diverse kurz- und langkettigen Strukturen aus, die mit Hilfe einer Zählkammer quantitativ analysiert worden sind.
1 Background and motivation
The aim of functionalization of microcolloids is to impart the ability of self-assembly as well as a later self-replicating to the artificial materials. An excellent example in the nature is DNA strand and its polymerase chain reaction to build up more chains analogue to a master chain. Considering a DNA strand as two cross-linked chains, each chain consist of many small building blocks, “nucleotides”, which are bonded to the next ones by irreversible cross-linking between their phosphodiester backbones. If necessary, each DNA strand is able to be opened and so form a new chain analogue to the first chain. The nucleobase of a DNA unit serve as reversible cross-linker between the first and second chain\(^1,2\). Based on this knowledge, a new model for functionalization of microcolloids was developed. The concept of this idea was to transfer the structure of DNA nucleotides to the spherical microcolloids. To this end, three functional regions, “patches”, must be generated on the surface of particles. A directional interaction between patches can enable the ability of self-assembly as well as replicating, which can be controlled by reversibility of these interactions (Figure 1-1). On this approach, the first step is to prepare patchy particles with two patches and further a third patch can be generated on their surface.

\[ \text{Figure 1-1} \quad \text{scheme of DNA polymerase chain reaction to form new strands by self-replicating of one master strand. Analogue to the single chains of a DNA strand, two identical strands will be formed via reversible and irreversible cross-linking between nucleotide units by their nucleobase and phosphodiester backbone, respectively (a). Conceiving the structure of a nucleotide to the spherical microcolloids to impart the ability of self-replicating to artificial materials with two green patchy regions for irreversible interactions to build a master chain of particles and a third black patch for its reversible cross-linking (b).} \]
2 Introduction and literature review
Recently, patchy particles have been attracting growing attention in material science due to their anisotropic surface properties\textsuperscript{[34]}. Compared to colloidal particles with uniform properties, they have the ability of directed self-assembly to build new functional materials, which might be used in photonics, electronics, or sensor applications\textsuperscript{[5-8]}. Furthermore, there are a variety of potential applications based on their anisotropy, such as switching of displays, self-healing materials, or drug delivery\textsuperscript{[9-13]}.

A directional chemical or physical interaction between patchy particles leads to the formation of specific complex structures, e.g., chains and rings, 2D and 3D structures like squares, pyramids, tetrahedra, twisted shapes and even diamonds, multi-layers, polymer-like chains, DNA or RNA string-like helices, and some of them have been fabricated over the past years\textsuperscript{[14-20]}. Since not all but some of these structures have been fabricated yet, considerable interest has focused on their formation mechanism and the corresponding interactions between patchy particles\textsuperscript{[21-28]}. This led to a number of theoretical works, which aimed to predict the form and three-dimensionality of the resulting structures by changing the patch parameters\textsuperscript{[29-31]}.

For example, R. Guo and his co-workers showed in the results of their simulation that the presence of three patches on the surface of each building block is needed to achieve DNA-like helices. Furthermore, the size and the spatial distribution of patches play a crucial role in the final configuration, e.g., the diameter of the helices\textsuperscript{[32,33]}. Furthermore, it was shown in theoretical works of E. Bianchi that patchy particles could be self-assembled to clusters which morphology depends on the size and number of patches for different types of patchy particles, such as DNA- or protein-like systems. They used either Monte Carlo simulations or developed optimization ideas based on evolutionary algorithms\textsuperscript{[30,34-44]}.

Apart from the simulations, preparation of multi-patchy particles with more than two patches in a one-step process was realized by colloidal fusion of a liquid core out of a pressure-deformed cluster of particles. The obtained patchy particles were able to form polyhedral configurations such as tetrahedral structures. The disadvantage of this method is the limited applicability and the lack of chemical and physical diversity of the obtained patches\textsuperscript{[45]}.

Additionally, it was reported, that microcontact printing (\(\mu\)CP) as a quick, cheap and efficient method, ensures the transfer of the ink molecules to the colloids via polydimethylsiloxane
(PDMS) stamps with high reproducibility with respect to the patch parameters\textsuperscript{[46]}. It is also possible to precisely control most of the parameters influencing the resulting patch parameters, like ink concentration, ink layer thickness on the stamp, stamping force and duration, adhesion of the ink to the stamp and particle surface, polarity and solvency of the auxiliary solvent used for the release of patchy particles etc. On the other hand, relatively small yield of resulting patchy particles and significant technical difficulties of this method in the application to the sub-micrometer particles or for the production of patchy particles with more than two patches should be mentioned as disadvantages of the proposed \mu CP method\textsuperscript{[17-56]}. 

In \mu CP, chemical or physical interactions between the ink and particles can be used to attach patches onto the surface of colloidal particles, e.g. reversible electrostatic interactions or irreversible covalent bonds\textsuperscript{[51-55]}. For example, \mu CP was established for the attachment of an amino-functionalized ink onto the synthesized polymer particles via epoxy ring opening reaction (ERO)\textsuperscript{[56-58]}. It was also shown that polymeric inks with high molecular weight, e.g., branched polyethyleneimine (PEI) could be electrostatically attached to the surface of the silica particles. A variety of patches from 2D and 3D patches to striped patterned patches were formed via microcontact printing with wrinkled PDMS stamps\textsuperscript{[40,49,59]}. Despite their shape diversity, all these patches were uniform in other properties and identically charged. It was also reported by O. Cayre that one positively charged patch made of ODTAB can be generated on the negatively charged sulfate latex particle\textsuperscript{[60]}. 

Inverse patchy colloids (IPC) were considered as a new type of heterogeneously charged particles in theoretical and experimental investigations\textsuperscript{[61,62]}. The term "inverse" here refers to the repulsive interactions between the patches and attractive interactions between patchy and patch-free regions of the particles. In a work of P. D. J. van Oostrum, silica patchy particles with two identically positively charged patches on their poles made of APTES and a negatively charged equatorial belt were prepared. Their electrostatic interactions and self-assembly to form clusters was studied using simulation methods\textsuperscript{[64,69]}. 

The strength of the electrostatic interactions between patchy particles can be controlled by effective charge heterogeneity between patchy and non-patchy surfaces. To this end, a specific ion is bonded to the surface of the patch or particle\textsuperscript{[63,64]}. For example, the surface charge density of the patch can be changed by protonation; hence the aggregation of patchy
particles in solution can be manipulated by pH-regulation in protic media to initiate particle-assembly in a desired way. In simulations using charged patchy particle models (CCPM), it was also found that the electrostatic interactions between patchy proteins can be controlled by change of ionic strength. Despite a variety of theoretical investigations on the prediction of the interactions generally between two charged spheres and also in specialized form of two charged patchy particles, there have not been many experimental studies in this regard\textsuperscript{65-67}.

Conceiving the idea of using patchy particles for biological applications tends to more investigations on patchy particles made of bio-organic substrates, which are compatible with living systems. In this sense, microcolloids such as polymer particles with epoxy groups were functionalized via covalent bonding of positively charged amino groups on their surface, e.g., proteins and enzymes\textsuperscript{60,66,68}.

In our work, we first focused on the synthesis of epoxy-functionalized polymer particles and generation of covalently bonded patches on their surface by μCP. Furthermore, we used sandwich μCP for creation of bi-patchy particles. Afterwards we put our main effort on the generation of either single charged or two oppositely charged patches, made of high molecular weight polyelectrolytes on the surface of polymer particles. We used comprehensive statistics and theoretical calculations to study the self-assembly behavior of patchy particles driven by their electrostatic interactions.
3 Theory and methods
3.1 Patchy particles

Patchy particles are defined as patterned anisotropic nano- or micro-sized particles with functionalized regions on their surface, "Patches", which lead to their directional interactions with other materials [14]. This enables their self-assembly to target structures of higher complexity. Covalent bonds or electrostatic attractions are employed as irreversible or reversible interactions for controlled particles self-assembly. Epoxy ring opening reaction, as one type of click chemistry can be used for generation of patches. A high molecular weight polymer with a large number of amino groups, e.g. PEI, can react easily with epoxy groups on the surface of particles.

Among a variety of possible materials for particle synthesis, MF particles showed the advantage of their IEP at almost neutral pH. This property can be used for creation of either positively or negatively charged patches on the surface of MF microparticles. Since preparation and characterization of patchy particles was performed in ethanol-water medium, zeta potential of MF particles was also measured as function of pH in water and pH\textsubscript{e} in ethanol-water (90:10). The resulting diagrams also show the IEP at pH 7.1 and pH\textsubscript{e} 8.9 respectively (Figure 3-f). PMVEMA and PEI, as strong and multivalent polyelectrolytes, induce a considerable electrostatic attraction to the MF particles, which results in the creation of patchy particles with a good yield and patches with a high surface charge density and stability.

3.2 Microcontact printing

Kumar and Whitesides introduced microcontact printing (μCP) as a type of soft lithography method in 1994 [68]. Comparing the approaches established for fabrication of patchy particles, such as Emulsion-Templating, metal vapor deposition, Liquid Core Colloidal Clusters (LCC) and so forth, μCP as a moderated, not expensive equipped method allows us to transfer the ink molecules to the colloids as a patch and to reproducibly control over the patch size [53, 54, 91].

The transfer of the ink onto the surface of microcolloids occurs at the interface of target colloids and an elastomeric stamp, usually made of polydimethylsiloxane (PDMS). The stamp,
coated with a uniform and thin layer of the ink via spin coating, will be then pressed against a mono-layer of colloids (Figure 3-2).

Figure 3-1 Zeta potential of the MF particles as a function of pH and the corresponding IEP at pH 7.1 in water (a), scheme of generation of negatively and positively charged patches on the MF particles (b) (Images reused from Ref. [89]).

Figure 3-2 Scheme of micro contact-printing: An inked PDMS stamp is pressed against a monolayer of particles. The transfer of ink molecules to the surface of the particles leads to creation of the patches. In case of “sandwich” micro contact-printing, two inked PDMS stamps are used to create two patches on the opposite poles of the particles.
The mono-layer of particles can also be generated by various methods such as, drop casting, convective assembly, Langmuir-Blodgett-film transfer and spin coating\textsuperscript{[70-78]}. In μCP, the size of patches can be controlled by variation of the pressure applied to the system and the rigidity of the PDMS stamp, however it is possible, that the size and form of the patches varies from particle to particle in a batch\textsuperscript{[46]}. Furthermore, μCP allows the generation of multi patches on the colloids in a one-step process via sandwich-μCP.

