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Synthesis of Inverse Patchy Particles

by

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A master thesis submitted for the degree

Diplom-Ingenieur (Dipl.-Ing.)

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Vienna, September 2014

Abstract

A fundamental comprehension of self-assembly processes is critical for understanding ongoing processes in nature and allows the design of new materials. Of special interest are colloids with patterned areas on their surface (patches), responsible for strong anisotropic interactions and specific self-assembly behaviors. Particles with a heterogeneous surface charge act as inverse patchy colloids (IPCs) (1). The term inverse refers to the repelling forces between two patches and the attraction between a patch and a region of opposite charge on the surface of a neighboring particle, which is in contrast to conventional patchy colloids whose patches are normally attracted to each other. A simple case of an IPC is a colloid with two charged polar patches and an oppositely charged equatorial region. The self-organization of such particles with respect to their overall charge, patch size and substrate charge was recently simulated, showing a variety of interesting structures. The experimental investigation of the behavior of IPCs with different patch properties (i.e. size, overall charge) in response to changing external factors (i.e. temperature, pH) could be a huge step towards understanding and controlling self-assembly to a certain extent. A synthesizing method for IPCs has therefore been developed in the present work. By masking the equatorial region of 2 µm silica particles with a polystyrene membrane, only the polar caps were accessible to modification with (3-aminopropyl)trimethoxysilane and rhodamine B isothiocyanate and were thus made both fluorescent and positively charged. After polymer dissolution with dichloromethane and acetone, free IPCs with a negatively charged equatorial area and two polar caps exhibiting a positive charge were obtained. The size of the patches varied from particle to particle, which restricts the significance of the investigation of their assembling-behavior. It is therefore necessary to develop either a method to separate the IPCs according to their patch size in several batches or to improve the developed method to synthesize more monodisperse particles.

Zusammenfassung

Ein grundlegendes Verständnis von Selbstanordnungsabläufen ist entscheidend um Prozesse, die in der Natur ablaufen, zu verstehen und die Entwicklung neuer Materialien zu ermöglichen. Von speziellem Interesse sind Kolloide mit lokalen Oberflächendekorationen (Patches), die für starke anisotropische Wechselwirkungen und eine spezielle Selbstanordnung verantwortlich sind. Eine besondere Gruppe dieser Partikel, so genannte *inverse patchy colloids* (IPCs), zeichnet sich durch eine heterogen geladene Oberfläche aus (1). Der Ausdruck *invers* bezieht sich dabei auf die Abstoßung zweier gleichgeladener Patches und die elektrostatische Anziehung zwischen einem Patch und einer Region mit entgegengesetzter Ladung auf einem benachbarten Partikel. Dieser Effekt steht im Gegensatz zu konventionellen Kolloiden mit heterogener Oberflächendekoration, deren Patches sich normalerweise anziehen. Ein einfacher Fall eines IPCs ist ein Kolloid mit zwei geladenen, polaren Patches und einer gegensätzlich geladenen Äquatorialregion. Die Selbstanordnung solcher Partikel in Bezug auf ihre Gesamtladung und Patchgröße sowie die Ladung des Substrates wurden kürzlich simuliert und zeigten eine Vielfalt interessanter Strukturen. Die experimentelle Untersuchung des Verhaltens dieser Kolloide mit unterschiedlichen Patcheigenschaften (z. B. Größe, Gesamtladung) in Abhängigkeit verändernder externer Faktoren (z. B. Temperatur, pH-Wert) könnte ein großer Schritt in Richtung Verständnis und bis zu einem gewissen Grad Kontrolle über Selbstanordnungsprozesse sein. Daher wurde im Zuge dieser Arbeit eine Methode zur Herstellung dieser IPCs entwickelt. Dadurch, dass die Äquatorialregion von 2 µm Silikapartikel mit einer Polystyrolmembran maskiert wurde, konnten selektiv nur die polaren, frei zugänglichen Regionen der Partikel mit (3-Aminopropyl)trimethoxysilan und Rhodamin B Isothiocyanat modifiziert werden, wodurch sie fluoreszent und positiv geladen wurden. Nach der Auflösung des Polymers mit Dichlormethan und Aceton konnten freie IPCs mit einer negativ geladenen Äquatorialregion und zwei polaren Regionen mit einer positiven Ladung gewonnen werden. Die Patchgröße variierte von Partikel zu Partikel, wodurch die Aussagekraft von Untersuchungen zur Selbstanordnung reduziert wird. Es ist daher notwendig entweder eine Methode zur Auftrennung der IPCs anhand ihrer Patchgröße in verschiedene Fraktionen zu entwickeln oder die entwickelte Synthesemethode zu verbessern um monodispersere Partikel zu produzieren.

Acknowledgement

I would like to thank Prof. Erik Reimhult for his advice and for assigning this task to me. I especially want to express my gratitude to Dr. Peter van Oostrum for his guidance throughout this work, for his expertise and enthusiasm and for coming up always with new ideas. I also like to thank Marcel Hans for the collaboration in this project and all colleagues for their support.

My special thanks go to my friends, who were always willing to distract me and showed me that it is important to have a life outside of work and university. I do not know where I should start thanking my family, but I hope they know how grateful I am for always supporting me and caring so much.

Table of Contents

List of Tables

List of Figures

List of Abbreviations

APTMS	(3-aminopropyl)trimethoxysilane
BIBB	α-bromoisobutyryl bromide
DCM	dichloromethane
DDAB	didodecyldimethylammonium bromide
DEE	diethyl ether
EHA	2-ethylhexyl acrylate
ICPs	inverse patchy colloids
MADQUAT	poly(2-dimethylamino)ethyl methacrylate) methyl chloride qarternary salt
MMA	methyl methacrylate
P4VP	poly(4-vinylpyridine)
P4VP-PS	poly(4-vinylpyridine-co-styrene)
PMMA	poly(methyl methacrylate)
PMT	photomultiplier tube
PPO	phenylbis(2,4,6-trimethylbenzoyl)phosphineoxide
PS	polystyrene M_W 192 000 Da
PS 1	polystyrene M_W 1 000 Da
PS 35	polystyrene M_W 35 000 Da
RITC	rhodamine B isothiocyanate
SEM	scanning electron microscope
tBA	*tert*-butyl acrylate
THF	tetrahydrofurane

1. Introduction

In the past few years a lot of attention has been given to the synthesis of micron sized particles showing anisotropic interactions. These anisotropic effects may arise from an anisotropic particle shape but can also be the result of a physically or chemically patterned surface of colloidal particles. The latter are also referred to as patchy particles and normally expose a limited number of discrete attractive regions, called patches, responsible for their special bonding characteristics (2; 3). Due to models based on patchy particles, it was possible to describe the behaviour of simple molecules like water (4) or more complex compounds like globular proteins in solutions (5; 6; 7). Patchy particles and their self-assembly properties were widely studied from both a theoretical as well as an experimental point of view. By now theoretical models to predict self-assembly scenarios have been developed and patchy particles with well-defined arranged patches could be synthesized (2; 8). These anisotropic colloids serve as good models to study self-assembly processes, as particles which would normally be repulsive or neutral assemble into ordered structures due to their attractive patches (3). These spontaneous processes are reversible and the formed structures are usually in an equilibrium state (9). As self-assembly is ubiquitous in nature it is important to develop a good understanding for it. Some well-studied examples are: the protein assembly of virus capsids i.e. of tobacco mosaic virus (10; 11), the self-assembly of S-layer proteins (12) or the formation of DNA helices from single-stranded oligonucleotide chains, which can be used as the basis for designing three-dimensional novel nanostructures (13; 14). In industry, the self-assembly of colloids into well-defined structures can be the basis to create novel and cost effective materials. Nanoparticles with self-assembly properties were applied i.e. in the development of optically and electrochemically based biosensors, electronic nanocircuitries (15; 16) or photonic devices (17).

A novel form of patchy particles was recently defined as *inverse patchy colloids* IPCs (1). These heterogeneously charged particles are characterized by their repulsive patches, which are attracted to patch-free regions of neighboring particles. Assuming a simple case, where particles have two charged polar patches and an equatorial region of the opposite charge; the polar patches are attracted to the equatorial region of adjacent particles and repelled by other patches. These competing and anisotropic interactions

possibly lead to new interesting self-assembly scenarios. The effective pair potential between IPCs was theoretically studied and a coarse-grained model was developed (1). The self-organization of IPCs, with two polar patches of different extension and various overall particle charges between two parallel, horizontal neutral or charged walls under confinement was simulated. Between two neutral walls, the particles either arranged in disordered aggregates (ring-like structures) or in microcrystalline domains (branched network with triangular particle structures), mainly depending on the opening angle of the patches. The bottom wall charge in combination with the overall charge of the particles influenced the assembly of the inverse patchy colloids in such a way that leads to either complete inhibition of aggregation, to an arrangement of gel-like structures or to the formation of crystalline domains with locally disordered structures. It was suggested that by changing the pH or the salinity it is possible to modify the assembling patterns (18).

To experimentally study self-assembly of IPCs, this work reports a method to synthesize particles with two positively charged polar patches and a negatively charged equatorial region. A polymer solution of polystyrene (M_W 192 000 Da) dissolved in diethyl ether was spread over hexagonally closed-packed silica particles (D = 2 µm) on an agarose-gel (2 %) at -20 °C. After solvent evaporation silica particles were embedded in a polystyrene membrane in a way that they were protruding from both sides of the polymer. The substrate exposed patches were referred to as the bottom patches whereas the air exposed particle sites were termed as top patches. The gaps between PS and the silica particles were filled with styrene and the polymer-free caps of the particles were modified with (3-aminopropyl)trimethoxysilane (APTMS) and rhodamine B isothiocyanate (RITC). Thus, after polymer dissolution free IPCs were achieved (Figure 1). It might be possible to demonstrate various arrangements of these IPCs by changing the patch sizes, the overall particle charge and external factors like pH or temperature. The synthesis of IPCs contributes to the understanding of self-assembly and might lead to novel applications.

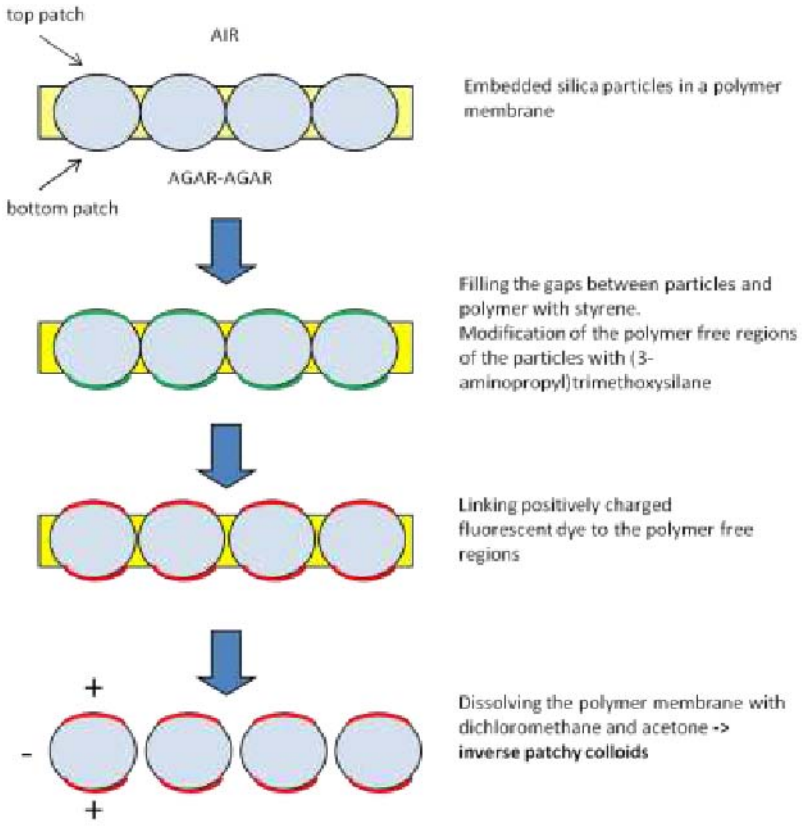

Figure 1: Schematic method to modify silica particles embedded in a polymer membrane to create inverse patchy colloids.

This report provides an overview of the steps leading to the development of this method. It starts with a description of experiments carried out on liquid surfaces to create a composite membrane of silica particles and polymer (3.1.1), inspired by a technique to synthesize porous polymer membranes (19). Chapter 3.1.2 includes the replacement of water by agar-agar and agarose due to particle losses to the liquid substrate and the replacement of monomer by polymer, as a result of cross-linking problems (3.1.2.2). The final method to modify the polymer-free caps is described in more detail in section 3.2.

2. Materials and Methods

2.1. Materials

In Table 1 all chemicals used for this work are listed. All of them were utilized without any further treatment and were stored at room temperature, with the exception of particles, rhodamine B isothiocyanate and monomers, which were stored at 4 °C.

Table 1: Chemicals used for the experiments.

