Extending in situ liquid phase transmission electron microscopy to etching of silica and the swelling-collapse of temperature sensitive polymer microgels

Albert Grau-Carbonell
Extending *in situ* liquid phase transmission electron microscopy to etching of silica and the swelling-collapse of temperature sensitive polymer microgels

Uitbreiding van *in situ* vloeistoffase transmissie-elektronenmicroscopie naar het etsen van silica en het zwellen en ineenkrimpen van temperatuurgevoelige polymeer microgels

(met een samenvatting in het Nederlands)

Proefschrift

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Albert Grau Carbonell

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Promotor: Prof. dr. Alfons van Blaaderen
Copromotor: Dr. ir. Marijn A. van Huis

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Cover: Artistic representation of a dedicated liquid cell TEM holder.
In the field of observation, chance favors the prepared mind.
Louis Pasteur
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Chapter 1

Introduction

1.1 Origins of electron microscopy

Seeing is believing. Or sometimes, seeing is discovering. Through the study of the natural world, scientists realized that the understanding of many fields would come from the observation of reality at different length scales. The quest to develop instrumentation able to image very small or distant phenomena has led to a myriad of technological advancements. Of such developments, we will focus here on electron microscopy as it was the technique of choice for most experiments described in this thesis.

Back in the late 1500s efforts to observe nature beyond the capabilities of the naked eye started. Although lenses had been used to manipulate light, for instance to correct vision, they were not used in scientific endeavours due to the low magnifying power of the lenses of the moment. In those times in the Netherlands Zacharias Janssen and his father Hans Jenssen started stacking lenses to improve the total magnifying power of optical set-ups, but such compound set-ups did not reach relevant magnifications. It would be Antonie van Leeuwenhoek that would improve the magnifying power of a lens up to 270x magnification, which earned him the title of Father of Microscopy. This jump in magnifying power set the beginning of the use of lenses to observe nature’s tiniest details. A copy of such first microscopy crafted by van Leeuwenhoek’s hands is on display at the University Museum in Utrecht.

Microscopy techniques were developed further and by the 1900s the optical limit dictated by light diffraction was about to be reached. The development of magnetic lenses by Hans Busch led Ernst Ruska and Max Knoll to develop in 1931 the first transmission electron microscope (TEM), which would achieve resolutions higher than light, by transmitting electrons instead of light through a sample. The first commercial TEM was launched in 1938 (Siemens) but it would take until 1942 for Ruska to develop the electronics needed to be able to precisely scan a sample with a focused electron beam and collect and quantify the resulting signal generated by scattered electrons, producing the first scanning transmission electron microscope (STEM). With the introduction of digital cameras and image processing in 1986 by a Japanese lens company, light microscopy and electron microscopy became pillars of the scientific enterprise.

1.2 Electron microscopy in liquids

Nowadays, most TEM imaging is performed on samples suspended in vacuum. A thin sample is prepared and introduced in a vacuum chamber where an electron beam passes through the sample. Although this configuration allows imaging with optimal resolution, it limits the ability of conven-
tional TEMs to observe liquid phase processes at the nanoscale since most liquids rapidly evaporate in vacuum. Efforts have been made to keep liquids from evaporating inside TEM chambers. A simple strategy is to drop-cast liquids with low vapour pressures (in the range of a few Pa) able to withstand evaporation inside a conventional TEM, such as ionic liquids. Unfortunately, this approach strongly limits the array of liquids one can study. Environmental TEMs (ETEM) are TEMs with a system of pumps and apertures to gradually change the pressure along the column, resulting in increased pressures around the sample. ETEM widens the list of liquids that one can image but still shows strong limitations with common high vapour pressure liquids (e.g. water, isopropanol, vapour pressures in the range of kPa). Encapsulation of liquid is then the strategy of choice for most in situ experiments, as it allows working virtually with any liquid chemically compatible with the confining cell. Two main technologies have been developed to encapsulate liquids inside the TEM high vacuum: Chip-based Liquid Cells (LC) and Graphene Cells.

In this thesis all in situ liquid phase experiments have been performed by means of chip-based LC transmission electron microscopy (LC-TEM, Figure 2.3). Standard LC-TEM set-ups encapsulate the liquid between two silicon chips assembled in a dedicated liquid cell holder. The microchip-encapsulated liquid is imaged through two silicon nitride (Si$_x$N$_y$) imaging windows. Such windows are thin enough to be almost electron-transparent but robust enough to withstand the pressure difference between the liquid and the high vacuum of the microscope. The thickness of the liquid sample is determined by the silicon-chip architecture. Typically one of the chips contains spacers with precise thickness that to a large extent determine the resulting separation between chips. Dedicated liquid cell holders are typically equipped with microfluidic capabilities allowing researchers to flow liquids through the cell in real time during experiments. State-of-the-art holders also incorporate electronic capabilities allowing features varying from heating of the liquid volume to small-scale electrochemistry experiments.