Fabrication of bi-patchy particles can be achieved by printing of the particles with two inked flat stamps. Furthermore, to generate three or even four patches in a one-step process, flat PDMS stamps can be replaced with wrinkled stamps. Applying of PDMS wrinkles has opened a new way to generate pattern-like multi-patches and more on the surface of colloids in one single step (Figure 3.3)\textsuperscript{[59]}.  

![Diagram of patch generation](image)

**Figure 3.3** Generation of two, three and four patches on the particle surface via μCP using flat and wrinkled PDMS stamps in a one-step process (Image adapted with modification from Ref. \textsuperscript{[59]}).

### 3.3 Analytical techniques

#### 3.3.1 Fluorescence microscopy

Over the past years, many attempts to improve microscopy have been focused on increase of contrast between the object of interest and its background. For example, fluorescence microscopy has become an irreplaceable microscopy in some research fields, such as biochemistry due to this advantage that it reveals only the interesting object on its dark background\textsuperscript{[74]}. 

-15-
Fluorescence occurs, when a tagged probe absorbs the light. As a result, the light becomes excited and then emitted at specific wavelengths. The difference between exciting and emitted wavelengths, considered as Stokes shift, is the individual property of each fluorescence material. This is owed to the molecules, named fluorophores, which are used to label the object. According to Stokes shift, the specimen is illuminated with a filtered light of the excitation wavelength of the fluorophore and the return light is also filtered at its shifted emission wavelength, which is usually longer than the excitation wavelength [75]. To prevent overlapping of the exciting and emitted light beams a dichroic mirror is required to separate the light paths. This splitter mirror is in the microscope so located that it receives and reflects the paths at 45°. A filter cube with very high selectivity is always used to pre-select the exciting wavelengths. Another one permits only the specific section of return light with the longer shifted wave length to pass into the detector, for example, one photon in 10,000 of wrong excitation lights illuminated to the specimen and emitted lights returned from its fluorescence molecules (Figure 3-4)[76].

![Figure 3-4](https://www.fluorescencemicroscopes.com-25.04.19)

**Figure 3-4** Scheme of fluorescence microscopy (a), image of a filter cube (b). A filtered light of the excitation wavelength of the fluorophore is illuminated to a dichroic mirror and reflected towards the tagged probe. The return light is also filtered at its emission wavelength and will be detected and analyzed to make the visualization of the object of interest (Image (a) modified from Ref.[77] and image (b) modified from https://www.fluorescencemicroscopes.com-25.04.19).
3.3.2 Scanning electron microscopy

The surface properties of an object of interest such as morphology, geometry, and roughness can be characterized by scanning electron microscopy (SEM). The principle of this technique is the acceleration of electrons in a vacuum from a thermionic, Schottky or field-emission cathode to an anode by a voltage difference in a range of 0.1 to 50 keV. Bombarding of the specimen with the accelerated electron beam leads to an elementary atomic interaction between electrons and the specimen at its surface, which is resulted in the elastic and inelastic scattering of electrons. The detectors receive the final signal, consisting of a collection of emitted electrons from the at different energy levels, i.e., secondary electrons (SE) with lower energy of 2-5 eV and backscattered electrons (BSE) with an energy of 50 eV (Figure 3-5)[78]. At the end, a computer reveals the surface structure of the sample via analyzing the stored data obtained from the detector. It is to mention, that specific samples with less or no conductivity must be sputtered with a noble metal, e.g., platinum or gold before imaging.

![Figure 3-5 Principle of SEM: An electron beam is accelerated between the cathode and the anode by the voltage difference. The elastic and inelastic interactions between electrons and specimen lead to the emission of secondary electrons (SE) and backscattered electrons (BSE), which will be transmitted as a signal to the detector for imaging (Image modified from Ref. [78]).](image-url)
3.3.3 Transmission electron microscopy

In the conventional transmission electron microscopy (TEM or CTEM), an electron beam is transmitted through a thin section of the specimen. Here, the difference to SEM is that the electrons pass the sample and the internal properties of the object are then represented by absorbing and scattering of electrons. The high resolution of imaging even in a sub-nanometer range is possible due to the shorter wavelength of the electrons than photons in visible light, which makes this microscopy more accurate compared to light microscopy. In order to passage of the electrons through the object, the thickness should not exceed a limit of 60-80 nm. For low voltage transmission electron microscopy (LV-TEM) of polymeric materials with a voltage of some units of kV, the object must be cut into ultra-thin sections with a thickness of 30-60 nm via a diamond microtome knife. To this end, the embedded object in a resin matrix, usually epoxide or methacrylate, is fixed on the arm of a specialized instrument, which moves slowly on the edge of the diamond microtome for the precise generation of ultra-thin slices (Figure 3-6).

![Figure 3-6](image)

*Figure 3-6* Scheme (a) and a sample (b) of embedded object, which is fixed on the arm of the machine. The arm moves in a vertical motion against the diamond knife and cut the object into ultra-thin sections (Image (a) modified from Ref. [81] and image (b) from [http://wims.rutgers.edu-25.04.19](http://wims.rutgers.edu-25.04.19)).

Another possible approach to produce thin sections for TEM is freezing and cutting of the object by liquid nitrogen at Cryo-temperatures. In Cryo-TEM, the electron beam passes through the frozen sample. An electromagnetic lens creates the magnified image of the internal structure of the sample on the detector via scattered electrons (Figure 3-7).
**Figure 3-7** Scheme of sample preparation for Cryo-TEM: The sample is dropped on a holder, mounted to be fixed and then frozen with liquid nitrogen. The opening of the holder leads to a random fractioning of the object (a) and the image processing by the passage of the electrons through the sample (b) (image (a) based on Ref. 83 and image (b) modified from Ref. 82).

### 3.3.4 Dynamic light scattering

As a tool for the measurement of the hydrodynamic diameter of the colloids, usually dispersed in water, dynamic light scattering (DLS) is based on the scattering of the light by the random movement of the particles in a suspension, called as Brownian motion.

At a known temperature, the hydrodynamic diameter ($d_H$) of the particles is calculated using Stokes–Einstein equation 5-1:

$$d_H = \frac{kT}{3\pi\eta D}$$  \hspace{1cm} (5-1)

Where $k$ is Boltzmann’s constant, $T$ is temperature, $\eta$ is the viscosity of the dispersant and $D$ is diffusion coefficient.

Here, particles are illuminated by a laser beam, which interferes with the corresponded reflected light from the particles. Due to the destructive or constructive phase addition of the illuminated and scattered light beams, the intensity of the signals, which are received by the
detector, fluctuates. The intensity fluctuations are more often for smaller particles because of the higher movement rates. The intensity of the signals obtained from a particle at \( t_0 \) is compared with itself at a later time, \( t_r \), to measure the relation between them. In this sense, the correlation coefficient varies from 1 to 0 in a time period of 0 to infinity. The size distribution as a function of the relative intensity, total volume or the number of particles can be obtained by various algorithms of the correlation coefficient. The difference between these three functions will be more obvious if the plot shows more than one peak in a mixture of particles with different size distribution. The ratio between two peaks will be 1:1000 by changing the function from number to volume due to the proportionality of \( d^3 \) (\( V=4/3\pi r^3 \)) and 1:10^6 by converting to the intensity of scattering because of the Rayleigh approximation (Figure 3-8)[85,86].

![Diagram showing the principle of DLS](image)

**Figure 3-8** Principle of DLS: Destructive or constructive phase addition of illuminated and scattered light beams leads to intensity fluctuations, which occurs more often for particles with a smaller diameter. A correlation coefficient serves as a comparison of the intensity of the signal received from a particle at starting time with itself at a later time. Size distribution as a function of the number, volume and intensity can be obtained by processing of the correlation curve (Image based on Ref. [87]).

### 3.3.5 Brunauer-Emmett-Teller theory

In 1938, Stephen Brunauer, Paul Hugh Emmett, and Edward Teller (BET) developed a theory for calculating the specific surface area and the porosity of the materials based on the adsorption of gases, e.g., nitrogen on the internal and external surfaces[88].
The plot of the volume of the adsorbed nitrogen \( (V_a) \) as a function of relative vapor pressure \( (P/P_0) \) at a constant temperature is named BET-isotherm. According to IUPAC classification, six types of isotherms are possible, which are corresponding to the various categories of the materials with different porosity (Figure 3-9).

![BET isotherm diagram](image)

**Figure 3-9** Principle of BET: Relation between the volume of the adsorbed/desorbed gas molecules on the external and internal surfaces of the material and the partial vapor pressure is called BET-isotherm. IUPAC classification introduces six types of isotherm with different degree of porosity (Image modified from Ref. [89]).

Furthermore, calculation of the specific surface area, which for highly porous materials is usually larger than the geometric surface area offers adequate information about the porosity degree\(^{[90]}\).

For an accurate measurement, samples must be purified by degassing and heating in vacuum over night to remove the rest solvent and other gases, which can also be adsorbed.

### 3.3.6 Mercury intrusion porosimetry

Similar to BET theory, which evaluates the data of nitrogen adsorption isotherms, mercury intrusion porosimetry (MIP) also serves as a characterization technique of the porous materials, such as the pore size distribution, the total volume of the pores and the specific surface area\(^{[91]}\). From one side of the container, mercury is intruded into the porous material with external pressure, and from the other side vacuum pulls it and removes the rest air. The pressure difference between ingoing mercury and outgoing vacuum, which is needed to
overcome the surface tension of the non-wetting mercury, is used to calculate the pore diameter ($d_p$) using Washburn's equation 5-2:

$$\Delta P = \frac{2\gamma \cos \theta}{d_p} \quad (5-2)$$

Where $\Delta P$ is the pressure difference, $\gamma$ and $\theta$ are the surface tension and the contact angle of mercury respectively.

The plot of the pressure difference as a function of the volume of mercury, which is used to cover all of the pores, represents different pore diameter in case of more than one plateau (Figure 3-10).

Figure 3-10 Scheme of Mercury intrusion porosimetry: Mercury is pushed into the porous material, and vacuum removes the air and works against the surface tension of mercury. In the resulted pressure-volume plot, two plateaus correspond to the large and small pores respectively (a). The non-wetting properties of mercury due to its contact angle of more than 90° (b) (image based on Ref. [93,94]).