Product name	Source
Particles	
Non functionalized Silica microspheres size: 2 µm	BANGS LABORATORIES, Fishers, IN, USA
Micro particles based on silicon dioxide size: 2 µm	SIGMA-ALDRICH, St. Louis, MO, USA
Initiator	
Phenylbis(2,4,6-trimethylbenzoyl)phosphineoxide 97 %	SIGMA-ALDRICH, St. Louis, MO, USA
Surfactants	
Didodecyldimethylammonium bromide 98 %	SIGMA-ALDRICH, St. Louis, MO, USA
Poly(2-dimethylamino)ethyl methacrylate) methyl chloride quaternary salt	SIGMA-ALDRICH, St. Louis, MO, USA
Monomers	
2-Ethylhexyl acrylate 98 %	SIGMA-ALDRICH, St. Louis, MO, USA
Methyl methacrylate ≥ 98.5 %	SIGMA-ALDRICH, St. Louis, MO, USA
tert-Butyl acrylate 98 %	SIGMA-ALDRICH, St. Louis, MO, USA
Polymers	
Poly(4-vinylpyridine) M_W 60 000 Da	SIGMA-ALDRICH, St. Louis, MO, USA
Poly(4-vinylpyridine-co-styrene)	SIGMA-ALDRICH, St. Louis, MO, USA
Poly(4-vinylphenol) Mw 25 000 Da	SIGMA-ALDRICH, St. Louis, MO, USA
Poly(vinyl alcohol) Mowiol® 56-98 Mw 195 000 Da	SIGMA-ALDRICH, St. Louis, MO, USA
Poly(acrylic acid) Mw 1 800 Da	SIGMA-ALDRICH, St. Louis, MO, USA
Poly(methyl methacrylate) Mw 350 000 Da	SIGMA-ALDRICH, St. Louis, MO, USA
Polystyrene Mw 1 000 Da	SIGMA-ALDRICH, St. Louis, MO, USA
Polystyrene Mw 192 000 Da	SIGMA-ALDRICH, St. Louis, MO, USA
Polystyrene Mw 35 000 Da	SIGMA-ALDRICH, St. Louis, MO, USA
Polyvinylpyrrolidone Mw 1 300 000 Da	SIGMA-ALDRICH, St. Louis, MO, USA
Solvents	
2-Propanol ≥ 99.5 %	CARL ROTH, Karlsruhe, Germany
Acetone 99.5 %	CARL ROTH, Karlsruhe, Germany
Chloroform ≥ 99.8 %	SIGMA-ALDRICH, St. Louis, MO, USA
Dichloromethane ≥ 99.9 %	SIGMA-ALDRICH, St. Louis, MO, USA
Diethyl ether ≥ 99.5 %	SIGMA-ALDRICH, St. Louis, MO, USA
Ethanol absolute ≥ 99.8 %	CARL ROTH, Karlsruhe, Germany
Ethanol 96 %	CARL ROTH, Karlsruhe, Germany
Ethyl acetate ≥ 99.5 %	SIGMA-ALDRICH, St. Louis, MO, USA

Petroleum ether 30–50 °C	SIGMA-ALDRICH, St. Louis, MO, USA
Tetrahydrofurane ≥ 99.5 %	CARL ROTH, Karlsruhe, Germany
Others	
(3-Aminopropyl)trimethoxysilane 97 %	SIGMA-ALDRICH, St. Louis, MO, USA
Ammonia solution 25 %	MERCK, Darmstadt, Germany
Agar-Agar, bacteriological	OXOID, Basingstoke, United Kingdom
Agarose universal	PEQLAB, Erlangen, Germany
α-Bromoisobutyryl bromide 98 %	SIGMA-ALDRICH, St. Louis, MO, USA
Benzophenone ≥ 99 %	MERCK, Darmstadt, Germany
Glycerol, 99 %	
Rhodamine B isothiocyanate	SIGMA-ALDRICH, St. Louis, MO, USA
Trichloro(1H,1H,2H,2H-perfluorooctyl)silane 97 %	SIGMA-ALDRICH, St. Louis, MO, USA
Trimethoxy(octadecyl)silane 90 %	SIGMA-ALDRICH, St. Louis, MO, USA

2.2. Methods

2.2.1. Bright-field microscopy

Principle: With a bright-field microscope it is possible to produce a magnified image of a specimen using visible light and a system of lenses. Light is emitted by a lamp below the sample, which diffracts the light when passing through the sample. The objective lens collects that diffracted light and produces a magnified real intermediate image which is transformed into a real final image by the ocular and eye (20). In Figure 2 a typical construction of a bright-field microscope and the process of magnification of a specimen are shown.

Sample preparation: Samples were analyzed by a microscope (Nikon Eclipse ME600) with 50x or 100x magnification lenses without any further treatment.

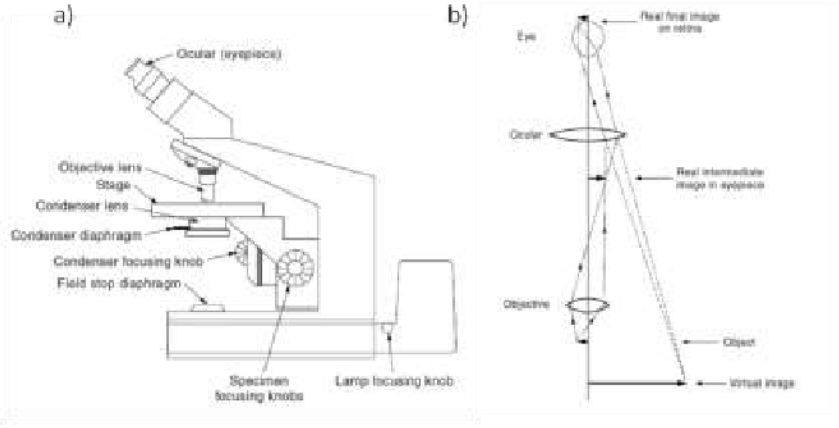

Figure 2: a: Structure of a bright-field microscope b: Magnification of an object in a bright-field microscope (20)

2.2.2. Scanning electron microscopy

Principle: Scanning electron microscopy (SEM) enables the generation of images showing the topography of samples in a micrometer to nanometer range. Basically, a SEM consists of an evacuated electron column and a control unit. An electron gun produces an electron beam, which is focused by coils. The electrons impinge on the sample, whereby signals are generated (e.g. secondary electrons) that are collected and detected. The beam scans the specimen point by point in a raster scan pattern to create an image of the surface (21).

Sample preparation: Samples from top of a liquid (water or agar-agar 0.2 %) were collected with the help of aluminum foil strips. To investigate the potential top patches of particles a piece of aluminum was briefly dipped into the sample container. To analyze the bottom patches the liquid surface was touched with a bent stripe (Figure 3). The dried aluminum pieces were stuck on a SEM mount with an adhesive carbon tab on top. Likewise small pieces of samples from solid surfaces were put after freeze-drying with a pair of tweezers either with the air exposed side (top patch) or the agar-agar/agarose exposed side (bottom patch) pointing upwards onto the carbon tabs.

The specimens were coated with a 3–6 nm gold film by a Leica EM SCD005 sputter coater and analyzed under high vacuum by a FEI Inspect S50 scanning electron microscope.

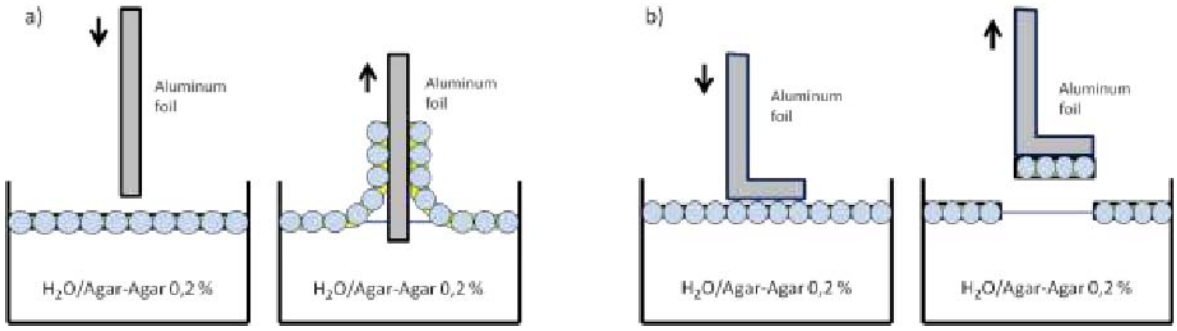

Figure 3: Method for taking samples for SEM imaging from a liquid surface to investigate a) the top air-exposed patch or b) the bottom or water/agar-agar (0.2 %) exposed patch of particles.

2.2.3. Fluorescent labeling

Principle: A common principle found in literature was applied to fluorescently dye the surface of the silica particles (22; 23). Particles modified with (3-aminopropyl)trimethoxysilane (APTMS) expose reactive amine groups on their surface, which can be conjugated to the cationic rhodamine B isothiocyanate (RITC), a fluorescent

dye (Figure 4). Thereby, the sites of the particles, which are free of membrane, become fluorescent and positively charged (Figure 1).

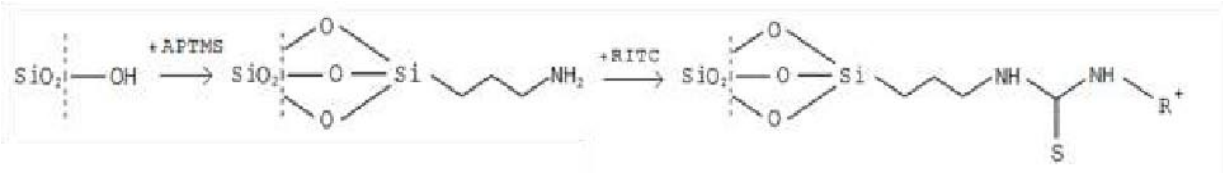

Figure 4: Modification of the silica particle surface with APTMS and RITC.

Sample preparation: The modification with APTMS was performed with different concentrations and exposure times in water or ethanol or via vapor deposition for 8 h at 100 °C (see chapter 3.2). Afterwards, the samples were dyed with a RITC solution for at least 3 h (one spatula tip of RITC in 100 mL Ethanol, absolute) and were washed several times with Ethanol.

2.2.4. Fluorescence microscopy

Principle: A fluorescence microscope is an instrument that produces a magnified image of a specimen due to the emitted fluorescent light from excited molecules and a combination of dichroic mirrors, lenses and filters. Usually, samples are labeled with a fluorochrome or fluorescent dye like fluorescein or rhodamine. Electrons of those fluorescent molecules are excited to a higher state of energy when they absorb photons of a specific wavelength. For the excited electrons to reach their ground state again, photons of a longer wavelength (due to some energy losses) are emitted (20).

Sample preparation: Fluorescent labeled samples were pipetted on microscope cover slips. After drying, a drop of glycerol was added on top and the cover slips were put upside down onto microscope slides. They were fixed with melted wax at the edges, so that they could not slip out of place. The samples were analyzed by a Nikon Eclipse TE2000-S microscope after adding a drop of oil on top of the cover slip.

2.2.5. Confocal microscopy

Principle: Confocal microscopy enables high contrast images of fluorescent samples or reflective surfaces by point scanning the specimen with a laser beam and a pinhole aperture in front of a detector. It is a type of fluorescence microscope that generates and detects signals, which are then built up point by point by a computer to form an image. It

is possible to create a three-dimensional illustration of a sample, by changing the microscope focus along the z-axis in small steps (20). Figure 5 shows the optical pathway of a laser beam in the scan head and the mechanism of raster scanning.

Sample preparation: Samples were prepared as described in section 2.2.3. A Leica SP5 II Laser Scanning Confocal Microscope with a 561 nm multi argon laser source and the objective HCX PL APO CS 63x/1,40–0,60 OIL was used for analysis.

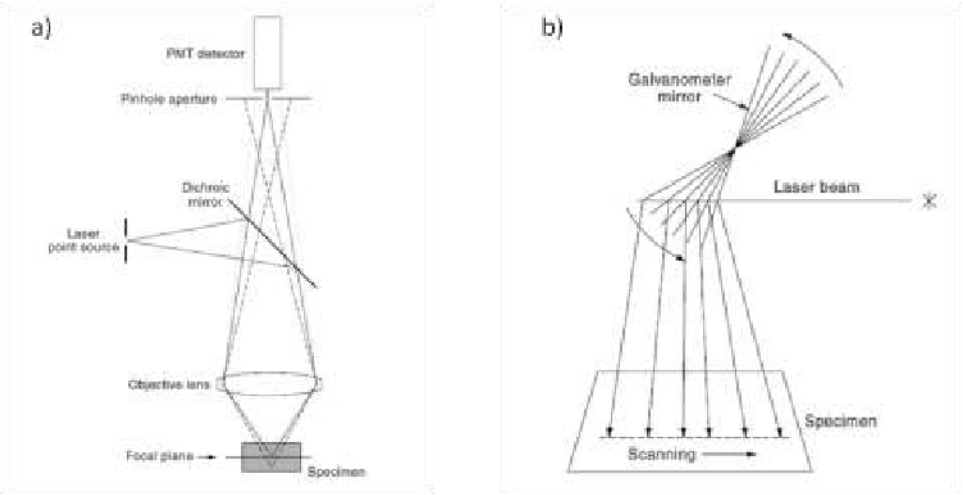

Figure 5: Optical path and mechanism of raster scanning of a confocal microscope. a) Optical path of a laser beam in a confocal scan head. Only the emitted fluorescent light of one point of the sample passes the pinhole aperture and is perceived by the photomultiplier tube (PMT) detector (straight lines). The emitted photons of other spots not at the focal plane are not detected (dashed lines). b) Scheme of a raster scanning mechanism. Usually two vibrating mirror are scanning the x- and y-axis (20).

2.2.6. Calculations

2.2.6.1. Mass of particles forming a closed packed monolayer

If particles form a closed-packed monolayer, the area of one particle can be calculated as the area of a parallelogram with the side D, which is equal to the diameter of a particle (Figure 6). The mass of particles added to get such a monolayer is equal the mass of one particle times the number of all particles (equation 1). The total surface area A of the interface is divided by the area of one particle ($\frac{\sqrt{3}*D^2}{2}$). The mass of one particle is given by its density times its volume ($\frac{\pi*D^3}{6}$) (19). The particles calculated with equation 1 cover approximately 91 % of the whole substrate surface.

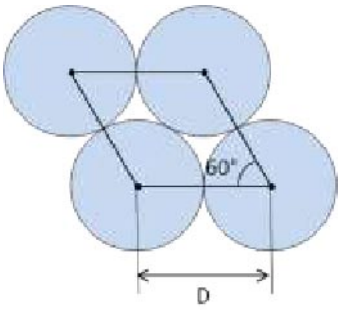

Figure 6: Schematic illustration of hexagonally closed packed silica particles. The area of one particle is calculated by the area of a parallelogram with the side length D.

$$M = N * m = A * \frac{2}{\sqrt{3}*D^2} * \rho * \frac{\pi*D^3}{6} = \frac{\pi}{3*\sqrt{3}} * \rho * D * A \quad (1)$$

M	mass of particles that form a closed-packed monolayer
m	mass of one particle
N	number of all particles in a close-packed monolayer
A	surface area
D	diameter of the particles
ρ	density of the particles

2.2.6.2. Opening angle α of a patch

The opening angle α, shown in Figure 7, is calculated by the arcsine of the patch radius divided by the particle radius (equation 2).

$$\sin \alpha = \frac{d_p/2}{D/2} \quad (2)$$

α	patch opening angle
d_p	diameter of the patch
D	diameter of a particle/distance between two touching particles

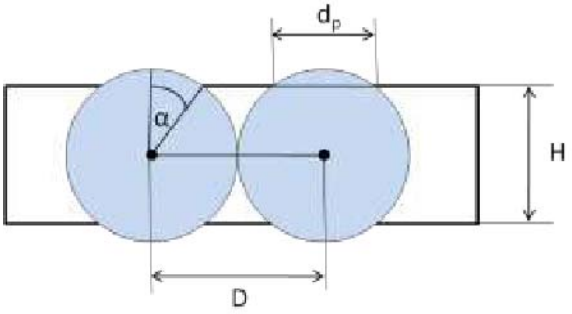

Figure 7: The opening angle α is calculated by the diameter of the patch and the diameter of the particle which is equal to the distance of two touching particles sticking in a membrane of height H.