3.3.7 Zeta potential measurement

Considering a sphere surrounded by an electrical double layer, zeta potential (ZP) is the potential of the most external layer, which differs from the surface potential (Figure 3-11). The ZP of the particles can be indirectly calculated using the measured electrophoretic mobility. The Smoluchowski equation 5-3 shows this relation:

$$U_E = \frac{2 \varepsilon z f(K_d)}{3\eta} \quad (5-3)$$
Where $U_e$, electrophoretic mobility, $z$, zeta potential, $f(K_z)$, Henry’s function and $\eta$ is viscosity. According to Hückel and Smoluchowski approximation, $f(K_z)$ is 1 and 1.5 respectively[^96].

**Figure 3-11** Electrical double layer of a positively charged sphere, introducing the zeta-, the stern- and the surface potential (a) the principle of ZP measurement using electrophoretic light scattering (b) (Image (a) modified from Ref. [^97] and image (b) modified from Ref. [^98].)

A possible approach to measure the electrophoretic mobility is based on the scattering of the light by the movement of particles in an electric field. By applying the field, particles, which are mounted between two electrodes, flow in the capillary tube of the cell due to their surface charge.

The detector receives a side beam of the original light and scattered beam, and then compares the frequency of the scattered light with the frequency of the original light as a reference. The difference, which is named “Doppler shift”, is determined by analyzing of the received data. Finally a computer calculates the ZP using a series of mathematical equations[^98].

### 3.3.8 Scanning force microscopy

The surface topography of an object of interest can be mechanically characterized by scanning force microscopy (AFM). It is a high-resolution type of Scanning Probe microscopy (SPM) and was invented by a group of scientists of IBM in 1982[^99].
The principle of this technique is that a tip, attached to a micro-cantilever spring, shortly lever, scans the probe over its surface (Figure 3-12). For the mechanical scanning, a piezoelectric moves the sample in a raster pattern in respect to the tip. Two type of tapping-modes for scanning of the sample surface are possible: contact and non-contact modus.

**Figure 3-12** Simplified scheme of AFM imaging via scanning the probe and tapping of the surface of the sample using the tip on the cantilever (Image modified from Ref. [100]).

The advantage of applying contact modus is the improvement of the resolution, named atomic resolution, of the resulted AFM-images. In the contact modus the interatomic forces between the sample and the tip are short range and more controlled, while in a non-contact modus the long range interactions, 10-100 nm, such as van der Waals, electrostatic or magnetic exist. The interactions between atoms of the sample and the tip lead to the deflection of the lever. To follow the movements, a laser beam is focused on the cantilever and a photo detector receives the scattered beam. The detector digitizes the deflections or movements in a z-stack as a function of x and y and send the results to a computer. A wide range of precise topographic and height information in a nm resolution is obtained by processing the data in the computer[101].
4 Epoxy-functionalized particles for covalently bonded patches
4.1 Synthesis and characterization

The aim of synthesis of the epoxy-functionalized particles is to generate covalently bonded patches on their surface. The particles can be attached to the polymeric ink by means of one type of click chemistry, epoxide-ring opening (ERO) reaction. For reproducible fabrication of patchy particles with a reasonable yield, the synthesized particles should meet some essential criteria, such as spherical form, low or better no porosity, low polydispersity and finally a high density of epoxy groups on the surface of the particles.

In this work, preparation of polymeric particles with epoxy functional groups was based on the polymerization of glycidylmethacrylate (GMA). To this end, three approaches are followed: first of all, for better thermal and chemical stability, highly cross-linked poly (glycidyl methacrylate-co-divinyl benzene) P(GMA-co-DVB) particles are synthesized by precipitation polymerization\textsuperscript{[102,103]}. In the second approach, polyvinylpyrrolidone (PVP) is used as a stabilizer for seeded dispersion polymerization\textsuperscript{[104-106]}. At last, pre-formed polystyrene (PS) particles are modified by the formation of P (GMA) shell around them via dispersion polymerization\textsuperscript{[107]}. The particles obtained by these three methods and a batch of commercially available epoxy-functionalized melamine formaldehyde (MF) particles were used for preparation of patchy particles via covalent bond with amino groups.

4.1.1 PGMA-co-DVB particles

Polymer particles with epoxy functional groups on the surface are the product of precipitation polymerization of GMA with DVB as a cross-linker.

\[
\begin{array}{c}
\text{H}_2\text{C} & \text{CH}_3 \\
\text{O} & \text{O} \\
\text{CH}_3 \\
\text{C}=\text{C} \quad \text{O} \\
\text{O} \quad \text{O} \\
\text{AIBN / Tol / ACN} \\
60^\circ\text{C, 24 h} \\
\end{array}
\begin{array}{c}
\text{(GMA)} \\
\text{(DVB)} \\
\text{(PGMA-co-DVB)}
\end{array}
\]

According to a modified literature approach, GMA and DVB with a ratio of 25:75 polymerize in a mixture of acetonitrile-toluene and so form particles upon a gentle movement of reaction mixture\textsuperscript{[156]}. 

-27-
For a successful fabrication of the polymer particles, the stirring method and the proper ratio of the monomer and the cross-linker play a key role\cite{108}. Using a magnet stirrer results in a double-shaped forms of the particles and a lower concentration of DVB causes the formation of large particulate aggregates. A homogenous stirring of the reaction mixture is achieved by the gentle movements in the incubator. The obtained particles in the incubator are spherically shaped with an average diameter of $4.5 \pm 2.5 \mu m$; however an improvement of polydispersity index to the values less than 0.3 by adjusting the reaction parameter such as temperature and movement speed was not successful (Figure 4-1).

\textbf{Figure 4-1} SEM-images of the obtained PGMA-co-DVB particles at lower concentration of DVB, a ratio of (GMA: DVB) 50:50, in incubator leads to bulky polymer particles (a) whereas a ratio of (GMA: DVB) 25:75 using magnet stirring end up with double-shaped particles (b). The particles obtained at a ratio of (GMA: DVB) 25:75 in incubator are spherical (c), although their size distribution show a high polydispersity (d).
The porosity of the particles was characterized by nitrogen adsorption isotherms with the following evaluation based on BET theory. According to IUPAC classification, the obtained BET isotherm of PGMA-co-DVB particles is a type II isotherm, which corresponds to the non-porous or macro-porous materials (Figure 4-2)[109]. The average diameter and total volume of the pores, which was measured by BET, showed the non-porosity of the particles (Table 4-1).

**Figure 4-2** BET-isotherm of PGMA-co-DVB particles (a) and its comparison with BET-isotherm “type II” from IUPAC classification corresponded to the materials, which are either not porous or have macro-pores (b) (Image (b) modified from Ref. [109])

**Table 4-1** Size and pore volume measured by BET of PGMA-co-DVB particles show the non-porosity of the particles. In contrast to these values, comparison of the $S_{BET}$ 9.0 m²/g with the calculated $S_{porous}$ 1.33 m²/g shows a huge difference.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Unit</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>cumulative pore volume (Gurvich) $V_4$ at $P/P_0 = 0.98$</td>
<td>mL/g</td>
<td>0.008</td>
</tr>
<tr>
<td>specific surface area $S_{BET}$</td>
<td>m²/g</td>
<td>9.0</td>
</tr>
<tr>
<td>micro-pore volume</td>
<td>cm³/g</td>
<td>0.004</td>
</tr>
<tr>
<td>mesopore surface area</td>
<td>m²/g</td>
<td>2.4</td>
</tr>
</tbody>
</table>
The BET specific surface area of the particles “$S_{BET}$” (equation 6-1), which is equal to the total surface area per particles mass unity (e.g. one gram) was compared with their calculated geometrical surface area “$S_{geom}$”.

$$S_{BET} = \frac{\text{total surface area}}{\text{mass of the particle}}$$

(6-1)

Considering a particle as a sphere, the geometrical surface area can be calculated as a product of surface area of a single particle and the number of the particles composing a weight unity of them, i.e. one gram of these particles. The mass of a single particle “$m_p$” is calculated by the known density and volume (equation 6-2).

$$m_p = \frac{4}{3} \pi r_p^3 \cdot \rho_p$$

(6-2)

Where $r_p$ is the radius and $\rho_p$ is the density of a particle.

Number of particles per 1 g of particulate powder is related to the mass of a single particle (1/$m_p$). For a total number of particles “$N$”, $S_{geom}$ is equal to the area of a single particle “$A_p$” multiplied by $N$. Similarly, the area of a single particle is related to its radius as $A_p$ is equal to $4 \pi r^2$ (equation 6-3).

$$S_{geom} = N \cdot A_p = \frac{A_p}{m_p} = \frac{3}{r_p \rho_p}$$

(6-3)

Considering the average diameter of 4.5 μm and the density of 1 g/cm$^3$ in (equation 6-4), $S_{geom}$ is found to be 1.33 m$^2$/g. Thus, the comparison of the geometrical and the BET specific surface areas of PGMA-co-DVB particles show a huge difference, which could be a consequence of their non-compact internal structure.

Characterization of the interior morphology of the particles by ultra-thin section TEM and Cryo-SEM shows that the BET-N$_2$ adsorption is affected by porous internal structure of particles. This may be a result of the harsh measurement conditions of this method, for example applying a vacuum at high degassing temperature of 50 °C, which causes a number of holes in the particles by removing of the residual solvents, unreacted monomers or oligomers. The cap-like shaped particles compared to originally sphere-shaped particles,
observed by SEM, is another example of the shape-deformation of the particles through nitrogen adsorption. This may explain the contradiction of the calculated $S_{\text{geom}}$ with the obtained $S_{\text{BET}}$ from the evaluation of the isotherm of type II (Figure 4-3).

**Figure 4-3** SEM, ultra-thin section TEM and Cryo SEM images of the PGMA-co-DVB particles before and after BET-N$_2$ sorption (a-c, left to right, respectively).

### 4.1.2 PVP seeded PGMA particles

Polymerization of the GMA monomers in presence of PVP as stabilizer was performed in ethanol and optimized by increase of the reaction time. Physical and chemical properties of the resulting particles were characterized. Because of the chemical reactivity of used ethanol for ERO during formation of particles, the density of epoxy groups on the surface of resulting particles was quantified.
To study the effect of time, every 2 hours a sample of obtained particles was observed by optical microscopy. After the first 2 hours, some double-shaped particles are formed, which disappeared after 16 hours (Figure 4-4). The polymerization in ethanol for 16 hours yields almost monodisperse particles with an average diameter of 2.2 ± 0.3 μm (PDI = 0.02).