2.2.6.3. Amount of polymer needed to get a desired α

Starting point for the calculation is the height H of the polymer layer which is given by two times the cosine of the opening angle α times the radius r of a particle ($H = 2 * \cos\alpha * r$), see Figure 7. The volume V_{pol} of the polymer needed to form a layer with height H is the area *A* of the gel surface multiplied by *H* ($V_{pol} = A * H$), not taking into account the presence of particles in the polymer membrane, wherefore less polymer is required. Considering this fact, the volume *V* of polymer needed is V_{pol} minus the volume of all parts of colloids sticking in the polymer V_{col}. The volume of the two spherical caps V_{cap}, not covered by the polymer, is calculated by the equation: $V_{cap} = 2 * \frac{h^2 * \pi}{3} * (3r - h)$. By subtracting *H/2* from the particle radius the height h of a spherical cap is obtained (h = r – H/2). V_{col} is the total volume of one particle V_{par} ($\frac{\pi * D^3}{6}$) minus the volume of the spherical caps V_{cap} ($V_{col} = V_{par} - V_{cap}$). V_{col} times the number of particles covering the surface n subtracted from V_{pol} results in the actual volume of polymer required to get a desired opening angle α ($V = V_{pol} - N * V_{col}$). A prerequisite for this calculation is that bottom and top patches of the particles are equal.

$$V = A * H - N * (\frac{\pi * D^3}{6} - 2 * \frac{h^2 * \pi}{3} * (3r - h)) \quad (3)$$

V	volume of the polymer to get patches with an opening angle α
A	surface area
H	height of the polymer membrane
N	number of particles
D	diameter of a particle
h	height of a spherical cap
r	radius of a particle

2.2.7. Particle preparation

2.2.7.1. Preparation of purchased silica particles

Purchased, relatively uniform silica particles having a diameter of 2 µm were washed two times with ethanol and dried under a stream of gaseous nitrogen. The mass of

particles forming a hexagonal closed-packed monolayer (equation 1) or covering 80 % of the substrate surface were calculated, resuspended in ethanol and sonicated for 15 min.

2.2.7.2. Hydrophobization of silica particles

Silica particles (D = 2 µm) were washed two times in absolute ethanol and two times in tetrahydrofurane (THF). Thereafter, approximately 300 µL of α-bromoisobutyryl bromide (BIBB) was added to about 1000 µL of THF with particles and stirred for one hour to modify the surface of the colloids (Figure 8 a). Then the particles were washed thoroughly four times with ethanol. For safety reasons, the solution had been diluted with ethanol (approximately 1:1) before the first washing step. The particles were dried under a gaseous nitrogen stream. Modified particles were resuspended in ethanol and sonicated for 15 min. To stabilize the hydrophobic coating of the particles, for some experiments colloids modified with BIBB were boiled in ethanol for 10 min before they were dried under a gaseous nitrogen stream (Figure 8 b).

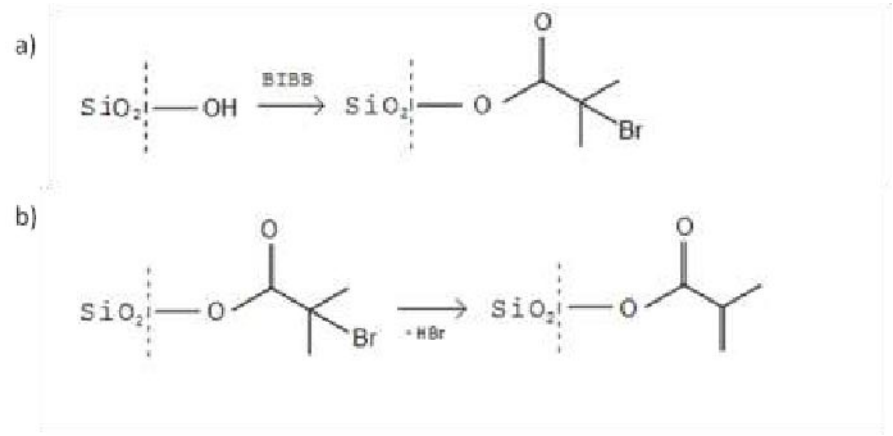

Figure 8: Hydrophobic surface functionalization of silica particles. a) Modification of silica particles with BIBB by stirring in THF for 1 h. b) Stabilization of the coating by boiling hydrophobized silica particles in ethanol for 10 min.

3. Results and Discussion

The aim of this work was to develop IPCs by masking parts of the surface of closed-packed silica particles with a polymer membrane, leaving only two smaller regions on the opposite sides of the particles accessible to silanization and fluorescent labeling. By arranging particles in 2D structures on a water/air interface, the bottom area of the particles was screened by water. Thus, a deposited polymer could not adhere to that surface leaving the bottom side of the particles available to chemical modification. The properties of the polymer along with the structure of the substrate surface and the particle arrangement determined the size of the top patches.

According to an interesting method, monolayers of silica particles could be used to create composite membranes consisting of polymer and particles, the latter protruded from the membrane on both sides. After particle dissolution, porous polymer membranes could be obtained (19). However, the idea of our work was to embed particles into a polymer, which should be dissolved instead of the particles after the modification of the polymer-free regions. To begin with, silica particles (D = 2 µm) were hydrophobized either by the adsorption of hydrophobic surfactants (3.1.1.1) or by linking α-bromoisobutyryl bromide (BIBB) to the surface (3.1.1.2). The particles in a solvent mixture were spread together with monomer and photoinitiator on top of a water or liquid agar-agar gel (0.2 %) surface or into a petroleum ether layer on top of a water phase. Particles with adsorbed surfactant fell into the water phase, whereas BIBB modified particles mainly stayed on the water/air interface but were not evenly distributed and the used monomers did not fill the spaces between them. The experiments and their most important outcome are summarized in Table 2.

Table 2: List of most important experiments and their outcome carried out on liquid substrates (water or agar-agar gel 0.2 %).

basic approach	specific feature or amendments to basic approach	outcome
A mixture of particles (D = 2 µm), monomer, ethanol, petroleum ether, photoinitiator and surfactant were pipetted at room temperature on a liquid substrate (in cell culture plates d = 1.6 cm). After solvent evaporation the samples were irradiated at 254 nm for 20 min	▪ hydrophobized particles by an adsorbed surfactant	▪ particle loss to the water phase ▪ monomer not attracted enough to silica
	▪ pipetting the mixture in a 2 mm petroleum ether layer on top of the liquid surface	

A mixture of **BIBB modified particles** (D = 2 µm), monomer, ethanol, petroleum ether, photoinitiator and surfactant were pipetted at room temperature in a 2 mm petroleum ether layer on top of a liquid substrate (in glass beakers d = 0.8 cm). After solvent evaporation the samples were irradiated at 254 nm for 20 min	▪ pipetting the mixture directly on the liquid surface without a petroleum ether layer	▪ particle loss ▪ early polymerization
	▪ pipetting the mixture in a 2 mm petroleum ether layer on top of the liquid surface	▪ inhomogeneous particle distribution ▪ early polymerization
	▪ smaller particles D = 870 nm	
	▪ no photoinitiator	▪ early polymerization and monomer drops
	▪ benzophenone in the water phase	▪ particle loss ▪ bubbles in the water phase
	▪ at 4 °C in the dark	▪ particle loss ▪ inhomogeneous monomer and particle distribution
	▪ at 4 °C in the dark ▪ pinned interface	▪ inhomogeneous particle distribution
	▪ at 4 °C in the dark ▪ pinned interface ▪ pipetting BIBB modified particles in a petroleum ether layer ▪ preventing the solvent from evaporation for 3 h ▪ adding monomer and photoinitiator to the petroleum ether layer	▪ most parts of the surface covered with particles ▪ inhomogeneous monomer distribution

On solid agar-agar gels (2 %), particle loss could be excluded but the simultaneous application of particles, solvent and monomer resulted in disordered particle structures (3.1.2.1). Therefore, particles in ethanol were first pipetted on top of the gel surface. After complete solvent evaporation, the gels were frozen at -20 °C. Then the particles were pre-wetted with solvent for 3–6 h before a polymer solution was pipetted on top and prevented from evaporation for another 0.5 h. Polymer was used instead of monomer because of polymerization problems, which arose at -20 °C (3.1.2.2). Experiments performed with the polymer polystyrene (M_w 1, 35 and 192 kDa) dissolved in diethyl ether resulted in the formation of composite membranes with silica particles sticking out on both sides of the polymer. Most important experiments and their outcome are listed in Table 3. The samples were modified with APTMS and RITC in distilled water and absolute ethanol respectively (see 3.2). Before the specimens were treated with APTMS the monomer styrene was added to fill possible gaps between the polymer-

network. The polymer was dissolved in dichloromethane and acetone after fluorescent labeling, resulting in particles with two clearly fluorescent patches. The experiments are described in detail in the following chapters.

Table 3: List of most important experiments and their outcome carried out on solid substrates (agar-agar or agarose gels).

basic approach	specific feature or amendments to basic approach	outcome
Monomer and photoinitiator were either applied together with the particles by **spin coating** or pipetted on top of dried particles on agar-agar gels (2 %) at room temperature in plastic petri dishes d = 5.4 cm. Samples were irradiated at 254 nm	▪ applying particles by spin coating	▪ inhomogeneous particle distribution ▪ radial arrangement
Particles, monomer, photoinitiator, surfactant and solvent pipetted on solid agar-agar gels (2 %) at room temperature. After solvent evaporation the samples were irradiated at 254 nm.	▪ amount of particles used covering 80 % of the substrate surface	▪ inhomogeneous particle distribution
Particles, covering 80 % of the substrate surface, in ethanol were applied on solid agar-agar gels (2 %) in plastic petri dishes (d = 5.4 cm) at room temperature. After ethanol evaporation the gels were **frozen at -20 °C**. Monomer and photoinitiator in ethanol were applied on the particles at -20 °C. After solvent evaporation the samples were irradiated with UV light and freeze-dried over night.	▪ applying monomer and photoinitiator on dried particles	▪ very thin polymer membrane
	▪ higher agar-agar concentrations (4 and 6 %)	▪ rough substrate surface ▪ inhomogeneous particle/monomer distribution
	▪ increased monomer concentration	▪ thin polymer membrane, ▪ remaining polymer formed drops
	▪ monomer and photoinitiator in diethyl ether applied on dried particles	▪ promising results ▪ DEE dissolves the petri dish (PS) ▪ polymerization problems
	▪ all steps at room temperature ▪ monomer and photoinitiator in ethanol or petroleum ether	▪ petroleum ether: monomer drops ▪ ethanol: particle relocation
Mixture of polymer dissolved in solvent and particles in ethanol pipetted on agar-agar gels (2 %) in hydrophobized glass petri dishes (d = 2.9 or 3.5 cm) at **room temperature**.	▪ polymer: PS or PMMA ▪ solvent: DCM or DEE	▪ inhomogeneous particle distribution ▪ polymer drops
Particles (80 %) in ethanol were applied on solid agar-agar gels (2 %) in hydrophobized glass petri dishes (d = 2.9	▪ polymer: poly (4-vinylphenol), poly(acrylic acid), Mowiol® or polyvinylpyrrolidone ▪ solvent: ethanol	▪ inhomogeneous polymer structure
	▪ polymer: PS (Mw 192 kDa) or PMMA ▪ solvent: DCM (with and without surfactant)	▪ hypothesized Cassie-Baxter state

or 3.5 cm) at room temperature. After ethanol evaporation the gels were frozen at -20 °C. Polymer dissolved in a solvent was applied on the particles at -20 °C. After solvent evaporation the samples were freeze-dried over night.	▪ polymer: PS; solvent: DEE ▪ pre-wetting dried particles with DEE at -20 °C before applying the polymer solution	▪ some particles with a bottom patch smaller than 90°
	▪ polymer: PS ▪ solvent: DEE with surfactant	▪ completely covered particles ▪ hypothesized Cassie-Baxter state
	▪ polymer: PS or PMMA ▪ solvent: THF, acetone, methyl ethyl ketone, ethyl acetate, chloroform or DCM ▪ pre-wetting with the solvent at -20 °C	▪ rough polymer surface and/or ▪ hypothesized Cassie-Baxter state
	▪ polymer: P4VP ▪ solvent: ethanol	▪ completely covered particles
	▪ polymer: P4VP mixed with PS or PMMA or co-polymer P4VP-PS ▪ solvent: DCM or chloroform	
Particles (80 %) in ethanol were applied on solid agar-agar gels (2 %) in hydrophobized glass petri dishes (d = 2.9 or 3.5 cm) at room temperature. After ethanol evaporation the gels were frozen at -20 °C. **Co-polymer P4VP-PS mixed with PS (1:1) dissolved in DCM (with surfactant)** was applied on the particles at -20 °C. After solvent evaporation the samples were freeze-dried over night.	▪ all steps at room temperature	▪ many top patches 50-60° ▪ hypothesized Cassie-Baxter state
	▪ all steps at room temperature ▪ sonication of the samples after polymer solution was applied	▪ irregular shape and size of the top and bottom patches ▪ many particles completely covered
	▪ sonication of the samples in an ice water bath after polymer solution was applied	▪ irregular shape and size of the top and bottom patches
	▪ pre-wetting with DCM and surfactant for 5 h at -20 °C ▪ prevention of evaporation of the polymer solution for 0.5 h	▪ irregular shape and size of the top and bottom patches ▪ some bottom and top patches around 55°
Particles (80 %) in ethanol were applied on solid **agarose gels (2 %)** in hydrophobized glass petri dishes (d = 2.9 or 3.5 cm) at room temperature. After ethanol evaporation the gels were frozen at -20 °C. The dried particles were **pre-wetted with DEE** at -20 °C, before **PS dissolved in DEE** was applied. The polymer solution was **prevented from evaporation for 0.5 h**. After solvent evaporation the samples were freeze-dried over night.	▪ polymer: PS Mw 1 or 35 kDa ▪ solvent: DEE ▪pre-wetting with DEE for 3 h	▪ large pieces of composite membranes with only a few irregularities ▪ many top and bottom patches < 90° ▪ irregular shape and size of top and bottom patches ▪ PS Mw 192 kDa: IPCs were observable by confocal microscopy after chemical modification
	▪ PS Mw 192 kDa ▪ solvent DEE ▪ pre-wetting with DEE for 6 h	

3.1. Embedding particles in a polymer membrane

3.1.1. Liquid substrates

3.1.1.1. Particles hydrophobized by the adsorption of a surfactant

A crucial step in synthesizing IPCs by masking a part of the particle surface, is the assembly of hydrophilic silica particles in a closed-packed monolayer on a water/air interface. Hydrophobization of the particles with covalently binding hydrophobic chemicals (i.e. 3-(trimethoxysilyl)propyl methacrylate) is not useful, as it prevents the modification with (3-aminopropyl)trimethoxysilane (APTMS). Instead, one of the two surfactants poly(2-dimethylamino)ethyl methacrylate) methyl chloride quaternary salt (MADQUAT) or didodecyldimethylammonium bromide (DDAB) was added to the solvent mixture. These cationic surfactants adsorb on the negatively charged surface of the silica colloids and therefore should hydrophobize the particles.

To obtain a polymer membrane soluble in various solvents after surface modification, monomer molecules (methyl methacrylate MMA) forming only two bonds during polymerization were applied. The experiments were performed with 2 µm colloids, which are easy to observe in optical microscopes. Furthermore, after the first approaches chloroform was replaced by petroleum ether, because of chloroform's high density and tendency to form a drop in the middle of the water surface.

Purchased particles were prepared according to 2.2.7.1 and mixed with the following constituents:

- solvent: chloroform (volume ratio of ethanol to chloroform 1:1, mass ratio of colloids to solvent 1:100)
- photoinitiator: phenylbis(2,4,6-trimethylbenzoyl)phosphineoxide PPO (mol ratio of photoinitiator to monomer 1:100)
- monomer: methyl methacrylate (volume of a 20 µm thick cylinder with a diameter equal to the water surface)
- surfactant: MADQUAT (1.5 $mg * mL^{-1}$)

The mixture was sonicated for 15 min and spread on a water surface in a d = 2 cm glass vessel. After solvent evaporation the samples were irradiated at 254 nm for 20 min.

SEM images (Figure 9) indicated that some particles were sticking out of a membrane showing an opening angle of 50–60° for the top and bottom patches. This result seemed to be very promising. Though, it was not sure if the method for analyzing the particles with the SEM (2.2.2) worked and showed the top and bottom side of the membrane. In addition, it has to be pointed out that the clusters of particles shown in Figure 10 were hardly present on the water surface. One reason for finding just a few clusters of particles may be the formation of a chloroform drop in the middle of the water surface during solvent evaporation. Therefore, particles concentrated in the middle, whereas the edge of the sample was almost free of particles and nearly only consisted of polymer (Figure 10).

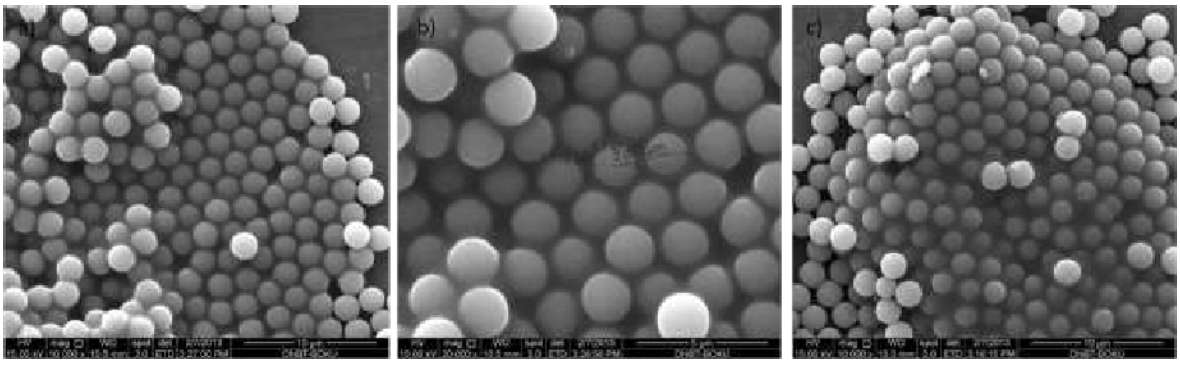

Figure 9: SEM images of silica particles in a 20 µm PMMA membrane spread by an ethanol/chloroform mixture, showing probably the air (a and b) and water exposed side (c) of the sample with patches between 50–60°.

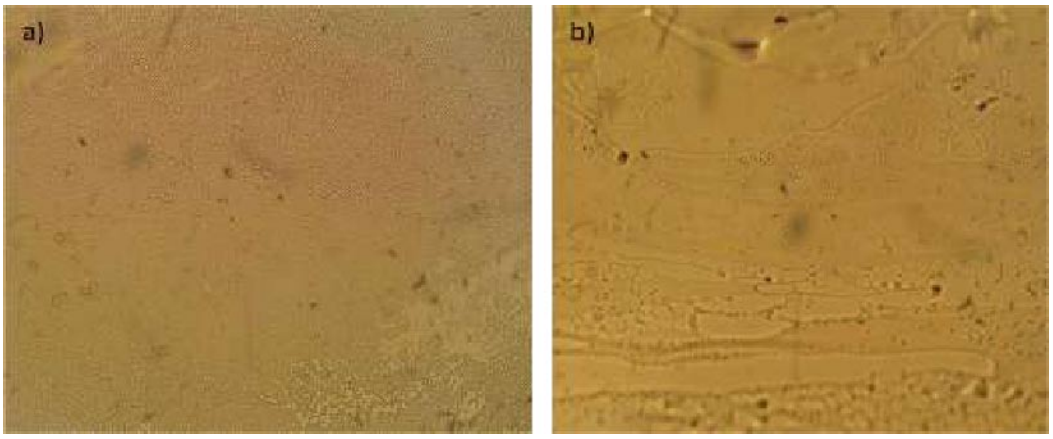

Figure 10: Bright-field microscopy images of silica particles in a PMMA membrane applied from an ethanol/chloroform mixture at the center (a) and the edge (b) of a water surface. Most particles arranged in the center of the sample, whereas the edge stayed almost free of particles.

Hence, chloroform was replaced by petroleum ether as a solvent, which has a lower density. In contrary to petroleum ether, ethanol is miscible with water very well. So, the

ratio of ethanol to petroleum ether in the solvent mixture was decreased to 1:5, to avoid that a large amount of ethanol promotes particles falling into the water. Ethanol could not be left out completely, as particles were not stable in pure petroleum ether. The amount of monomer was reduced to the volume necessary to form a 1 µm thick layer. Plastic cell culture plates (diameter of the wells 1.6 cm) with a treated polystyrene surface to make the plastic more hydrophilic, served as containers for experiments on top of a water surface as well as on top of a viscous agar-agar gel (0.2 %). The latter was used both in its gelled and in its liquid (not yet gelled) state. It was noted that particles tended to fall very easily from the air-water interface into the water phase. Thus, even minor vibrations led to fewer particles on the surface, which should be reduced by using agar-agar with a higher viscosity than water. As this natural polymer gels at 32.0–37.5 °C (24) petroleum ether (boiling point: 30–50 °C) when pipetted onto the liquid gel evaporated very fast. This was presumably too violent for the particles resulting in an almost particle free surface. A too rapid solvent evaporation could also lead to a reaction induced phase separation and further to the formation of a cross-linked polymer with an inhomogeneous morphology (25). Additionally, samples prepared on gelled, cold agar-agar and water surfaces did not show promising results. On both substrates mainly monomer droplets with the exception of a few particle clusters were located on the surface before UV irradiation (Figure 11 a).

Samples were also prepared by pipetting a 2 mm petroleum ether layer on top of the substrate surface and then adding the mixture of particles, solvent, monomer, photoinitiator and surfactant leading to a modestly better result. The particles had more time to arrange on the interface. Colloids formed clusters distributed over most parts of the surface (Figure 11 b), but the outcome was not satisfactory.

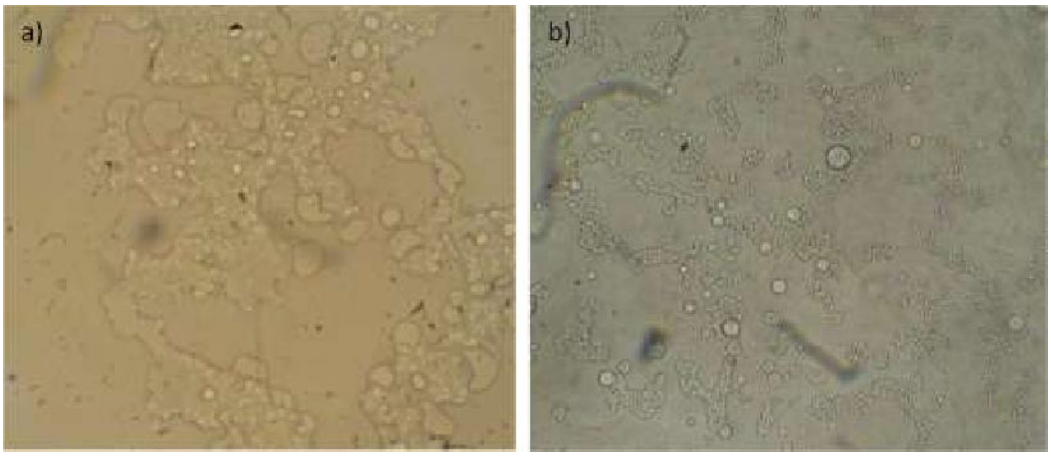

Figure 11: Mixture of ethanol, petroleum ether, MMA, MADQUAT, PPO spread on top of a water surface without (a) and with (b) a barrier of petroleum ether. Some 2D particle clusters and monomer drops were seen on both water/air interfaces.

Experiments with the surfactant DDAB in a concentration of 0.1 $mg * mL^{-1}$ showed similar results. The added surfactants probably reduced the surface tension of water too much, so that the main part of the particles did not stay at the interface. It was striking that MMA still preferred to form droplets rather than to creep between the particles. For that it can be assumed that MMA is not attracted well enough to the silica particles to fill the space between the colloids. Probably there were too few colloids for a particle-assisted wetting or the particle surface or monomer was not suitable (26). Therefore, further experiments described in section 3.1.1.2 were performed with the monomers tert-butyl acrylate (tBA) and 2-ethylhexyl acrylate (EHA) and coated silica particles.

3.1.1.2. Particles modified with α-bromoisobutyryl bromide

Since particles with adsorbed surfactant mostly fell into the water or viscous agar-agar gel (0.2 %) silica colloids were coated with α-bromoisobutyryl bromide (BIBB) to hydrophobize them. In contrary to a modification with 3-(trimethoxysilyl)propyl described by YAN and GOEDEL (2004) one with BIBB can be removed with acids like sulfuric acid, allowing subsequent modification with APTMS. For a possibly better particle distribution the amount of the solvent mixture was increased to 1:200 (mass ratio of colloids to solvent) and the amount of monomer (MMA, tBA and EHA) decreased to one third of the mass of the colloids, following the experiments of YAN and GOEDEL (2004).

Spreading the hydrophobized colloids directly on top of a water surface in small glass beakers (d = 0.8 cm) did not yield a satisfactory amount of particles on the interface. Therefore, further experiments were carried out by the above (3.1.1.1) described method

of first applying a layer of petroleum ether into which then the mixture of solvent, monomer and photoinitiator was pipetted. Clearly, an improvement was achieved. At first the water surface was almost completely covered with particles (Figure 12 a and c), but polymerization of the monomer started already before the entire solvent evaporated. BIBB is a known initiator for atom transfer radical polymerization (27; 28) and therefore likely induces the early polymerization under the described experimental conditions. As a result, the cross-linked polymer probably initially swelled and then shrank after the remaining solvent had evaporated completely, causing a wrinkled polymer (29; 30) and a strongly distorted surface which in turn led to a great loss off particles (Figure 12 b and d). The wrinkling of the polymer started near the container wall and continued in increasingly attenuated form towards the middle. SEM pictures showed that most particles were not arranged in a hexagonal pattern. Particles were either free of polymer or covered on one side completely. Between the various monomers no perceptible difference was noticed.

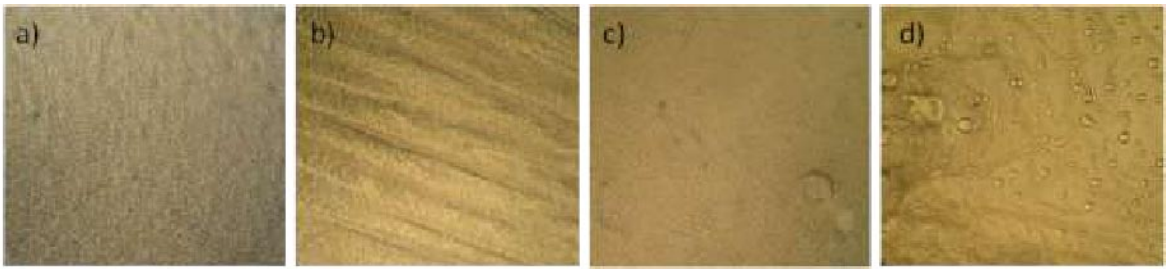

Figure 12: Bright-field microscopy images showing samples directly after pipetting hydrophobic particles, ethanol, petroleum ether, photoinitiator and MMA (a) or tBA (c) into a petroleum ether layer on a water surface. Initially, particles seemed to be homogeneously distributed but after complete solvent evaporation a wrinkled pattern formed b: sample with MMA; d: sample with tBA.

The same experiment was performed with smaller particles (D = 870 nm) produced following Stöber's method (31) and hydrophobized as described above, since the smaller the colloids the higher the probability that they form a close-packed monolayer with fewer defects (19). Presumably, because of the early polymerization, particles were arranged in some regions in multiple layers, whereas some spots, mainly located at the edges, were completely free of particles. It was not possible to observe composite membranes. As no improvement could be demonstrated, further approaches were performed with 2 µm particles, which are easier to detect in optical microscopes.

Consequently, three problems had to be worked out: an increased monolayer formation, a better monomer distribution and the inhibition of the early polymerization.

In order to prevent the latter, no photoinitiator was added to the particle mixture. Depending on the monomer, samples showed either monomer drops (MMA) or a partially patterned surface (tBA and EHA), indicating that polymerization occurred too early (Figure 13). In other regions of the sample monomers tBA and EHA also formed drops. None of the monomers distributed over the whole surface and filled the gaps between the particles. Close to the glass beaker wall particles fell into the water, presumably at the time when the last bit of solvent evaporated, whereas at other spots particles arranged in multiple layers. By adding benzophenone to the water (mol ratio of monomer to benzophenone 100:1 and 1000:1) early polymerization could be inhibited, but bubbles formed in the water phase, which were probably causing the observed particle loss. Another approach was performed by preparing samples at 4 °C in the dark, showing a better outcome. Many particles and almost no wrinkled polymer were found on the water/air interface.

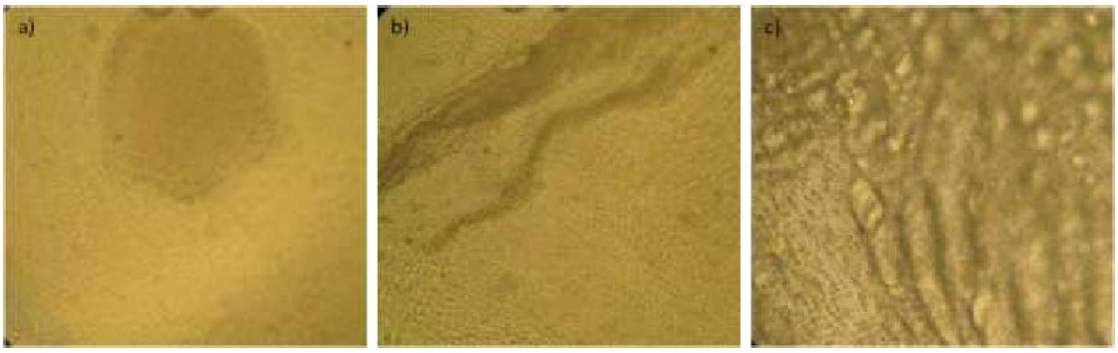

Figure 13: Experiments performed without photoinitiator showed either monomer drops with MMA as the monomer (a) or several patterned structures on the surface indicating early polymerization with tBA and EHA as the monomer (b and c respectively).