Figure 4-4 Optical microscope images of the PVP seeded PGMA particles after a reaction time of 2 hours (a) and 16 hours (b) dispersed in water (left) and dried (right).
The distribution and size of internal pores of the particles were characterized by Mercury intrusion porosimetry (Table 4-2). The volume of mercury intruded in the particles pores as function of applied pressure shows two plateaus, which correspond to different groups of small and large pores with diameter of approximately 100 and 1000 nm, respectively. The value of 1 μm for the pore size may refer to the pores between particles, which could not be occupied (Figure 4-5).

Table 4-2 Size and volume of the pores of the PVP seeded PGMA particles measured by Mercury intrusion porosimetry. The average pore diameter refers to the small pores of the particles.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Unit</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>cumulative pore volume</td>
<td>[mm³/g]</td>
<td>577</td>
</tr>
<tr>
<td>total pore surface</td>
<td>[m²/g]</td>
<td>16.7</td>
</tr>
<tr>
<td>average pore diameter</td>
<td>[nm]</td>
<td>138</td>
</tr>
<tr>
<td>Porosity</td>
<td>[%]</td>
<td>28</td>
</tr>
</tbody>
</table>

Figure 4-5 measured volume of mercury adsorbed by the PVP seeded PGMA particles as a function of applied pressure (a) and scheme of the pores “of” and “between” the particles (b).
To obtain further information about the porosity of the particles, their interior structure was characterized using Cryo-SEM and ultra-thin section TEM. It is suggested, that PVP serves as a core for obtained particles with a shell of PGMA with epoxy groups on their surface\cite{104}. In ultra-thin section TEM, the PVP core can be observed as a dark circle with a lighter shell, which represents PGMA layer, which might be a result of higher electron density of PVP than PGMA. Large pores with a diameter of 1 µm, which were measured by Mercury intrusion, are not observed by Cryo SEM. The SEM images also show a lower polydispersity compared to the P (GMA-co-DVB) particles (\textbf{Figure 4-6}).

\textbf{Figure 4-6} SEM (a), Ultra-thin section TEM (b) and Cryo SEM (c) images of the PVP seeded P (GMA) particles.

Because the synthesis of particles was carried out in an ethanolic medium, the epoxy groups on their surface could partially react with ethanol. To check this, the real density of epoxy groups on the surface of the particles was measured via ERO reaction by the titration with Sodium thiosulfate ($\text{Na}_2\text{S}_2\text{O}_3$)\cite{110}.

\[
\begin{array}{c}
\text{PGMA} + \text{Na}_2\text{S}_2\text{O}_3 + \text{H}_2\text{O} \xrightarrow{\text{ERO}} \text{OH} \quad \text{S}_2\text{O}_3^- + 2\text{Na}^+ + \text{OH}^-
\end{array}
\]

By addition of $\text{Na}_2\text{S}_2\text{O}_3$ the initial pH value of the particle dispersion increases to a constant value, where the titration curve reaches a plateau by addition of approx. 400 µl (\textbf{Figure 4-7}). From the volume of used $\text{Na}_2\text{S}_2\text{O}_3$ and the change of the pH value the fraction of the surface covered with epoxy groups "$\varphi$" can be calculated.
Figure 4-7 change of the pH of the particle dispersion during the titration of PVP seeded PGMA as a function of the volume of used Na₂S₂O₅.

S_{geom} of particles and the number of epoxy group “N_{epoxy}” are calculated by means of equation 6-5.

\[ N_{epoxy} = \frac{S_{geom} \cdot \varphi}{a_{epoxy}^*} = \frac{3 \cdot m_p \cdot \varphi}{r_p \cdot \rho_p \cdot a_{epoxy}} \quad (6-5) \]

Where \(a_{epoxy}^*\) is the surface area of an epoxy ring.

Since \(N_{epoxy}\) is proportional to the number of resulting OH⁻ groups “\(N_{OH^-}\)”, the molar amount of OH⁻ groups “\(V_{OH^-}\)” is obtained by means of equation 6-6.

\[ V_{OH^-} = \frac{N_{epoxy}}{N_A} = \frac{3 \cdot m_p \cdot \varphi}{r_p \cdot \rho_p \cdot a_{epoxy} \cdot N_A} \quad (6-6) \]

Where Avogadro number “\(N_A\)” is \(6.0 \times 10^{23} \text{ mol}^{-1}\).

Finally, \(\varphi\) can be then calculated by substitution of the final volume of the particle dispersion “\(V_{fin}\)” and \(V_{OH^-}\) (equation 6-7).

\[ C_{OH^-}^{fin} = \frac{V_{OH^-}}{V_{fin}} = \frac{3 \cdot m_p \cdot \varphi}{r_p \cdot \rho_p \cdot a_{epoxy} \cdot N_A \cdot V_{fin}} \Rightarrow \varphi = \frac{26800 \cdot C_{OH^-}^{fin} \cdot V_{fin}}{m_p} \quad (6-7) \]
As mentioned above, the side reaction of ethanol with epoxy groups at high temperatures during the polymerization might be a reason for the small $\phi$ value of 0.005%.

### 4.1.3 PGMA modified PS particles

Preparation of the PS particles was carried out by the PVP seeded dispersion polymerization of styrene in ethanol. Hexadecanol serves here as a hydrophobic agent to eliminate the Ostwald-ripening due to its non-solubility in water. This can increase uniformity and decrease polydispersity of the obtained particles\[^{112}\].

\[
\begin{align*}
\text{H}_2\text{C} & \quad \text{(S)} \\
\text{H}_2\text{C} & \quad \text{(PVP)} \\
\text{H}_2\text{C} & \quad \text{(Hexadecanol)} \\
\text{CH}_3\text{CH}_2\text{CH}_2\text{OH} & \quad \text{(PS)}
\end{align*}
\]

These particles were then modified with PGMA to create an epoxy-functionalized polymeric layer on the surface of the PS particles. Sodium tetraborate "Borax" as buffer agent maintains the pH-value of the polymerization mixture at a constant value\[^{118}\].

\[
\begin{align*}
\text{(PS)} & \quad \text{(GMA)} \\
\text{KPS / SDS / Borax / Water} & \quad \text{(PGMA-PS)}
\end{align*}
\]

After modification of the PS particles with PGMA, some ellipse-shaped particles are formed, which were not observed in the dispersion of the original PS particles (Figure 4-8). One possible reason for this effect might be the result of removing of the residual solvent in the PS particles and subsequently the formation of ellipse-shaped particles during their modification with PGMA at high temperature for long time of reaction. Comparison of the dispersions of the original and modified PS particles in water with the PGMA particles seeded with PVP as reference particles shows, that over time both dispersions of the original and
modified PS particles consist of three layers, the top layer of supernatant (water) and two layers of particles with different densities\textsuperscript{114,115}. The thickness of the layer of the PS particles with lower density, which float in water, was decreased after modification with PGMA (Figure 4-9). To obtain more information about interior structure of the PS particles before and after modification with PGMA ultra-thin section TEM imaging was performed, which also reveals the presence of the pores, which might have been filled with solvents used for the polymerization of initial PS particles (Figure 4-10).

**Figure 4-8** Optical microscope image of initial PS particles (a) and the modified PS particles with PGMA show ellipse-shaped particles (b).

**Figure 4-9** Dispersion of the initial (a) and modified PS particles (b) as well as the PVP seeded PGMA particles (c). Additionally to the supernatant; formation of two layers of the particles over time shows the density difference of the PS particles in a same batch.
Figure 4-10 Ultra-thin section TEM images of initial (a) and modified PS (b) particles with PGMA show some pores, which might have been filled with solvent and leads to formation of ellipse-shaped particles after modification with PGMA.

4.2 Fabrication of patchy particles

4.2.1 PGMA particles

For a solvent-free reaction between epoxy groups on the surface of the particles and amino groups of PEI a high temperature of 120°C was applied by using an oven for a total time of 4 hours\cite{56}. After deposition of the inked PDMS stamp on the particles a pressure set up was used to keep a regular pressure during μCP (Figure 4-11).

Figure 4-11 Pressure set up used for the application of a constant pressure on the particles during μCP, after placing the inked stamp on the monolayer of particles at the lower plate, the upper plate of the press machine is screwed down.
Functionalization of PGMA-co-DVB particles with polyethyleneimine (PEI) as polymeric ink via μCP as well as sandwich printing for preparation of mono- and bi-patchy particles was successfully performed (Figure 4-12).

![Image](image-url)

**Figure 4-12** Fluorescence microscopy image of mono- (a) as well as bi- (b) patchy PGMA-co-DVB particles with patches made of branched PEI (M<sub>n</sub> 600-1000 kDa) and their self-assembly.

Due to the polydispersity of the obtained patchy particles (see Figure 4-1) the quantitative analysis of the yield and their self-assembly behavior was not further studied, although the fluorescence microscopy images of patchy particles showed a tendency of patch-patch interactions. This might be a result of hydrogen-bond or van der Waals forces between PEI patches. After the functionalization of the particles surface with PEI upon μCP, the SEM images show patchy-like traces despite a quite low initial density of the epoxy groups on the surface of the PGMA particles (Figure 4-13).

### 4.2.2 Epoxy-MF particles

Due to the high density of epoxy groups on the surface of these particles, μCP was performed at RT. Commercially prepared MF particles with an average diameter of 4.88 ± 0.07 μm and high density of the epoxy groups on their surface (440 mmol epoxy groups per 1 g of particles) were also used for preparation of patchy particles via covalent bond. Fluorescence
microscopy images of patchy particles show the successful transfer of the polymeric ink onto the surface of the particles (Figure 4-14).

Figure 4-13 SEM images of the functionalized PGMA particles with branched PEI ($M_w$ 600-1000 kDa) show some traces of patches on the surface.

Figure 4-14 Fluorescence microscopy images of epoxy-functionalized MF patchy particles with patches made of PEI at neutral (a) and basic (b) pH.
To test the covalent character of bonding between amino and epoxy groups, the pH of the particle dispersion was increased from neutral to basic\textsuperscript{\cite{116}}. An electrostatic interaction between MF particles and dissociated positively charged PEI can be eliminated by increase of the pH value above the isoelectric point of PEI and patches will be detached from the particles surface. In contrary, in a basic medium non-dissociated but covalently bonded PEI patches will still remain attached to the surface of epoxy-functionalized particles. As a Blanc sample, non-functionalized MF patchy particles were used, which are electrostatically bonded to the branched PEI. This was then compared with the epoxy-functionalized patchy particles with patches made of the same branched PEI. Fluorescence microscopy images of the two samples show that at a very alkaline pH, the patches on the surface of the epoxy-functionalized MF particles was maintained (Figure 4-15).

\textbf{Figure 4-15} Fluorescence microscopy images of non-functionalized MF patchy particles with patches made of PEI at neutral (a) and very alkaline (b) pH
5 MF particles with electrostatically bonded patches
This part is divided into two subsections: First, the reproducible preparation and characterization of mono-patchy MF microparticles with oppositely charged single patches, made of PMVEMA or PEI was investigated. Qualitative and quantitative analysis of the self-aggregation behavior of resulting mono-patchy particles as a function of pH for each type of patches was performed and the results were compared with theoretical calculation of electrostatic forces between patchy particles, based on a simplified electrostatic model.

The second part concerns the fabrication of bi-patchy MF microparticles with two oppositely charged patches on their poles made of PMVEMA and PEI via sandwich microcontact printing. Spontaneous self-assembly of bi-patchy particles in solution was studied using comprehensive reliable statistics of variable chains formed via electrostatic interactions between oppositely charged patches.

These interactions between not only mono-patchy but also bi-patchy particles, which acts as driving force for their self-aggregation could be eliminated or weakened by increasing the ionic strength.

5.1 Mono-patches made of PMVEMA or PEI

The successful attachment of PMVEMA and PEI onto the surface of the MF particles was confirmed by fluorescence microscopy. For improved resolution and contrast, the refractive index mismatch between patchy particles and solution was minimized by re-dispersing them in 

\[ \text{ICH}_3\text{Br} \]

with an index of 1.64, although the pH-adjustment in such a nonpolar and aprotic substance was impossible, so that ethanol-water (90:10) was used as solvent in the following (Figure 5-1)\textsuperscript{117,118}.

5.1.1 Patch thickness and the yield of \( \mu \text{CP} \)

AFM analysis of the stamp and patchy particles after \( \mu \text{CP} \) and subsequent release in acetone yields reliable information about the thickness and the form of the resulted patches\textsuperscript{119}. Patchy particles with patches made of PMVEMA were fixed on a PDMS stamp, and scanned with AFM (Figure 5-2).
**Figure 5-1** Fluorescence microscopy images of mono-patchy particles with patches made of PMVEMA labelled with Rhodamine 6G (a), and PEI labelled with FITC dispersed in ethanol (b) and in ICH2Br (c) (Image reused from Ref. 68).

**Figure 5-2** AFM images of patchy particles with patches made of PMVEMA (Mw 1980 kDa) fixed on a PDMS stamp after release in acetone (a) and the section height profile of the PDMS stamp after µCP represents the holes in the PMVEMA layer (b-c). AFM image of the same PDMS stamp after release in ethanol does not show holes (Images reused from Ref. 68).
The release of patchy particles in a solvent occurs, because the adhesion of PMVEMA to the MF particles is stronger than its adhesion to the PDMS stamp due to the attractive electrostatic interaction at their interfaces. Here the polarity of the solvent used for release plays a key role in the thickness of the resulted patches. In a solvent with a higher solvency for PMVEMA, e.g., ethanol, the cohesion of PMVEMA in the film layer is weaker than its adhesion to the MF particles, which results in a reduced thickness of patches. In contrast to ethanol, in a solvent with lower solvency, e.g., in acetone, the thickness of the patches increases. AFM images of the PDMS stamp after release in acetone show circular holes. These reveal the parts of polymer layer, which were transferred as patches to particles surface. With the assumption that the depth of these holes is equal to the thickness of the resulting patches after release, the thickness of patch from the measurements of 100 holes in PMVEMA film (Mw 1980 kDa) was 90 ± 15 nm[69].

The thickness of the patches and the percentage of patchy particles relative to the total number of particles (yield of μCP) were influenced by the concentration and the molecular weight of the polymeric ink. In general, to determine the yield of the bi-patchy particles by sandwich μCP, a sample of the obtained patchy particles was observed by fluorescence microscopy, and the particles were counted manually. The yield was then calculated from the number of the bi-patchy particles divided by the total number of non-, mono- and bi-patchy particles.

To check, if the calculated yield of this arbitrarily chosen small sample can represent the yield of the whole batch, a Hemocytometer counting chamber was used[120]. Using the counting chamber, the total number of particles in each batch ($n_{batch}$) can be determined. Since the initial number of the untreated MF particles ($N$), which were drop-casted on the object carrier and used for μCP is known, the accuracy of the investigated statistics can also be defined.

According to the literature, the size of the sample of a population is related to the reliability of the statistics. For the desired precision ($ε$) the sample size can be calculated by a known number of the population (equation 7-1)[121].

$$n_{batch} = \frac{N}{1 + N(ε)^2}$$  \hspace{1cm} (7-1)
Two independent samples of the PMVEMA mono-patchy particles were prepared. The calculated yield using a counting chamber was 91%, which was in good agreement with 90% yield found by counting the small sample. In the counting chamber, 1 µL of the dispersion of the obtained particles were deposited and counted. This was then multiplied by the total volume of 50 µL, to calculate the value of \( n_{\text{batch}} \). The value of \( N \) can be calculated by division of total mass of the drop-casted MF particles by mass of a MF particle. By drop-casting of 5 µL of a 1 wt % dispersion of the particles, the total mass of the used MF particles was \( 5 \times 10^{-5} \) g. Using the diameter of 5.17 µm and the density of 1.51 g cm\(^{-3}\), the mass of a particle was calculated to be \( 109.83 \times 10^{-12} \) g. Replacing the obtained values of \( n_{\text{batch}} = 15334 \) and \( N = 455249 \) in equation 7-1, the precision was calculated to be \( \pm 0.008 \) (~99%).

With increasing the concentration of PEI from 1 to 3 wt %, the yield rises from 75 to 85 %. In contrast to PEI, higher concentration of PMVEMA results in lower thickness of patches as well as lower yield of patchy particles. Increasing the concentration of PMVEMA solution up to 3 wt % decreases its pH value to 2.0, which is very close to the isoelectric point of SiO\(_2\) layer on the top of the PDMS stamp (Figure 5-3). As a result, the adhesion of PMVEMA to PDMS is stronger than its adhesion to the MF particles, which reduces the yield from 84 to 24 % (Table 5-1).

![Graph showing the influence of PMVEMA concentration on film thickness and pH value](image)

**Figure 5-3** Influence of the concentration of PMVEMA on its film thickness and pH value, an increase from 0.5 to 3 wt % leads to the increase of polymeric film thickness from 10 to 90 nm as well as a decrease of the pH of the polymer solution from 2.6 to 2.0 (Image reused from Ref. 809).
Table 5.1 Dependence of the yield of \( \mu \)CP and the thickness of the polymer film on the concentration of the ink. The solution of PMVEMA (\( M_w 216 \) kDa) and branched PEI (\( M_w 600-1000 \) kDa) at different concentration were spin-coated on a silicon wafer, scratched by a needle and the height difference was measured by AFM.

<table>
<thead>
<tr>
<th>Conc. (wt %)</th>
<th>PMVEMA Thickness (nm)</th>
<th>PMVEMA Yield (%)</th>
<th>PEI Thickness (nm)</th>
<th>PEI Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>21 ± 3</td>
<td>76</td>
<td>25 ± 3</td>
<td>75</td>
</tr>
<tr>
<td>2</td>
<td>49 ± 4</td>
<td>84</td>
<td>46 ± 3</td>
<td>79</td>
</tr>
<tr>
<td>3</td>
<td>-</td>
<td>24</td>
<td>76 ± 2</td>
<td>85</td>
</tr>
</tbody>
</table>

The increased density of functional carboxyl and amino groups, and the reduced solubility of the high molecular weight polymeric ink during the release result in a higher yield as well as the thickness of obtained patches, which is independent of the type of polymer. Preparation of patchy particles using PMVEMA with approximately two times higher molecular weight (\( M_w 1980 \) kDa) than PEI leads to the formation of patches with an approximately 25 % higher thickness. This effect has also been mirrored in the yield of patchy particles. A ten times higher molecular weight of PMVEMA with a concentration of 1 wt % causes an improvement in the yield from 76 to 90 %.

5.1.2 Effect of PDMS treatment with ethanol

To study the effect of PDMS oligomers on its surface polarity, the stamps were treated with ethanol after the completion of the cross-linking. The residual non-cross-linked oligomers in the PDMS can diffuse to the surface of the stamp and decrease the efficiency of the plasma activation, i.e., reduce the hydrophilicity [Figure 5.4][123].
Figure 5-4 Scheme of the effect of PDMS treatment with ethanol on its adhesion properties and the thickness of patches shows the decrease of patch thickness obtained by μCP with treated PDMS stamp (a). SEM images of patchy particles with patches made of branched PEI (Mw 600-1000 kDa) prepared by untreated (b) and treated (c) PDMS stamps shows the differences in the form and thickness of resulting patches (Images reused from Ref. [69]).

As a result, the adhesion of the ink to the PDMS stamp is weaker than its adhesion to the MF particles, which leads to the creation of patches with larger thickness. On the contrary, the treatment causes increased adhesion of the ink to PDMS stamp and decreased thickness of patches, subsequently. Comparison of the thickness of PEI patches made by μCP with treated and untreated PDMS shows a difference of ca. 30 nm. The yield of μCP with treated and untreated stamps were 76 and 79 % respectively, which means the modification of the stamps adhesion properties does not affect the yield significantly.
In order to remove residual oligomers, the stamps were treated with ethanol as a polar solvent for 6 hours. In this period the weight of PDMS stamps was increased about 5% due to its swelling through uptake of ethanol. After withdraw of the stamps from ethanol, their weight was decreasing over the next 6 hours because of ethanol release and evaporation. At the end after a total time of 12 hours, the ratio of the final weight of PDMS stamp to its initial weight (\( W_{\text{end}}/W_0 \)) was 99% that reveals the presence of quite small amounts of non-cross-linked oligomers, which were dissolved and removed (Figure 5-5)[124-126].

**Figure 5-5** Weight changes (\( w/w_0 \)) of PDMS stamp during and after treatment with ethanol over time where \( w_0 \) is the initial weight and \( w \) is the weight as a function of time. PDMS stamp was removed from ethanol after 360 minutes (Image reused from Ref. [126]).