An explanation for the irregular distribution of particles may be the relatively small diameter of the beaker (d = 0.8 cm), by which the water surface formed a concave meniscus. Since glass is polar, the nonpolar petroleum ether layer forms a convex meniscus. So, during evaporation a concave water/petroleum ether interface meets a convex petroleum ether/air interface surface (Figure 14 a). This change may be too violent for the particles to stay at the surface. It may also explain why wrinkling of the polymer starts at the edge of the sample, where the layer of solvent is thinner and thus might evaporate at first. In Figure 14 it can be seen that the petroleum ether/air interface is actually slightly concave and not convex. Nevertheless, because of the big meniscus of the water/petroleum ether interface the evaporation may start at the edge and particles

may concentrate due to gravity in the middle. In a hydrophobic glass the water/petroleum ether meniscus was just reversed (Figure 14 b), not minimizing the surface change during evaporation. Therefore, a pinned surface (Figure 14 c) was created, by filling glass beakers with ethanol and 2 µL trimethoxy(octadecyl)silane up to a specific level. Ethanol was prevented from evaporation by putting the glasses into a shallow water bath and putting up-side down a bigger beaker around the ethanol filled beakers in order to create a saturated atmosphere. After several hours the beakers were washed thoroughly first with acetone and then with ammonia to inhibit the reaction of possibly residual silane.

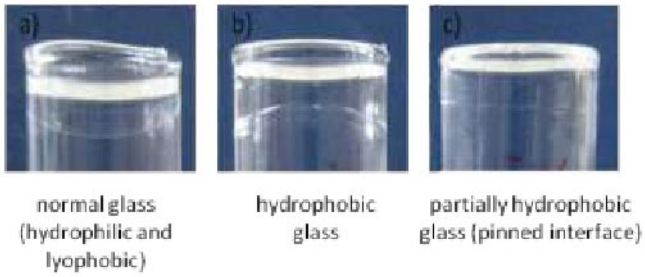

Figure 14: Water covered with a layer of petroleum ether in normal untreated glass (a), in a hydrophobic glass (b) and a glass with a pinned interface (c).

Indeed, the interface of water and petroleum ether was flat when water was filled to the exact level where glass changed from hydrophobic to hydrophilic and also stayed flat when the solvent evaporated and the particles, monomer and photoinitiator arranged on the surface. However, in contrast to previous experiments particles tend to concentrate near the glass wall whereas the middle stayed sometimes almost particle free.

To obtain an even monomer distribution the application method of particles and monomer at 4 °C was altered. Colloids were resuspended and sonicated in ethanol and then spread into a layer of petroleum ether, which was prevented from evaporation by an up-side down beaker in a layer of water. After about three hours the beaker was lifted and monomer with photoinitiator (mol ration of monomer to photoinitiator 100:1 or 1000:1) was added. At the bright-field microscope, samples appeared promising as most parts of the surface were covered with close-packed particles. The contrast between those closed-packed particles and their surrounding medium was lower than seen at sample sites with single particles, indicating that the space between the closed-packed particles was filled with polymer. However, SEM analysis showed that all monomer was

on top of the particles and not between them. Regardless the chemical composition of the monomer, it did not form a membrane spanning over the whole sample surface but was concentrated locally. Therefore, most colloids were completely free of monomer whereas some were entirely covered. The results were also not significantly changed by boiling particles in ethanol after coating with BIBB, which should make the coating of the particles more stable. The formation of monomer drops may be explained by the contact angles of modified silica colloids with the interface solvent-air and the interface solvent-water. If both contact angles are about 180°, the organic liquid forms drops without particles on the water surface. Particle-assisted wetting and for that a polymer membrane with particles penetrating both interfaces after polymerization occurs most likely at contact angles with values close to 90° (26), shown in Figure 15.

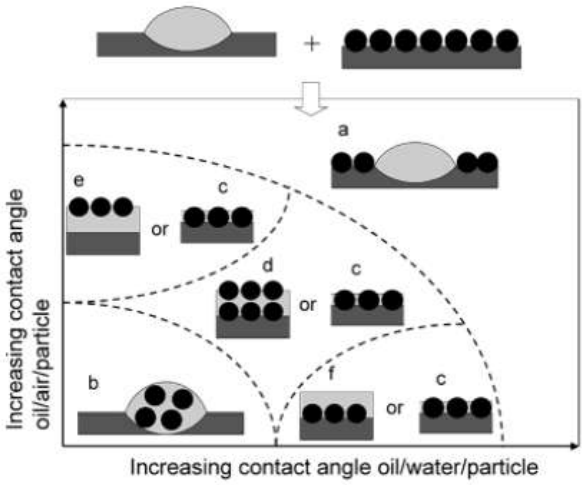

Figure 15: Depending on the contact angle, the organic liquid can either coexist next to silica particles (a) or can cover the particles completely (b). At intermediate contact angles various scenarios are possible (c–f) (26).

In addition to the fact that turbid spots, eventually monomer, were seen after evaporation on the coated glass surfaces, so that reproducibility was not given, no sufficiently even particle distribution was achieved with experiments carried out on liquid surfaces.

3.1.2. Solid substrates

The idea of applying particles on top of a solidified 2 % agar-agar gel was that although the agar-agar seems solid there is still enough liquid on top in which the particles can sink far enough to produce a composite membrane with colloids sticking out on both sides, but without losing particles into the water phase. For that, the size of the bottom patch

may be changed by varying the agar-agar concentration of the gel or by adding surfactants altering the surface tension. The size of the top patch may be influenced by the amount of polymer, by the contact angle between the polymer and silica or also by adding surfactants.

First experiments were carried out by spreading monomer and photoinitiator on top of already applied particles on a 2 % agar-agar surface. Difficulties caused by insufficient polymerization of the monomers had contributed to the replacement of monomer by polymer.

3.1.2.1. Polymer membrane built from monomer units

Agar-agar gels were prepared by melting 2 % agar-agar in distilled water in the microwave. After the solution cooled down to 55 °C (at least one hour in the water bath) it was poured into plastic petri dishes (d = 5.4 cm), to an amount sufficient to cover the bottom of the dishes. Then the gels were allowed to dry for 30 min at room temperature. Initially, uncoated particles, solvent, monomer and initiator were applied on the gels by spin coating (Specialty Coating Systems P6700 Series) the petri dishes at varying speeds (200–1400 rpm). The volume ratio of particles to liquid ranged from 1:10 to 1:40; the concentration of monomer, particles and photoinitiator was chosen as in previous experiments (3.1.1). Monomer and photoinitiator were either applied together with the particles or spread on top of the dried particles after spin coating. The suction of the spin coater was not strong enough to rotate gels in a petri dish faster than 1400 rpm and spin coating of gels without the petri dish led to deformations of the gels. It was found that spin coated particles in ethanol were not distributed over the whole surface, but were ordered in a mostly hexagonal closed-packed monolayer in the middle from which fewer particles radially arranged towards the petri dish wall (Figure 16). For particles only suspended in monomer and photoinitiator without additional solvent the velocity was apparently not high enough to spread them over the whole surface. Particles were present in multiple layers only in the middle of the gel.

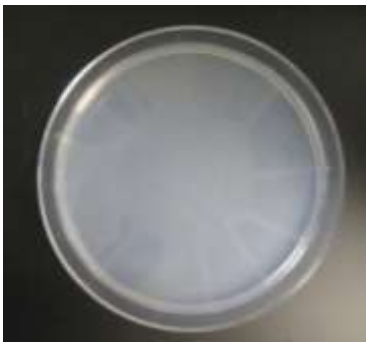

Figure 16: Particles in ethanol (volume ratio particles to ethanol 1:40) applied on an agar-agar gel (2 %) by spin coating. Initial speed was 200 rpm for 5 sec followed by 600 rpm for 60 sec. The particles arranged in a star-like shape on the agar-agar gels.

As no sufficient particle distribution could be obtained by spin coating, particles in ethanol (200 µL) were pipetted in several concentrations (30–90 %) directly on top of the gel. At concentrations smaller than 80 % particles were found either isolated or arranged in aggregates. Aggregates probably formed because of a balance between the repulsion of neighboring particles and a capillary attraction by means of an undulation of the three phase contact line around the colloids (32). A closed-packed monolayer should form with a concentration of 91 % particles (see 2.2.6.1) and indeed particles mainly arranged in that pattern. It was observed that also some multiple layers formed and therefore some areas with fewer loosely packed particles could be observed. Because of that, a concentration of 80 % was chosen for further experiments as then the occurrence of multiple layers was reduced.

When pipetting ethanol, petroleum ether, particles, surfactant, monomer and photoinitiator at once on the gel surface many multiple layers or particle free areas formed. Therefore, it was decided to spread particles in ethanol on the gel at room temperature and allow the solvent to evaporate until no ethanol smell was noticed anymore. Afterwards, monomer, surfactant and photoinitiator in petroleum ether were added. As seen before, monomer drops formed next to the particles and did not fill the spaces between them. This may be explained by the fact that the used monomers in petroleum ether did not have a favorable contact angle to wet the surface (26). A replacement of petroleum ether by ethanol led to a relocation of the particles on the gel surface, as ethanol is mixable with water.

Thus, agar-agar gels were frozen at -20 °C after particles had been dried to avoid mixing of water contained in the gel with ethanol. At the same temperature monomer,

photoinitiator and ethanol (300 µL) was added and the samples were after complete ethanol evaporation, irradiated for one hour. Thereafter, samples were freeze dried over night and membranes could be peeled off the gel. Images from bright-field microscopy before lyophilization indicated that particles arranged mostly in a closed-packed structure. An alteration of the contrast of the refractive index between some particles and the surrounding medium after UV irradiation suggested that at least some colloids were embedded in polymer. SEM analysis confirmed that observation (Figure 17), although the opening angles of the patches on the bottom side were larger than 90° and the polymer seemed to be very thin. No top patch could be measured because the analyzed sample pieces showed multiple layers of particles, which was not expected. These layers may be caused by adding ethanol and monomer before they reached -20 °C.

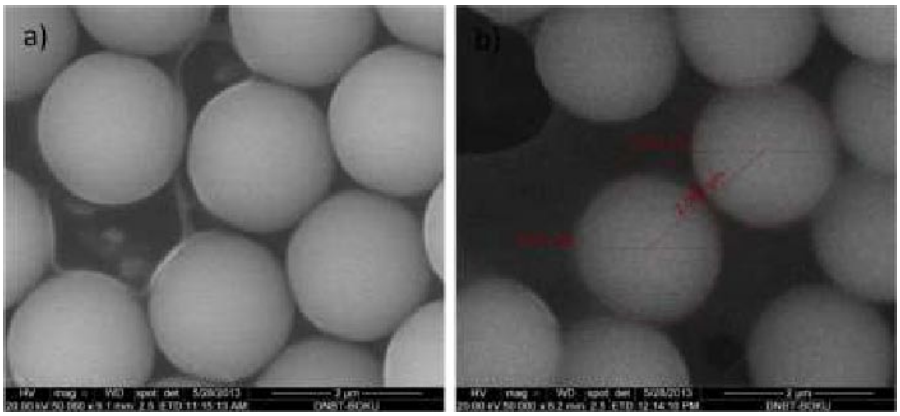

Figure 17: SEM images of samples prepared by pipetting particles in ethanol on an agar-agar gel (2 %). After ethanol evaporation gels were frozen at -20 °C and a mixture of ethanol, MADQUAT (0.1 mg * mL-1) and MMA (a) or EHA (b) were pipetted on top. After solvent evaporation and UV irradiation, samples were freeze dried and analyzed. A very thin polymer, in which some particles were sticking, formed.

Nevertheless, attempts were made to reduce the size of the bottom patch by using higher agar-agar concentrations (4 and 6 %). The higher concentration leads to harder gels with a presumably reduced water-particle contact. A smaller part of the particle surface might be covered by water and the polymer membrane might mask a larger particle area. The higher concentration resulted in an uneven surface, wherefore particles did not arrange in a close-packed structure and monomer concentrated locally. An increased monomer concentration did also not lead to smaller bottom patch sizes. The result was similar to that described above and showed the formation of a very thin polymer layer between the particles, whereas the remaining polymer was found in big drops.

A change of solvent led to an actual improvement in reducing the patch size. Instead of ethanol the monomer, photoinitiator and surfactant were mixed with diethyl ether (DEE) and applied on dried particles at -20 °C. The result, shown in Figure 18, looked very promising. Over a large area particles stuck out on both sides of a polymer membrane. However, it was not been taken into account that DEE dissolves polystyrene (PS). As the petri dishes used in this experiment were made out of PS, it could not be ruled out that the polymer seen in Figure 18 was a mixture of PS and PMMA.

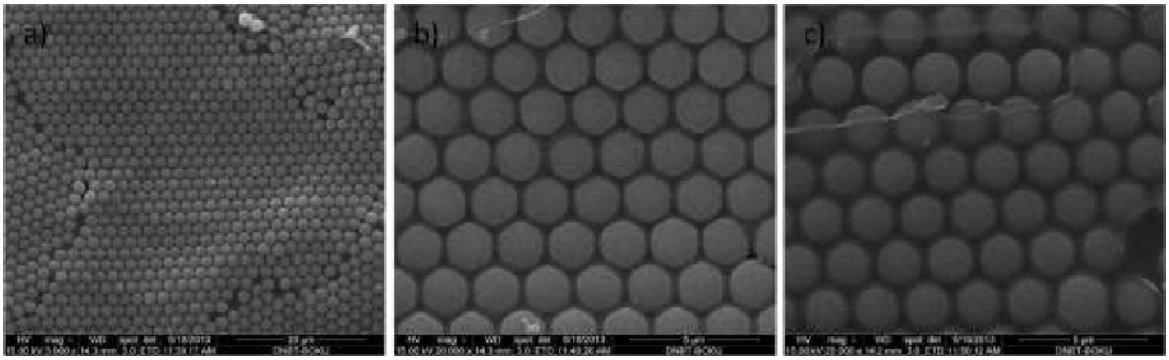

Figure 18: SEM images of the bottom (a and b) and top side of particles obtained after freeze-drying by experiments, in which MMA, MADQUAT (0.1 mg*mL^{-1}) and photoinitiator in DEE were spread on top of dried particles on an agar-agar gel (2 %) at -20 °C. Large areas with particles in a polymer membrane with top and bottom patches were found.

Attempts of reliably reproducing the results in glass petri dishes failed due to difficulties of monomer polymerization at -20 °C and wetting of the whole surface. The presence of compounds like oxygen, a polymerization inhibitor, might have affected the proper cross-linking of the small amount of monomer used in the experiments to produce the thin polymer layers. Hence, further experiments were carried out by using polymer solutions instead of monomer and photoinitiator.

3.1.2.2. Polymer membrane deposited from a polymer solution

Experiments were carried out in glass petri dishes (d = 2.9 and 3.5 cm) in order to not risk distorting the results by accidently dissolving the plastic container. The glass vessels were hydrophobized with Trichloro(1H,1H,2H,2H-perfluorooctyl)silane for a flatter water meniscus by adding 10 µL silane to the petri dishes in a bigger glass jar, which was closed and heated up to 100 °C over night. To achieve a better reproducibility, gels were prepared by pipetting 3 mL (d = 2.9 cm) or 4 mL (d = 3.5 cm) agar-agar solution (kept in a 55 °C water bath for at least one hour before use) at 37 °C in hydrophobic glass petri dishes. After 30 min, gels were transferred to the working space and were allowed to cool

down to room temperature for 15 min. Afterwards the amount of washed and dried particles covering 80 % of the gel surface were resuspended in 200 µL (d = 2.9 cm) or 250 µL (d = 3.5 cm) ethanol and sonicated for 15 min. Particles were pipetted on the gels, which were frozen at -20 °C for 20 min after ethanol evaporation. Polymer was dissolved in solvent and also brought to -20 °C. Thereafter, the polymer solution was spread over the particles and freeze-dried after the solvent had been evaporated completely.