### 5.2 Electrostatic interaction and force between mono-patchy particles

Depending on the pH of the solution, patches made of PMVEMA or PEI carry negative or positive charges, respectively. As mentioned above (see Figure 3-1), measurement of the zeta potential of the MF particles as a function of pH shows that the surface charge of the MF particles can also vary in a wide range of negative, neutral and positive values. Therefore, at a specific pH mono-patchy particles in a very rough approximation can be considered as simple, small dipoles, which are forced to aggregate in the solution due to their opposite surface charges. For example, in a moderately basic pH range, the surface of the MF particles...
is negatively and the patches made of PEI are positively charged due to their increased dissociation degree. Apart from single patchy particles and multi-particulate aggregates, some possible types of dimers were formed via patch-patch, particle-particle, and patch-particle interactions. Quantitative analysis of these dimers at different pH was carried out with the aim of assessing the controlled self-assembly of patchy particles in solution (Figure 5-6). Statistics show that patchy particles are predominantly assembled to dimers due to patch-particle interactions for both type of patches made of PMVEMA or PEI (Table 5-2).

![Figure 5-6](Image)

**Figure 5-6** Fluorescence microscopy images of mono-patchy particle-dimers with patches made of PEI (Mw 600-1000 kDa), which are formed via particle-particle (a), patch-patch (b), and particle-patch (c) interactions (Image reused from Ref. 89).

Interplay between charges of polymer patches and surface of the MF particles causes that at specific pH the largest number of dimers via patch-particle interactions are formed. At this point the electrostatic attraction between patches and particles surface also increases to its maximum. To quantify these interactions between mono-patchy particles, simplified model based on the theory developed by Hoffmann, Bianchi and Likos was used (see Appendix). According to this model, the interactions between mono-patchy colloids in the equilibrium state can be related to solely electrostatic forces (equation 7-2).

\[
F = \frac{1}{4\pi\varepsilon\varepsilon_0} \cdot \frac{Q_{\text{patch}} Q_{\text{particle}}}{r^2} \quad (7-2)
\]

Where \(\varepsilon\) and \(\varepsilon_0\) are the dielectric constants of ethanol-water medium and vacuum respectively, and \(r\) is the distance between corresponding point charges representing the interacting patches and patch-free particles (Figure 5-7).
Table 5-2 Statistics of the dimers considering three interaction types between patchy particles as a function of pH for PMVEMA as well as PEI patches

<table>
<thead>
<tr>
<th>PMVEMA</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>pH&lt;sub&gt;e&lt;/sub&gt;</td>
<td>n&lt;sub&gt;particle-patch&lt;/sub&gt;</td>
<td>n&lt;sub&gt;patch-patch&lt;/sub&gt;</td>
<td>n&lt;sub&gt;particle-particle&lt;/sub&gt;</td>
<td>n&lt;sub&gt;tot&lt;/sub&gt;</td>
</tr>
<tr>
<td>8.9</td>
<td>53 %</td>
<td>5 %</td>
<td>42 %</td>
<td>100 %</td>
</tr>
<tr>
<td></td>
<td>112</td>
<td>11</td>
<td>87</td>
<td>210</td>
</tr>
<tr>
<td>7.8</td>
<td>82 %</td>
<td>3 %</td>
<td>15 %</td>
<td>100 %</td>
</tr>
<tr>
<td></td>
<td>167</td>
<td>6</td>
<td>31</td>
<td>204</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>PEI</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>pH&lt;sub&gt;e&lt;/sub&gt;</td>
<td>n&lt;sub&gt;particle-patch&lt;/sub&gt;</td>
<td>n&lt;sub&gt;patch-patch&lt;/sub&gt;</td>
<td>n&lt;sub&gt;particle-particle&lt;/sub&gt;</td>
<td>n&lt;sub&gt;tot&lt;/sub&gt;</td>
</tr>
<tr>
<td>8.9</td>
<td>65 %</td>
<td>5 %</td>
<td>30 %</td>
<td>100 %</td>
</tr>
<tr>
<td></td>
<td>222</td>
<td>18</td>
<td>105</td>
<td>345</td>
</tr>
<tr>
<td>10.8</td>
<td>86 %</td>
<td>3 %</td>
<td>11 %</td>
<td>100 %</td>
</tr>
<tr>
<td></td>
<td>300</td>
<td>10</td>
<td>38</td>
<td>348</td>
</tr>
</tbody>
</table>

Figure 5-7 Fluorescence microscopy image (a) and the scheme of interaction forces (b) in a dimer formed of two mono-patchy particles, where the resulting force is a sum of attractive (F<sub>12</sub>, F<sub>23</sub> and F<sub>14</sub>) as well as repulsive forces (F<sub>13</sub> and F<sub>24</sub>) (Image reused from Ref. [89]).
The localized charges $Q_{\text{particle}}$ and $Q_{\text{patch}}$ can be calculated as a function of pH using equation 7.3 \[7.3\].

\[
U_E = \frac{Q}{6 \pi \eta a}
\]

(7.3)

Where $U_E$ is the electrophoretic mobility, $a$ is the radius of particle or patch, $\eta$ is the viscosity of the dispersion medium. These values of $U_E$ are related to the electrokinetic properties of MF particles, as well as colloidal particles coated by the corresponding polymers PMVEMA or PEI, respectively \[128-131\]. With the approximation that the thicknesses of the Stern and hydrodynamic layers of a particle are negligible, surface potential is equal to zeta potential. The relation between electrophoretic mobility and zeta potential was found by Smoluchowski (equation 7.4).

\[
U_E = \frac{2\varepsilon zf(K_a)}{3\eta}
\]

(7.4)

Where $\varepsilon$ and $\eta$ are dielectric constant and viscosity of the dispersion medium, respectively, $z$ is zeta potential, and $f(K_a)$ is Henry's function. For MF particles with a size of 5.17 $\mu$m, $f(K_a)$ is 1.5 according to Smoluchowski approximation \[132,133\].

The surface charge of patches rises by increasing the dissociated fraction of polymer molecules, i.e., protonation of PEI and deprotonation of PMVEMA. The statistic of dimers formed via patch-particle interaction was in a reasonable accordence with the theoretically estimated electrostatic force between patchy and non-patchy surfaces of particles (Figure 5-8).

Despite repulsive interactions between identically charged surfaces, other less frequently observed dimers were also formed via patch-patch and particle-particle interactions (see Figure 5-6). This might be a result of different non-electrostatic interactions, such as Van der Waals forces or the formation of hydrogen bonds. For dimer formation, collision of individual moving particles is necessary. If their kinetic energy is high enough to overcome the electrostatic repulsion between two surfaces of the same sign, formation of dimers driven by the above mentioned forces could take place.
Figure 5-8 Comparison of the calculated electrostatic force between patchy and non-patchy surface of particles and the percentage of dimers resulting from patch-particle interactions for PMVEMA (a) as well as for PEI (b) as function of pH values. The pH yielding the strongest electrostatic force leads to the largest number of dimers via patch-particle interactions (Image reused from Ref. [69]).
5.2.1 Self-aggregation behaviour of patchy particles in a solution with an increased ionic strength

It is well-known that electrostatic interaction between charged objects (ions, particles, etc.) in electrolyte media is strongly influenced by ionic strength, i.e., by electrolyte concentration\(^{134}\). In order to prove the effect of this factor on the electrostatically driven aggregation of patchy particles, we added a saturated solution of NaCl in ethanol-water (90:10) to the dispersion of each type of mono-patchy particles.

For a quantitative analysis of the self-aggregation of mono-patchy particles before and after addition of NaCl, a small sample of particles is observed by fluorescence microscopy, and the particles are manually counted. Here, the total number of particles does not represent the whole batch, but the particles in the small sample which were observed and counted (Table 5-3).

The dissociation of salt in ethanol-water medium leads to screening of electrostatic attractive interactions between the patchy particles. In accordance with this, the statistics, made on the basis of microphotographs, show that the fraction of single patchy particles, compared to the aggregates fraction strongly increased after addition of salt (Figure 5-9).

Table 5-3 Fraction of single patchy particles compared to the number of aggregated particles in ethanol-water (90:10) before and after the addition of NaCl. The percentage of single particles proportional to the total number of particles significantly increases.

<table>
<thead>
<tr>
<th></th>
<th>PMVEMA</th>
<th></th>
<th>PEI</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(\pi_{\text{single}})</td>
<td>(\pi_{\text{aggregates}})</td>
<td>(\pi_{\text{single}})</td>
<td>(\pi_{\text{aggregates}})</td>
</tr>
<tr>
<td>+ NaCl</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before</td>
<td>4 %</td>
<td>96 %</td>
<td>13 %</td>
<td>87 %</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>468</td>
<td>64</td>
<td>427</td>
</tr>
<tr>
<td>After</td>
<td>51 %</td>
<td>49 %</td>
<td>53 %</td>
<td>47 %</td>
</tr>
<tr>
<td></td>
<td>267</td>
<td>256</td>
<td>273</td>
<td>246</td>
</tr>
</tbody>
</table>
**Figure 5-9** Diagram shows that after addition of NaCl to the dispersion of PEI patchy particles leads to increase of ionic strength and consequently to elimination of the electrostatic interactions between patchy particles, so that the number of aggregated particles decreases.

### 5.3 Oppositely charged bi-patches on particle poles

#### 5.3.1 Characteristic and the yield of patchy particles

The obtained bi-patchy particles were characterized by various microscopy methods. To achieve a clear visualization of the patches for fluorescence microscopy, polymeric inks PMVEMA and PEI were labeled with Rhodamine 6G and Fluorescein isothiocyanate (FITC) tags, respectively. Information about the morphology and surface properties of the patches were obtained by scanning electron and scanning force microscopy (Figure 5-10).