Specimens were prepared with poly(4-vinylphenol), poly(acrylic acid), Mowiol® and polyvinylpyrrolidone as described above. The amount of polymer, forming a 1 µm membrane, was dissolved in 150 µL ethanol. After freeze-drying small irregular shaped sample flakes were obtained (Figure 19 a), instead of a smooth membrane. SEM analysis showed that particles concentrated locally and polymer formed filamentous structures and no planar membrane (Figure 19 b). Probably the ethanol melted the top ice layer so that particles could rearrange and concentrate locally. Poly(4-vinylphenol) dissolved in ethanol mixed with particles and pipetted on a gel at room temperature generated polymer drops (Figure 19 c).

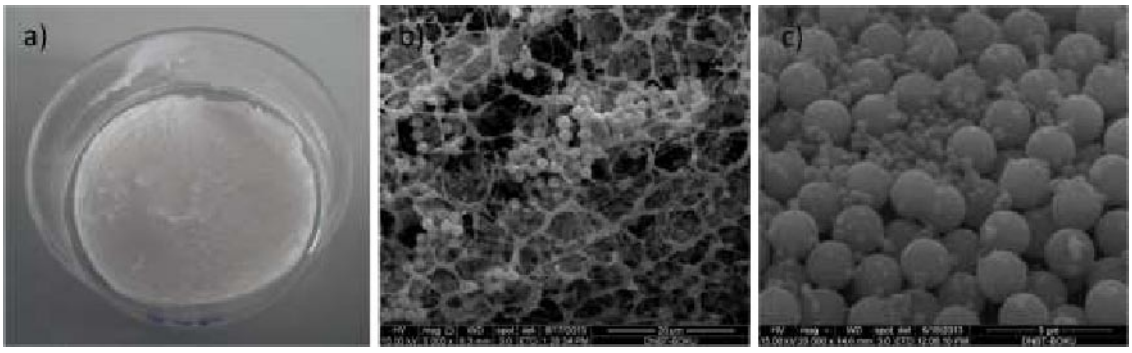

Figure 19: a: Sample showing polyvinylpyrrolidone dissolved in ethanol pipetted on particles on an agar-agar gel at -20 °C after freeze-drying; b: SEM image of the same sample; c: Poly(4-vinylphenol) and particles in ethanol pipetted on a gel at room temperature. The polymers did not form a smooth membrane but filaments or drops.

Better results were achieved by polystyrene with a molecular weight of 192 000 Da (PS) and poly(methyl methacrylate) (PMMA) dissolved in dichloromethane (DCM). The concentration of the prepared polymer solutions was 1 $g * L^{-1}$. Both polymers formed a smooth membrane, which was easy to peel off the freeze-dried agar-agar (Figure 20 a). SEM analysis revealed that PMMA was located on top of the particles leading to no top patch and bottom patches mostly larger than 90°, although some bottom patches were found to be around 61° (Figure 20 b and c). Experiments with PS generated several top patches around 50°. It seemed that many particles fell out of the polymer and hence

created holes in the membrane (Figure 21 a and b). The bottom patch of those particles actually embedded in a membrane was similar to experiments with PMMA, mostly larger than 90° (Figure 21 c). First it was speculated that the large bottom patch was the result of a too soft agar-agar gel, which did not prevent particles from sinking into the gel too far. A higher amount of agar-agar (4 and 6 %) though, only yielded in a more disordered particle assembly but not in a smaller patch size. It could be also ruled out that the large bottom patches were caused by expansion of the water of the agar-agar gels during the freezing process, as this effect was also seen at room temperature.

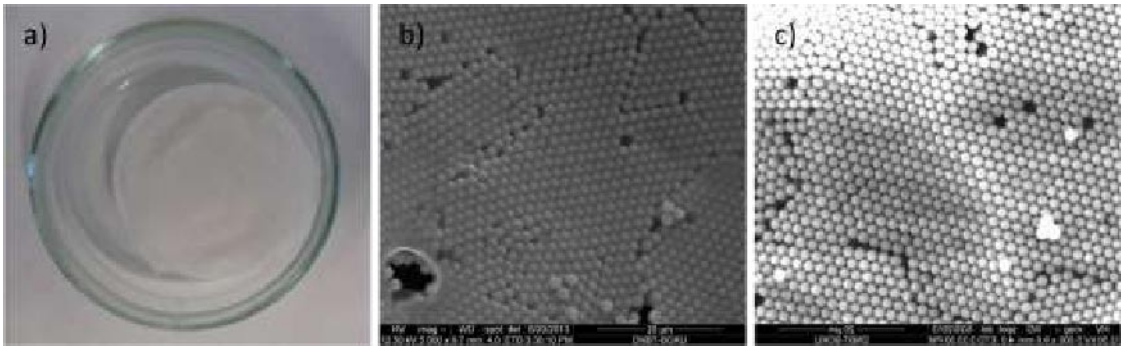

Figure 20: a: PMMA dissolved in DCM pipetted on particles on an agar-agar gel at -20°C after freeze-drying; b: SEM image of the air exposed side of the sample; c: SEM image of the agar-agar exposed side. The membrane after lyophilization was easy to peel off the gel, but it is clearly seen that the polymer formed a layer on top of the particles and did not fill the gaps between them.

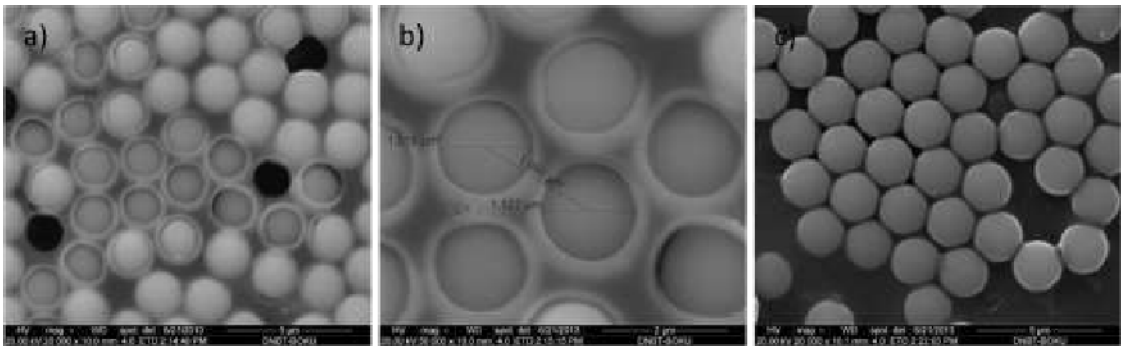

Figure 21: PS (1 µm) was dissolved in DCM and pipetted on dried particles at -20°C a and b: SEM images of the top patches; c: bottom patches. Some of the particles stuck out of the top face of the membrane resulting in a top patch of ~50°. Other particles did not adhere well to the polymer and fell out generating holes in the polymer. The bottom patch of the particles was mostly >90°.

Another theory was based on the observation that some colloids showed bottom patches smaller than 90°, whereas adjacent colloids appeared to be completely polymer-free. It seemed that the polymer solution did not wet all particles equally. The behavior of the polymer solution when pipetted on the dried particles may be explained by a hypothesized Cassie-Baxter state of the polymer solution on the particle monolayer. The Cassie-Baxter equation describes the contact angle of a liquid on a heterogeneous

surface. The pores between the particles may be filled with air and form gas pockets. So the solvent, when applied on top of the dried colloids, may contact two different surfaces, air and silica. An ultrahydrophobic surface is obtained as the contact angle over an air pocket is 180° (Figure 22) (33). Therefore, it does not wet the agar-agar exposed side of the particles and no polymer membrane can form there. Instead it forms lenses on top of the particles.

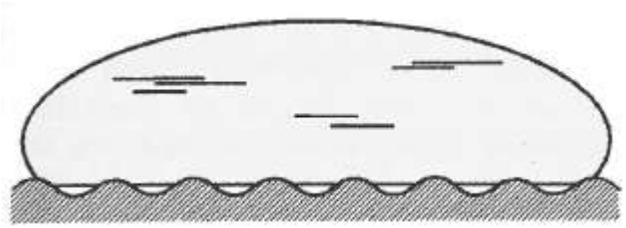

Figure 22: A liquid drop on a rough superhydrophobic surface exhibiting the Cassie-Baxter state (33).

Initial attempts to avoid this effect were carried out by pipetting polymer dissolved in DCM together with particles in ethanol at room temperature on top of an agar-agar gel. Therefore, no air pockets can form. After freeze-drying the gels, samples showed very small pieces of polymer and particles similar to those in Figure 19 a. SEM analysis of the small freeze-dried pieces showed that particles mostly did not arrange in a closed-packed structure and the polymer tended to form drops on top of the disordered colloids. Ethanol, which is a non-solvent for PS and PMMA, probably mixed with the solvent DCM and the polymer and induced phase separation, which can lead to a rough polymer surface and depending on the conditions to the formation of drops or fibers. This can occur when the ratio of DCM to ethanol reaches a critical point because DCM evaporates faster than ethanol (34; 35; 36).

For that, further experiments were performed on dried particles at -20 °C. The surfactant MADQUAT was added to the solvent to reduce the surface tension of DCM. The probability of a Cassie-Baxter state was therefore assumed to be lower, but experiments did not show a significantly better result. Furthermore, attempts were performed to wet the frozen gels with dried particles with pure DCM before the polymer solution was added. Therefore, DCM was pipetted slowly on top of the frozen agar-agar gels held at a ~45° angle. The solvent did not spread over the whole surface but ran down the sample in a small stream and for that the Cassie-Baxter state was not prevented.

As the results were not satisfying, experiments were performed with different solvents with a lower surface tension than DCM (27.8 $mN*m^{-1}$). In addition, the exact amount of polymer needed to get particles with two patches both with an opening angle of 40° (see equations in section 2.2.6.3) was used. PS dissolved in the solvents tetrahydrofuran (26.7 $mN*m^{-1}$), acetone (23.0 $mN*m^{-1}$), methyl ethyl ketone (24.0 $mN*m^{-1}$) or ethylacetate (23.2 $mN*m^{-1}$) did not lead to convincing results. Often a rough polymer surface was found. Better results were achieved with diethyl ether which has a very low surface tension of ~ 16.7 $mN*m^{-1}$ at 25 °C (37). To dissolve PS in DEE, which is a bad solvent for PS, 0.5 mg PS was vigorously vortexed, carefully heated up and sonicated in 1 mL DEE. The frozen gels with dried particles were wetted with DEE at -20 °C. DEE spread across the frozen surface and after some minutes PS in DEE was added. Various bottom patches between 60° and larger than 90° were seen. Gels prepared at -20 °C with pre-wetted particles showed marginally more particles with a bottom patch smaller than 90° than not wetted samples.

As DEE is badly mixable with water, polymer could also be pipetted on top of dried particles at room temperature, resulting in samples either with drops on top of the colloids (samples without surfactant), or completely covered particles (with surfactant). Nevertheless, some bottom patches were observable, although around 90°. It is clearly seen in Figure 23 that the polymer did not wet the bottom side of most particles but formed lenses on top. This might be an indication that the surface tension of DEE without surfactant at room temperature is still too high and therefore exhibited the Cassie-Baxter state (see Figure 22).

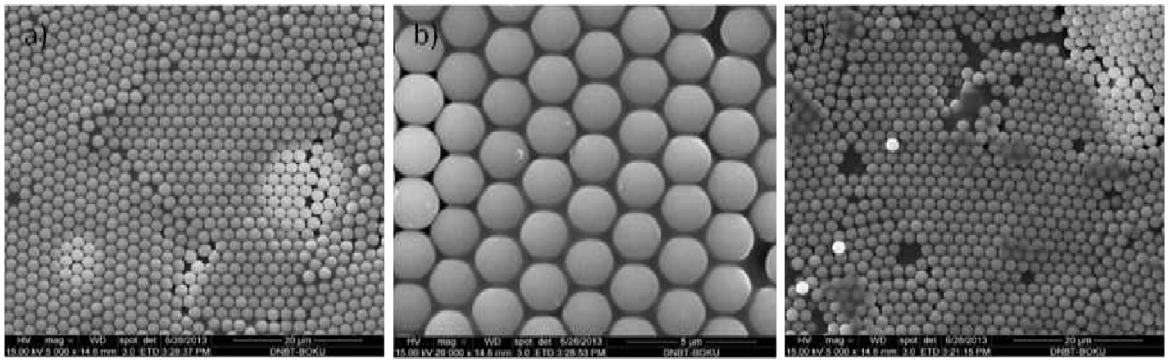

Figure 23: PS (1 µm) was dissolved in DEE and pipetted on dried particles at room temperature. a and b: bottom side of the sample; c: top side of the sample. The polymer did not spread over the whole sample surface but concentrated in drops. The polymer solution presumably were found to be in a Cassie-Baxter state on the particle surface not wetting the particles equally.

Based on the assumption that the dried particles form a superhydrophobic surface the fact that more bottom patches were achieved at -20 °C than at room temperature, may indicate a temperature-depending wettability of the surface. The contact angle of a water droplet on a superhydrophobic surface decreases with decreasing temperature, as water vapor from the drop can condense into the air pockets of a superhydrophobic surface, upon cooling (38).

In parallel, experiments with PMMA were carried out. As this polymer is not soluble in diethyl ether it was dissolved in acetone, tetrahydrofuran, ethyl acetate, chloroform or methyl ethyl ketone. The polymer in acetone formed many small droplets between the colloids. Although the polymer free regions of the particles were quite small (~50°) and the polymer seemed to be evenly distributed, the structure was not dense enough to prevent later binding of APTMS (Figure 24). In addition, it must be pointed out that all samples prepared with acetone only generated membranes difficult to peel off the dried gels. It seemed as if the polymer and particles moved into the agar-agar gel instead of forming a composite membrane on top. Analysis with the fluorescent microscope of all samples showed completely fluorescent particles and also very fluorescent polymer, although with SEM patches were observable. The polymers used so far, were presumably not adsorbing strongly enough on the surface of the silica particles to avoid APTMS from binding on the whole colloid surface.

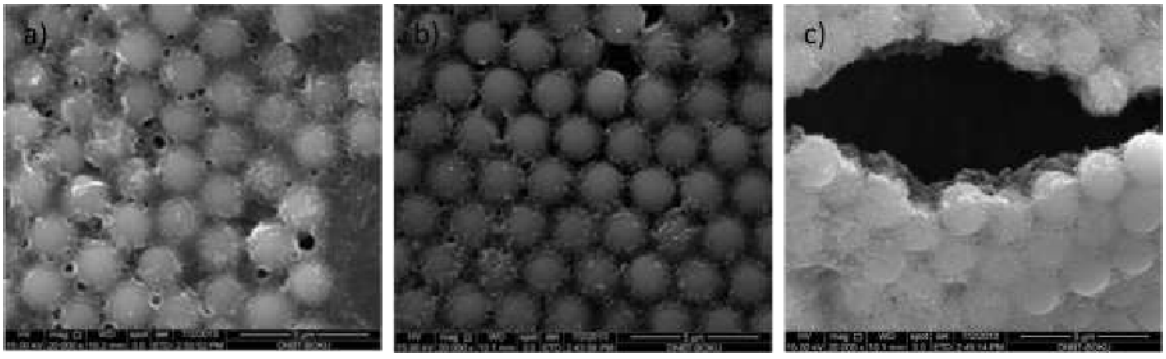

Figure 24: PMMA (1 µm) dissolved in acetone and pipetted on dried particles at room temperature. a: SEM image of the top patches; b and c: bottom face of the sample. The polymer formed a relatively thick membrane with many smaller wholes and wrinkles. The particles protruding from the polymer membrane showed patches around 50°

Therefore, another polymer poly(4-vinylpyridine) (P4VP) was used. The nitrogen of the pyridine forms a hydrogen bond with silanol groups and thus adsorbs on silica very well (39). As P4VP in ethanol covered the whole silica surface, the polymer was mixed with PS

or PMMA dissolved in DCM to eventually reduce the affinity a bit, showing similar results. The co-polymer P4VP-PS dissolved in DCM or chloroform did also not yield in a promising outcome.

Mixtures of co-polymer and additional PS in DCM in various concentrations (20–80 %) on top of dried particles at -20 °C or at room temperature were not leading to a satisfactory outcome either. Best results were achieved with a 50 % mixture of co-polymer and PS (M_w 192 000) with MADQUAT (0.1 mg * mL^{-1}) on top of dried particles at room temperature. Many top patches with an opening angle of 50–60° were observable, whereas other particles were completely covered with a polymer membrane. The bottom patch of the particles was either around 90° or larger. The co-polymer was not soluble in any solvent with a lower surface tension. Hence the polymer mixture was presumably in a Cassie-Baxter state and did not wet the bottom side of the particles. An improvement could be obtained by sonicating the samples immediately after the polymer solution was applied on top of the dried particles at room temperature for several seconds. Bottom patches were observable at SEM between 45° and larger than 90°. The appearance of the top sides of the particles varied a lot. Some particles were covered with the polymer whereas others showed a very small patch or one about 90°. Some of the found patches exhibited irregular shapes. Sonication of samples prepared at -20 °C in an ice water bath for 60 sec resulted also in a higher occurrence of bottom patches. However, those patches were of different sizes and irregular shape. Furthermore, the top side of the particles was often completely covered with polymer.

An even better result was obtained by preventing the polymer solution from evaporation for a certain time by putting a lid on the glass petri dish. After a soaking time of only 20 min slightly improved results were achieved for the bottom patches. More particles showed a bottom patch around 90° and did not look entirely polymer free. The top patch of the colloids was still most of the time 0°. Wetting the particles first with a solvent-surfactant solution (MADQUAT 0.1 mg * mL^{-1}) for 5 h and then allowing the polymer solution to wet for another 0.5 h at -20 °C resulted in some bottom and top patches around 55°, although similar to the sonication experiments the shape and size of the patches was mostly irregular. It is worth mentioning that the bottom side of the

particles was sometimes completely covered with polymer when wetted for several hours.

In order to gain a flatter gel surface and thus a more even size distribution of the patches, the purer gelling agent agarose was used, instead of agar-agar. The membranes were easier to peel off the agarose gel than off the agar-agar gel, after freeze-drying. It was also noticed that the amount of agarose in the petri dishes was critical for a satisfying result. A low amount of agarose in the petri dish (2 mL in the d = 2.9 cm petri dish) led to shrinking of the gel after lyophilisation, impeding to peel off the membrane. Almost no particle-polymer membrane on the dried gel was found by the application of great amounts of agarose (≥ 4 mL), which may be caused by a high portion of water in the substrate. Thus, sublimation during freeze-drying may disturb the membrane preservation.

To further decrease the occurrence of a Cassie-Baxter state, PS of a molecular weight of either 1 000 Da (PS 1) or 35 000 Da (PS 35) dissolved in DEE was used. The surface tension of PS decreases with decreasing molecular weight (40). Wetting with DEE for several minutes resulted in better results than achieved in any previous experiment. Preventing the solvent for 3 h and afterwards the polymer solution for 0.5 h from evaporation led to samples with large pieces of composite membranes with only a few irregularities (Figure 25 a and d). However, the size, amount and also the shape of the patches differed from each other. The size and shape of the particles was probably influenced by the gel surface and the particle arrangement. The shape of the patches seemed to be circular if particles arranged hexagonally close-packed, whereas it seemed to be square-shaped when the particles formed a square packed structure (Figure 25 e). If particles organize i.e. in a double layer the particles of the top layer may have only a top patch or no patches at all (Figure 26). The patch sizes probably also differed because of the agarose gel surface. It did not have a perfectly smooth and flat surface, but formed small pores and grooves. So, the particles were not all on the same height level when the polymer layer was added (Figure 25 f). Most bottom patches that could be measured were between 38° and 90° and the top patches between 37° and 63°.

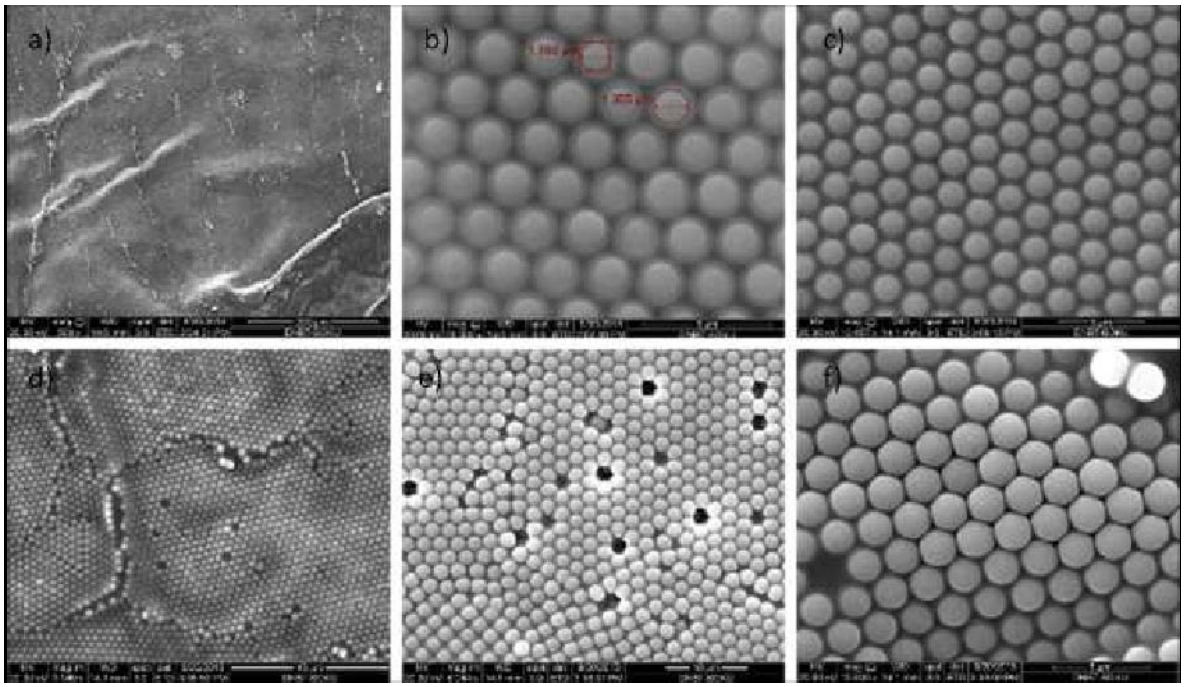

Figure 25: PS 1 dissolved in DEE and pipetted on dried particles wetted for 3 h with DEE at -20°C. The amount of polymer was calculated to form patches with an opening angle of 40°. a–c: top side of the particles; d–f: bottom side of the particles. Large areas of monolayers sticking in a PS membrane, showing only a view detects, could be obtained. The opening angles of the patches varied from particle to particle.

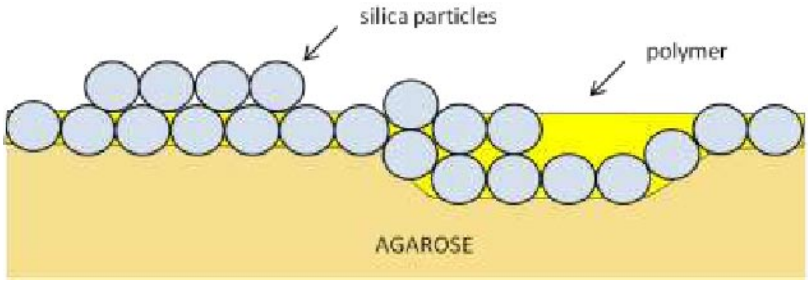

Figure 26: The particle arrangement and the gel surface influence the size and amount of patches on a particle. A groove in the gel surface might lead to a covered top side of the particle or a small top patch. A double layer might lead to entirely polymer-free particles or to particles with only a top patch.

Experiments with PS (M_W 192000 Da) and a wetting procedure of 6 h showed better results than obtained without wetting. Many top and bottom patches around 60° were found. If particles were wetted too long, i.e. overnight, the polymer was also found underneath the particles leading to some colloids looking out on one or both sides of the polymer, whereas others were entirely covered with polymer (Figure 27).

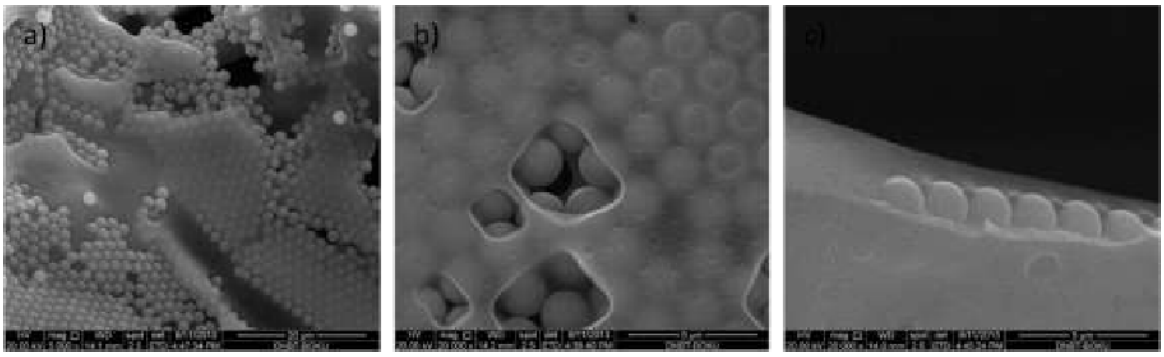

Figure 27: PS 35 dissolved in DEE and pipetted on dried particles wetted over night with DEE at -20 °C. The amount of polymer was calculated to form patches with an opening angle of 40°. a and b: images from the air exposed side; c: image form the agarose exposed side. The polymer was also found to cover the bottom side of many particles, leading to some particles completely surrounded by PS. Other particles showed one or two patches.

In summary, a prerequisite for creating a required composite membrane by applying a polymer solution on dried colloids was a uniform, flat substrate on which large 2D crystals of particles can assemble. A 2 % agarose gel showed good results. The hypothesized Cassie-Baxter state of the solvent-polymer solution on a monolayer of particles could be reduced by:

- performing the experiments at low temperatures
- using a solvent with a very low surface tension
- wetting the monolayer of silica particles before applying the polymer-solvent mixture
- using low molecular weight polymer

Best results were achieved by applying PS in DEE on pre-wetted particles at -20 °C. The composite membrane was easy to peel of the freeze dried gel and many particles showed a top and bottom patch around 60° or smaller. DEE is a solvent with a low surface tension (37) and PS does not adsorb on silica well enough to cover the complete particle surface. Using lower molecular weight PS (1 000 Da and 35 000 Da) reduced the wetting time (3 h) needed for a satisfying outcome, compared to PS of a molecular weight of 192 000 Da (6 h). The generation of a PS (192 000 Da) membrane with silica particles protruding from it, is schematically shown in Figure 28.

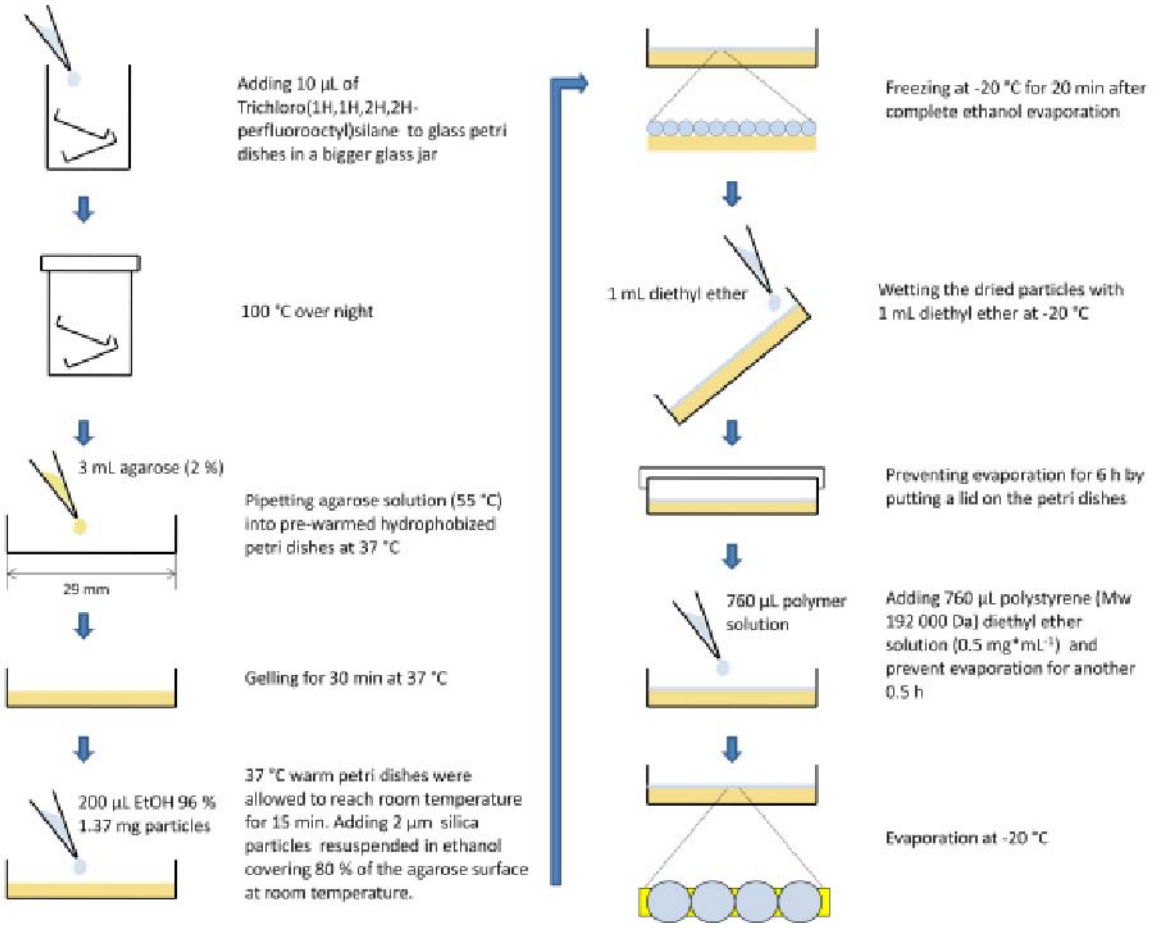

Figure 28: Applied method to embed silica particles in a polystyrene membrane. Agarose gels (2 %) were prepared at 37 °C in glass petri dishes (d = 29 mm), hydrophobized with trichloro(1H, 1H, 2H, 2H-perfluorooctyl)silane. Commercially, relatively monodisperse particles were washed two times with ethanol and dried under a gaseous nitrogen stream before they were resuspended in ethanol and sonicated for 15 min. The amount of particles covering 80 % of the gel surface was spread on top of the agarose gel. After ethanol evaporation the gels with mostly hexagonally closed-packed particle monolayers were frozen at -20 °C. After pre-wetting with DEE, the amount of PS in DEE forming a polymer membrane embedding particles with patches of α = 40°, was added. After evaporation composite membranes of silica particles and polystyrene were achieved.