To study the effect of the concentration of the polymer solutions used for μCP, on the yield of the bi-patchy particles, PMVEMA and PEI were equally concentrated at 1, 2 and 3 wt %. An increase from 1 to 2 wt % leads to a larger number of patchy particles, whereas by a further increase to 3 wt % the yield decreases (Table 5-4). The yield of the bi-patchy particles is a result of the yields of mono-patches: firstly PMVEMA and secondly PEI mono-patches. For example, reduction of the yield of the bi-patchy particles to 21 % at 3 wt % is the consequence of the small yield (24 %) of the PMVEMA mono-patches (see Table 5-1), despite of the relatively high yield (85 %) of the PEI patches at this concentration. The reason for the
reduced yield of PMVEMA mono-patchy particles was the fact that the pH of higher concentrated PMVEMA tends to more acidic values, which is very close to the IEP of the PDMS stamp (pH ~ 2)\textsuperscript{122}. Therefore the adhesion of PMVEMA to PDMS is stronger than its adhesion to the MF particles. Finally, as standards for further investigations, the polymer inks at 2 wt % were used due to the reasonable yield of the bi-patchy particles at this concentration\textsuperscript{69}.

![Figure 5-10](image)

**Figure 5-10** AFM image PMVMEA (a) and SEM image of PEI (b) mono-patches, as well as SEM image (c) and fluorescence microscopy image of bi-patches on a MF particle, which are colored red and green, respectively (d).

**Table 5-4** Yield of bi-patchy particles as a function of the concentration of the polymer solutions, PMVEMA and PEI, which was spin-coated on the stamp for µCP.

<table>
<thead>
<tr>
<th>Conc. (wt %)</th>
<th>Number of bi-patchy particles</th>
<th>Total number of particles</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>162</td>
<td>220</td>
<td>74</td>
</tr>
<tr>
<td>2</td>
<td>170</td>
<td>207</td>
<td>82</td>
</tr>
<tr>
<td>3</td>
<td>30</td>
<td>145</td>
<td>21</td>
</tr>
</tbody>
</table>
5.3.2 Electrostatic interactions between bi-patchy particles

The self-aggregation behavior of the bi-patchy particles in solution was followed by fluorescence microscopy. To avoid any influence of the surface charge of the MF particles on the interactions between patches, the pH value of the solution has to be maintained close to the IEP of MF particles. To this end, a mixture of ethanol-water (90:10) was used as dispersant. The pH 8.9 of this mixture corresponds to the IEP of the MF particles\(^69\). Because this medium possesses lower difference between its refractive index and the refractive index of the MF particles, the clarity of fluorescence microscopy images of the bi-patchy particles was improved.

Considering that the yield of the bi-patchy particles by sandwich μCP is lower than 100 %, the self-aggregation occurs in a mixture of mono- and bi-patchy particles. According to the probability rules, six cross-linking form of the patchy particles can occur through the accidental collision of their patchy and non-patchy surfaces. The possibility of each connection type will then be one-sixth (~17 %), however the possibility of crosslinking to the particles surface is higher than to the patches due to its larger surface area. Comparison of these theoretical estimations with the statistical values of the obtained patchy particles via sandwich μCP shows a huge difference in the fraction of the connection type (PMVEMA—PEI) (65 %), which are cross-linked via their oppositely charged patchy surfaces (Figure 5-11).

Bi-patchy particles build up in ethanol-water (90:10) some chain-like structures via electrostatic interaction between their oppositely charged (PMVEMA—PEI) patches. Their steric structure observed by fluorescence microscopy seem to be nearly to the branched, bent and linear chains, which consist of two, three (short) and more than three (long) bi-patchy particles (Figure 5-12).

The statistics of the formed chains via PMVEMA—PEI connection, concerning their length and shape, are summarized (Table 5-5). Considering bent chains as a simple kind of branched chains, the statistics show a higher fraction of long and branched chains. Thus, in the absence of external physical forces, e.g., mechanical, the only driving force for the self-aggregation of the bi-patchy particles to the chains via PMVEMA—PEI connection is the attractive electrostatic interaction between their oppositely charged patchy surfaces.
Figure 5-11 Fluorescence and optical microscope images of the self-assembly of the bi-patchy particles (a-b) as well as the scheme of the six possible connection types (c) formed via interactions between patchy and non-patchy surfaces of the particles; scale bars: 10 μm. Statistic of the six possible connection types in the aggregations of the MF patchy particles, obtained via sandwich μCP (d). The fraction of PEI patch interactions is larger than the fraction of PMVEMA interactions due to the higher yield of PEI patches.
Figure 5-12 Overlay of fluorescence and optical microscope images of the branched (a), bent (b) and linear (c) chain-like structures, which are formed via electrostatic attractions between oppositely charged PMVEMA and PEI patches. For a better visualization, connections between bi-patchy particles are represented with white lines, PMVEMA and PEI patches are additionally highlighted with red and green colored half-circles, respectively.

Table 5-5 Statistic of short (2-3 particles) and long (> 3 particles) chain-like structures as well as branched, bent and linear chains show that the largest number of long and branched chains. This reveals the presence of electrostatic interactions between oppositely charged patches, which leads to a large number of spontaneously formed long and branched chains.

<table>
<thead>
<tr>
<th>Chains</th>
<th>short</th>
<th>long</th>
<th>total</th>
<th>branched</th>
<th>bent</th>
<th>linear</th>
<th>total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fraction</td>
<td>34 %</td>
<td>66 %</td>
<td>100 %</td>
<td>42 %</td>
<td>39 %</td>
<td>19 %</td>
<td>100 %</td>
</tr>
<tr>
<td></td>
<td>102</td>
<td>196</td>
<td>298</td>
<td>129</td>
<td>120</td>
<td>58</td>
<td>307</td>
</tr>
</tbody>
</table>
5.3.3 Effect of ionic strength on self-aggregation behavior of bi-patchy particles

Self-aggregation of the bi-patchy particles via electrostatic interactions can be screened by change of ionic strength. By addition of a saturated solution of NaCl to the patchy particles, the electrostatic interactions between patchy particle will be reduced due to the dissociation of the salt, so that the long chains break down to form short chains and single particles (Table 5-6). As reference, the statistics of the short and long chains, as well as the single particles of the untreated MF particles, were compared to the statistics of the bi-patchy particles before and after addition of the NaCl solution. As expected, the number of single particles after addition of NaCl was increased. However, this value was essentially lower than the number of singlets in the reference sample. The residual small fraction of aggregated particles might reveal the presence of other attractive interactions such as hydrogen bonds or Van der Waals forces, although weak electrostatic interactions, which still exist between patchy surfaces of the bi-patchy particles. This effect could be seen either by optical as well as fluorescence microscopy or even with unaided eye (Figure 5-13).

Table 5-6 Statistic of the long and short chains, as well as the single particles in a dispersion of bi-patchy particles before and after the addition of the solution of NaCl. As a reference, the statistics were compared with a reference sample of untreated MF particles.

<table>
<thead>
<tr>
<th>Sample</th>
<th>long chains</th>
<th>short chains</th>
<th>single particles</th>
<th>total</th>
</tr>
</thead>
<tbody>
<tr>
<td>initial bi-patchy particles</td>
<td>224</td>
<td>130</td>
<td>9</td>
<td>363</td>
</tr>
<tr>
<td></td>
<td>62 %</td>
<td>36 %</td>
<td>2 %</td>
<td>100 %</td>
</tr>
<tr>
<td>increased ionic strength</td>
<td>78</td>
<td>167</td>
<td>96</td>
<td>341</td>
</tr>
<tr>
<td></td>
<td>23 %</td>
<td>49 %</td>
<td>28 %</td>
<td>100 %</td>
</tr>
<tr>
<td>reference sample</td>
<td>38</td>
<td>49</td>
<td>257</td>
<td>344</td>
</tr>
<tr>
<td></td>
<td>11%</td>
<td>14 %</td>
<td>75 %</td>
<td>100 %</td>
</tr>
</tbody>
</table>
Figure 5-13 Overlay of the optical and fluorescence microscopy images of the bi-patchy particles before (a) and after (b) addition of the solution of NaCl. Elimination of the self-aggregation can also be observed by the unaided eye. In solution, the white sediment of the self-aggregated long chains on the bottom of the Eppendorf tube (c) converts to a turbid dispersion of short chains and single particles by reducing of the ionic strength (d) statistic of long and short chains, as well as single particles in a dispersion of bi-patchy particles before and after addition of the solution of NaCl. As a reference, the statistics were compared with a sample of untreated MF particles (e).
6 Summary and conclusion
First, it is concluded, covalently bonded PEI patches could be generated on the surface of epoxy-functionalized particles. It could also be shown, that the patches are attached via covalent bond and not via electrostatic interactions. In a basic medium, despite non-dissociated state of PEI, the patches keep attached to the particle surface. Furthermore, zwitterionic mono- and bi-patchy MF microparticles with positively or/ and negatively charged patches made of PEI or/ and PMVEMA could be fabricated, respectively. Electrostatic interactions between patchy particles in medium induces their spontaneous self-assembly to form different aggregates. The morphology of these aggregates is still uncontrollable, but their formation could be controlled by change of pH and increase of ionic strength.

The second chapter dealt with preparation of mono-patchy particles and the effect concentration and the molecular weight of the polymeric ink and the influence of the modification of the adhesion surface properties of PDMS stamp on the yield and thickness of patches. It was also described, that self-aggregation behaviour of patchy particles via electrostatic interactions can be controlled by changing the pH, i.e., the charge difference between patchy and non-patchy surface of particles. At specific pH, where more than the half of ink molecules are dissociated, and the zeta potential of the MF particles is increased, the number of mono-patchy particle-dimers via patch-particle interaction rises to a maximum. This dependence of patchy particles self-assembly on the pH value in the dispersion medium can be satisfactorily described by a simplified model accounting only for electrostatic interactions between patches and particles taken as point charges. Additionally, changing the ionic strength of the medium by addition of salt led to an increased population of single patchy particles, which also confirms a main contribution of electrostatic forces in interactions between patchy particles.

In the third chapter, generation of oppositely charged polymeric patches on the surface of the polymer MF particles with a reasonable yield was described. The effect of the concentration of the polyelectrolyte ink on the yield of the bi-patchy particles was studied, and a standard concentration of 2 wt % was chosen for further investigations.

A variety of statistics were performed to understand the relation between electrostatic interactions and formation of chain-like structures by self-aggregation of bi-patchy particles.
Based on literature, it was shown that precision of these statistics is found to be 99% and they are reliable. To check, if the formation of the chain via oppositely charged patches is a result of the electrostatic interactions, the ionic strength of the dispersion was changed by the addition of a saturated solution of NaCl to the bi-patchy particles. According to the change of the physical properties of the dispersion and the statistics, the self-aggregation was eliminated or weakened to some extent due to the reduced electrostatic attractions, however comparison of these statistics with a reference sample of the MF particles show the higher number of short chains even after the addition of the salt. This may indicate that there are still other types of interactions between bi-patchy particles, e.g. hydrogen bond, van der Waals forces, etc., which were not influenced by a change of the ionic strength.
7 Experimental and materials
7.1 Materials

Poly(methyl vinyl ether-alt-maleic acid) (PMVEMA) with $M_w$ of 216 and 1980 kDa, branched Poly(ethylenimine) (PEI) with $M_w$ of 600-1000 kDa, Fluorescein isothiocyanate, Rhodamine 6G, and bromoiodomethane were supplied by Sigma Aldrich. Melamine formaldehyde particles with the average size of $5.17 \pm 0.09 \mu m$ were synthesized by Microparticles GmbH. The PDMS kit, Sylgard 184 – silicone elastomer, contained the Monomer and curing agent was obtained by Dow Corning. The standard buffers for calibration and adjustment of pH were obtained by Mettler Toledo.

7.2 Preparation of epoxy-functionalized particles

7.2.1 PGMA-co-DVB

1.5 ml (10 mmol) GMA, 4.5 ml (32 mmol) DVB and 100 mg (0.6 mmol) AIBN were added to a mixture of 260 ml acetonitrile and 40 ml toluene. The mixture was degassed by nitrogen bubbling for 30 min, heated up to 60 °C and gently shaken in incubator at a rate of 30 rpm overnight. The obtained suspension was centrifuged at 5000 rpm for 20 min. The supernatant was removed and particles were re-dispersed in ethanol and centrifuged three times at the same conditions. Finally, the polymer particles were transferred to a clean centrifuge tube and the residual ethanol was removed under vacuum. The dried particles were weighted and stored at RT for later use.

7.2.2 PVP seeded PGMA

1.76 ml (12 mmol, 17.6 wt % with respect to solvent mass) GMA, 315 mg (3 mmol, 25 wt % with respect to monomer mass) PVP and 25.2 mg (0.15 mmol, 2.0 wt % with respect to monomer mass) AIBN were dissolved in 10 ml ethanol and degassed for 30 min, heated up to 60 °C and the solution was mechanically stirred at 80 rpm for 24 hours. The obtained suspension was centrifuged at 5000 rpm for 20 min. The supernatant was removed and particles were re-dispersed in ethanol and centrifuged three times at the same conditions. Finally, the polymer particles were transferred to a clean centrifuge tube and the residual
ethanol was removed under vacuum. The dried particles were weighted and stored at RT for later use.

7.2.3 Determination of epoxy content on the surface of PGMA particles by titration

To define the density of the epoxy groups on the surface of the particles 2 ml of the particle dispersion with a concentration of 9.1 wt % and a pH value of 6.33 was titrated with Na$_2$S$_2$O$_3$ with a concentration of 1 mM and a pH value of 6.23. After completion of the reaction, i.e. reaching the constant pH value of 7.11, the final volume was 2.48 ml. The concentration of OH was calculated $106 \times 10^{-9}$ mol/l from the final pH value.

7.3 Microcontact printing

In this procedure, a Polymethylsioxane (PDMS) stamp, coated with the polymeric ink, was pressed against a monolayer of the MF particles. The obtained patchy particles were released in acetone or ethanol in an ultrasonic bath for 15 minutes. Then the dispersion of patchy particles was centrifuged for 3 minutes at 11000 rpm, the supernatant was removed, and the particles were washed three times in ethanol at 8000 rpm for 3 minutes. After the wash procedure, they were collected in an Eppendorf tube for further characterization.

7.3.1 Preparation of inked PDMS and monolayer of particles

To synthesize PDMS, a 10:1 mixture of monomer and crosslinker was poured into a rectangular Petri dish to form a polymer matrix with a thickness of approx. 3 mm. After letting the mixture rest overnight to remove air bubbles, it was heated up to 80 °C for 2 hours. Then the cross-linked PDMS was cut into 1 cm x 1 cm stamps and stored in a closed container for later use. The glass object carrier was also cut into 1 cm x 1 cm pieces, cleaned with ethanol in an ultrasonic bath for 5 minutes and dried in a nitrogen flow.

In order to improve the wettability of the substrates, PDMS stamp and object carrier were treated with air plasma for 1 minute at 0.2 mbar and a power of 100 and 300 W respectively. Then the stamp was spin-coated with 60 µL of the polymer ink at 4000 rpm for 1 minute,
whereas the concentration of the inks was varied from 1 to 3 wt %. The monolayer of the MF particles was prepared via drop casting of 5 μL of a 1 wt % aqueous particle dispersion on the object carrier and subsequent drying under nitrogen flow at RT for 10 minutes.

### 7.3.2 PDMS treatment with ethanol

Three PDMS stamp samples were treated in a container filled with ethanol for a total time period of 6 hours. During each measurement, samples were taken out of ethanol, dabbed with a lint-free tissue and weighted, while the used ethanol was replaced with fresh ethanol. After this time, the samples were moved to a clean Petri dish and weighted every 15 minutes for further 2 hours. The last measurement was then performed 4 hours later.

### 7.4 Preparation of dispersion medium at different pH

The aggregation behavior of patchy particles was investigated in ethanol-buffer solutions at different pH. A specialized electrode for the ethanol-based solution named EtOH-Trode was supplied by Metrohm. The calibration was carried out using water-based buffers at 25°C and pH 4, 7 and 10. To prepare the calibration solutions, ethanol was mixed with buffers at 10, 30, 50, 70, 80, 90 wt % for each pH. The concentration of ethanol in the dispersion of patchy particles for studying their self-aggregation behavior in the solution at different pH was maintained at 90 wt %. The pH values of media were measured before using.

### 7.5 Characterization

#### 7.5.1 Fluorescence microscopy

For a simple monitoring of the self-assembly behavior of patchy particles at different pH, fluorescence microscopy was used. To allow for a clear visualization of PEI and PMVEMA patches, patchy particles were labeled with FITC and Rhodamine 6G respectively. A stock solution of fluorescent dye in ethanol with a concentration of 5 μg / ml was initially prepared. 20 μL of this solution was added to the suspension of patchy particles in 80 μL ethanol and after 15 minutes centrifuged at 11000 rpm for 3 minutes. The supernatant was removed, and patchy particles were washed three times in ethanol at 8000 rpm for 3 minutes.
For the imaging, a Leica DMi8 microscope at the magnifications of 20, 40 and 63 was used, and the resulted images were processed via LAS X software provided by Leica.

### 7.5.2 Scanning force microscopy

Characterization of the qualitative and quantitative properties of patches, such as thickness and diameter, symmetry and form was provided by AFM. The imaging was done using a Bruker Dimension Icon with Tapping Mode in air and OTESPA tips ($k = 42 \text{ Nm}^{-1}$, $f_0 = 300 \text{ kHz}$) and the images were analyzed by Nanoscope Analysis software.

### 7.5.3 Scanning electron microscopy

Further information about the surface properties of patch such as morphology, roughness and also the patch diameter was provided by SEM. A Gemini SEM 300 (Fa. Zeiss) with an SE2-Detector at an acceleration voltage of 5 kV was used for imaging. The particles were sputtered using Platinum with a thickness of 4 nm.

### 7.5.4 Zeta potential measurement

A Nano ZS90 of Malvern was used to measure the zeta potential of the MF particles at 25 °C and different pH. As dispersant water and ethanol-water mixture (90:10) were chosen. The resulted values were processed by the Zetasizer-NanoApplication and averaged from three independent measurements.
Bibliography


Appendix

Sabapathy and co-workers have used a pair potential for the interaction between two mono-patchy IPCs derived on the basis of potential equation proposed by Hoffmann, Bianchi and Likos.

\[
\Phi(r_{ij}, \theta_{ij}) = Q_{ij}(r_{ij}, \theta_{i}', \theta_{j}') \frac{q_{e}}{\varepsilon} \left[ \frac{\exp(\kappa r_{ij})}{1 + \kappa r_{ij}} \right] \cdot \left[ \frac{\exp(-\kappa r_{ij})}{r_{ij}} \right] \quad (A1)
\]

Further simplification of this equation is possible taking into account the analysis made in the Ref. [54].

These authors calculated the electrostatic interaction energy of a pair of IPC particles at a fixed separation distance and at different orientations and showed that this energy is only weakly dependent on the kink angle between interacting IPCs in aggregates: The value of this function grows only by 50 \% in the physically reasonable range of kink angles from 50 to 120 degrees. Thus, the contribution of the orientation factor \( Q_{ij} \) to the entire interaction potential between IPCs is also quite low compared to the pH-dependent electrostatic interaction and with the pH-dependent effect of the ionic charge density distribution in the medium and thus can be neglected, i.e.:

\[
\Phi(r_{ij}, \theta_{ij}) \sim \Phi(r_{ij}) = \frac{q_{e}}{\varepsilon} \left[ \frac{\exp(\kappa r_{ij})}{1 + \kappa r_{ij}} \right] \cdot \left[ \frac{\exp(-\kappa r_{ij})}{r_{ij}} \right] \quad (A2)
\]

The term in the factorized potential accounting for the effect of the ions double layer could be simplified taking into account the very small thickness of the patches compared to the radius of the microparticles (IPCs), i.e. we can approximate that \( r_{ij} \) in \((A2)\) is practically equal to \( \sigma \) (where \( \sigma \) denotes the radius of patchy IPCs according to the Ref. [13]).

This approximation is valid because the statistics of the various particle aggregation shapes was made on the basis of the microscopic observations for equilibrium states
and therefore did not consider any parameters related to the aggregation kinetics when the patchy particles approach each other and \( r_{ij} \) is sufficiently larger than \( \sigma \) and can change dynamically during the aggregate formation.

Since,

\[
F_{r_{ij}} = q_p \cdot \left[ -\frac{\partial}{\partial r_{ij}} \left( \Phi(r_{ij}) \right) \right]
\]  \hspace{1cm} (A3)

After taking this derivative and substituting one gets:

\[
F_{r_{ij}^m} = \frac{q_p q_e}{\varepsilon} \frac{\exp(k\sigma)}{1 + k\sigma} \cdot \frac{\exp(-k\sigma)(1 + k\sigma)}{\sigma^2} = \frac{q_p q_e}{\varepsilon \sigma^2}
\]  \hspace{1cm} (A4)

Therefore, the final form of the simplified expression for the interaction force between patch and colloidal particle contains only the Coulomb term.