3.2. Chemical modification of the patches

The idea of synthesizing inverse patchy colloids was to chemically modify the polymer free patches in a way that they expose a positive charge whereas the equatorial region of the particles sticking in the polymer is by the nature of silica negatively charged. The positive charge should be obtained by chemically linking APTMS, a silane, with a positive charged amine group to silica. To visualize possibly inverse patchy colloids the particles were dyed with RITC, which should only bind to the reactive amine groups of APTMS and should make only the patches fluorescent. Afterwards, it was tried to dissolve the polymer, in which the particles were embedded, and the samples were analyzed with the fluorescent or confocal microscope.

To begin with, APTMS was applied on the patches by vapor deposition. The membranes were peeled off the agar-agar gels and were put in small glass vessels, which in turn were placed in a bigger glass jar. 10 µL of APTMS was added and the closed jar was put in the oven at 100 °C for 8 h. The membranes were allowed to cool down and 10 mL of RITC solution (one spatula tip of RITC in 100 mL of absolute ethanol) was added. After several hours the samples were washed with ethanol until the supernatant became colorless. Attempts to dissolve the membranes in various organic solvents (acetone, toluene, dichloromethane, chloroform, tetrahydrofuran) failed. Fluorescent and confocal microscopy revealed that mostly the polymer but also the polymer free particle surface was completely fluorescent. So, it remained unclear if the particles had patches or not. The few single particles that could be observed were entirely fluorescent and probably had not been in a membrane before APTMS was added. Membranes exposed to 100 °C for 8 h without the addition of APTMS were still soluble in i.e. DCM. Therefore, it was assumed that APTMS binding to the polymer molecules made the membranes insoluble.

As a result another method was performed to link APTMS to silica. Small sample pieces were put in 1 mL distilled water or ethanol and then APTMS in various concentrations (1, 0.1 or 0.01 %) for different exposure times (5 or 10 min) was added either at 4 °C or at room temperature. The reaction was stopped by adding a few drops of ammonia. The specimens were spun down and washed four times for 15 sec at 13400 rpm with ethanol to remove surplus APTMS. To dissolve the polymer, samples were vortexed and sonicated for 30 min with 400 µL DCM. As the density of DCM is higher than that of PS, 800 µL of acetone was added to reduce the density of the solvent. For that, not yet dissolved polymer was not swimming on top of the solvent, but formed a pellet after centrifugation. The pellet was resuspended in DCM and the procedure was repeated twice, but the membranes did not dissolve. Only a few more single particles not showing clear patches could be observed.

Based on the assumption that the polymer network is not dense enough and that the polymer does not adhere well enough to silica to avoid APTMS from binding between the polymer molecules and between silica and polymer, a filler material to the composite membranes was added to stuff any possible gaps. Samples with the polymer polystyrene were put in tubes with 1 mL ethanol. The monomer styrene (0.001 %) was added and

stirred for 1 h. The samples were washed three times with distilled water. Afterwards APTMS (0.001 %) was pipetted to the specimens at room temperature and the reaction was stopped after 1 min with ammonia. The samples were washed four times with distilled water (15 sec; 13400 rpm) and exposed to the fluorescent dye in absolute ethanol overnight. After washing with ethanol three times, parts of the polymer membranes were soluble after carrying out the above described dissolving procedure. Best results were obtained with particles that were embedded in a polystyrene (M_W 192 000 Da) membrane by dissolving the polymer in DEE and applying it on dried particles at -20 °C after 6 h pre-wetting. Although, many particles were still stuck in the polymer, some single particles with a clearly non fluorescent equatorial region could be observed by confocal microscopy (Figure 29 a). Some particle clusters were found (Figure 29 b), where it was not clear if particles were still stuck in polymer or if they aggregated after polymer dissolution due to attractive electrostatic forces. In contrary to expectations, the patches were larger than seen with the SEM (60–80°). An explanation might be the lower resolution of the confocal microscope than the SEM. In addition polystyrene might not adhere well enough to silica and styrene might not fill the gaps, so that APTMS could bind to a larger silica surface than hoped after SEM analysis. In accordance with observations from SEM, the patches of the particles were very polydisperse.

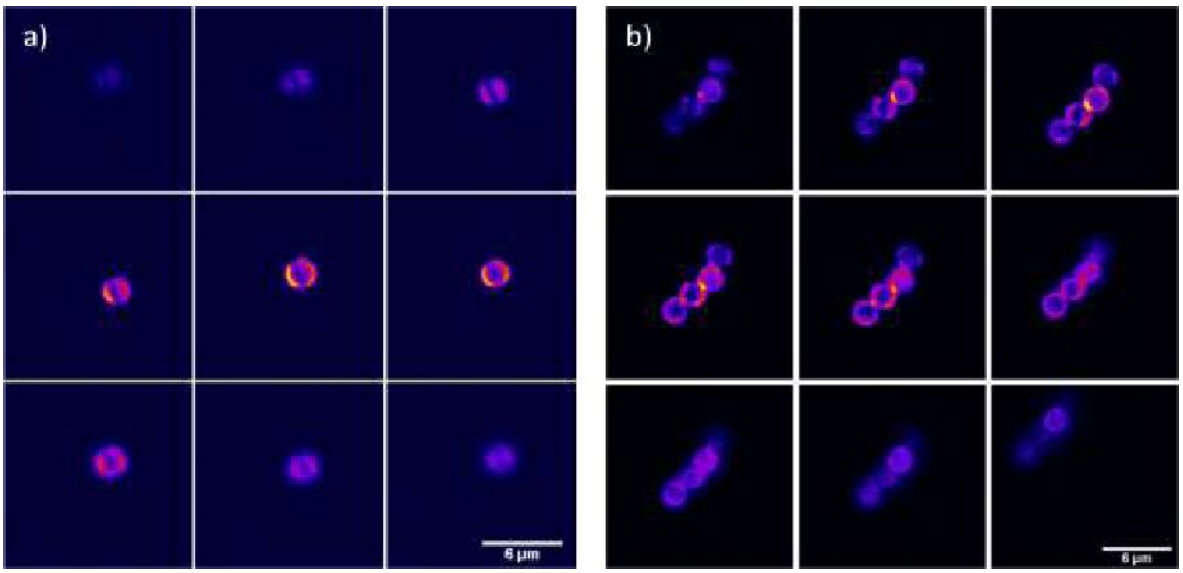

Figure 29: IPCs with two fluorescent patches, obtained by embedding silica particles in a polystyrene membrane (M_w 192 000 Da)

In conclusion, APTMS could not be applied on the polymer free parts of the particles by vapor deposition or by simply adding the chemical to the membranes in water or

ethanol. The achieved PS membrane (chapter 3.1.1.2) was not dense enough, wherefore APTMS could bind between the polymer molecules leading to completely fluorescent and insoluble membranes. This effect was significantly reduced by adding the monomer styrene as a filler material to the membranes in ethanol before treating the samples with APTMS. Best results were obtained with membranes consisting of PS 192 000 Da, as they seemed to be denser than the lower molecular weight PS membranes. After additional binding of RITC some particles could be obtained with two clearly fluorescent and therefore positively charged patches. The procedure of chemically modifying the polymer free regions of the silica particles is schematically shown in Figure 30.

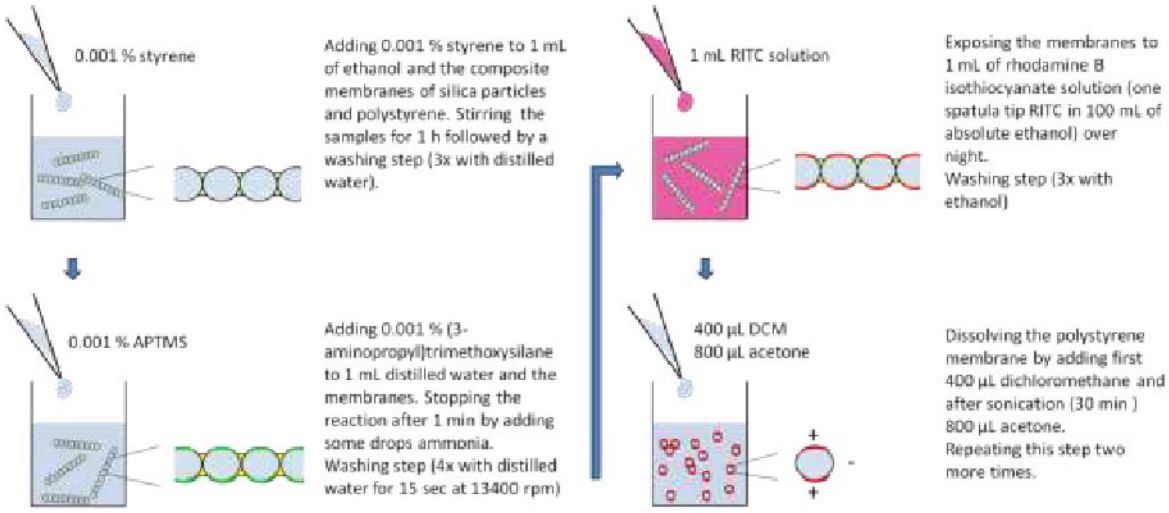

Figure 30: Applied method to positively charge the patches of silica particles sticking in a polystyrene membrane. The gaps between silica particles and PS were filled with styrene, so that APTMS only binds to the patches. Afterwards, the patches were modified with APTMS to which RITC is linked. Therefore, the patches became positively charged, whereas the equatorial region sticking in the polystyrene membrane remained negatively charged. By dissolving the membrane, free inverse patchy colloids were achieved.

4. Conclusion

For the first time, a method to synthesize inverse patchy colloids (ICPs) with two positively charged polar caps and an equatorial region of the opposite charge has been developed. The equatorial regions of 2 µm silica particles were masked by a PS membrane leaving the polar caps free of polymer and accessible to a modification with APTMS and RITC. The several steps of the synthesizing procedure are schematically shown in Figure 28 and Figure 30 describing the procedure of embedding particles in a polymer membrane and the chemical modification respectively. Best results were obtained by pipetting a solution of particles and ethanol on top of an agarose gel surface (2 %). After solvent evaporation, monolayers of particles were achieved. Thus, the agarose-exposed sides of the particles were partially covered by water contained in the gel, determining a minimum bottom patch size. To mask the equatorial regions, particles were pre-wetted for 6 h with DEE. PS (M_w 192 000 Da) in DEE was spread over pre-wetted colloids at -20 °. After subsequent evaporation of DEE, PS membranes with particles penetrating the polymer on both sides were obtained. Those membranes could be easily peeled off from the gels after lyophilization. The polymer-free sites of the particles were modified with APTMS, whereafter they exposed amine groups, which could be linked to the positively charged fluorescent dye RITC. PS was dissolved, leading to free IPCs, which were analyzed by confocal microscopy. With this method it was possible to produce polydisperse IPCs with relatively large patches (60–80°). The two patches on one IPC vary in size and differ from the patch sizes of other IPCs. Nevertheless they can serve as a preliminary material to investigate the self-assembly behavior of IPCs.

The substrate surface influenced the outcome of this method significantly. A 2 % solid agarose gel had as a substrate a great advantage in comparison to water or a more liquid gel, because it wetted the bottom part of the particle surface without losing hydrophilic particles to the liquid phase. Therefore, large 2D crystals of particles could be achieved. However, a disadvantage of a 2 % agarose gel was the occurrence of grooves and irregularities on the surface, probably causing the polydispersity in the patch sizes of the produced IPCs. Another crucial factor for the success of this method was the right choice of polymer solution, which replaced the use of monomer during the course of this work due to polymerization problems. The polymer should adhere well enough to silica to

prevent binding of APTMS to the whole silica surface but weak enough that the polymer does not cover the whole particle surface. The solvent for the polymer should have a low surface tension to reduce the hypothesized Cassie-Baxter state of the polymer solution on the particle monolayer. PS in DEE turned out to be a good option, although additional styrene had to be added to the membrane to fill gaps between the PS molecules and silica particles and PS.

5. Outlook

The finally produced IPCs can be used for a preliminary study of their phase behavior under different solvent conditions. To experimentally verify the theoretical model of how IPCs arrange in dependence on their overall charge, patch size and substrate charge (18), more monodisperse IPCs with specific patch sizes are needed. This might be achieved by either further improving the synthesis procedure itself or by separating the developed IPCs according to their patch sizes in several fractions. The latter might be realized by *Fluorescence Activated Cell Sorting*, a technique originally used in cell biology. Small droplets, containing only one particle, are generated from a flow of a particle suspension by vibrations. A measuring unit identifies the isolated, fluorescent particles and charges them according to the measured value. The particles are then sorted by deflection plates and can be collected in different vessels(41).

Altering the evolved method could include for example a change to a possibly smoother substrate like gelatin or carboxymethyl-agarose, which has a narrower gel network and smaller pores than underivatized agarose (42). In addition the use of carboxymethyl-agarose possibly leads to smaller bottom patch sizes due to a repulsion of silica particles from the negatively charged substrate surface. Another improvement of the synthesis technique might be the application of the polymer solution at even lower temperatures than -20 °C. The contact angle of water on a superhydrophobic surface decreases with decreasing temperature (38), which might be also the case for DEE. Thus, the hypothesized Cassie-Baxter state of the polymer solution on the dried particles below -20 °C is potentially further decreased and IPCs with smaller bottom patches might be achieved. As the bottom patches observed by SEM were mostly larger than the top patches, it might lead to more equal sized patches on one IPC. It possibly also leads to a more similar size distribution between the various IPCs, as the particles are wetted more uniformly. With some amendments to the described synthesis method it is probably possible to produce monodisperse IPCs, which can help to understand self-assembly processes of systems with heterogeneously charged components.

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