Every microscopy technique comes with its own limitations and LC-TEM is no exception. Electron-sample interactions are always relevant in EM and that is even more true for LC-EM. Electrons do not only interact with the micro- and nano-structures of interest but also the surrounding liquid media, forming radiolysis radicals and further adding complexity to the interpretation of LC-EM observations. The increased scattering through the liquid also imposes limits in resolution and decreases signal-to-noise ratios, specially at low electron dose rates.

### 1.3 Electron beam-sample interactions

Interactions between the incident electron beam and LC-EM samples are of paramount importance to optimal experimental design and interpretation. When electrons of an EM beam interact with a sample, elastic and inelastic interactions between electrons and atoms/molecules happen. Such interactions can lead to direct and indirect damage to samples of interest. Direct damage is characteristic of all EM observations at sufficient electron beam intensities (material dependent). For example, electron beam illumination can lead to the amorphisation of crystalline samples or the crystallisation of amorphous samples. When samples are imaged while immersed in liquid one must also account for the so-called indirect beam effects. Electrons interacting with the liquid media can create radiolysis radicals, which are typically active chemical species, that can interact with the material in the imaged area and diffuse and interact with all the surroundings. The possibility of generating active radical species on demand via electron beam illumination and observing teh resulting process in situ and in real time also provides unique research opportunities (e.g. reduction of metal ions in solution into nanoparticles). Depending on what liquid one is experimenting with, different radicals will be formed resulting in unique chemistry conditions. Multiple instances
of water radiolysis products being the main drivers of LC-EM observations will be found along the pages of this thesis (Chapters 3-5).

1.4 Colloids and nanoparticles

Colloidal systems are composed of particles with sizes ranging from a few nanometers ($10^{-9}$ m) up to micrometers ($10^{-6}$ m) dispersed in another medium and thus are small enough to undergo Brownian diffusion in it. Nanoparticles (NPs) on the other hand are particles ranging from a few nanometers up to around 100 nm. Although the size range of NPs is included in the size range of colloids, both categories are not the same. Nanoparticles can form colloidal dispersions if they are, for instance, dispersed in a solvent in which they are stable. However a system of NPs embedded in a solid matrix, or dried on a surface, would not be considered a colloidal system. Contrary to the common intuition that colloidal and nanoparticulate systems are advanced technological materials, they are extensively found in nature: Milk is a colloidal system consisting of protein and fat colloidal droplets dispersed in water, opals are stones made of ordered silica nanoparticles and the bright blue color of some butterflies arises from the reflection of specific wavelength of light with natural arrangements of nanoparticles on their wings. Colloids are, then, an everyday discreet companion. For instance, the use of nanomaterials by humanity, although in naive unawareness, dates back to the Iron Age when ceramics were mixed with asbestos fibers.

When the particle size diminishes, a relatively larger amount of material is exposed at the surfaces and the properties of materials switch from being dominated by the bulk properties of the material to its surface properties. Furthermore, below a particular limit size effects will also come into play (e.g., quantum confinement effects). Particles in the nano and colloidal range thus exhibit certain unique properties that make them interesting for many fundamental studies and an enormous variety of applications. For instance, colloids and nanoparticles dispersed in a liquid will undergo Brownian diffusion due to thermal fluctuations. If inter-particle interactions are also of the range of the thermal energy, particles will diffuse and interact until a thermodynamic equilibrium is reached.

1.5 Self-assembly (SA)

Self-assembly (SA) refers to the spontaneous ordering of a system consisting of a number of well defined components. SA happens at all length scales in nature, from the SA of biostructures inside a cell to the formation of inorganic materials to the formation of opals from silica nanospheres. In order for SA to happen, the building blocks need to be sufficiently monodisperse and interact weakly enough to not aggregate irreversibly. When these conditions are met, and under the right circumstances (e.g. pressure, number particle density), the building blocks will reach a given equilibrium state with positional or/and orientational order. The way a system self-assembles will reflect the properties of the building blocks: cubic colloids will SA into cubic crystals, while rod-like colloids will SA into phases with long range order in rod orientations and positions. The latter are known as liquid crystal (LC) phases. The reflection of the building-block characteristics on the SA, together with the wide range of shapes that can be achieved with colloidal synthesis, allows for the generation of many interesting SA structures. An example of the interplay between the structure of colloids and their SA can be found in Chapter 6.
1.6 Thesis outline

This thesis starts with a short review of the main features of transmission electron microscopy with a focus on liquid cell transmission electron microscopy (Chapter 2). Not only the operation of the microscopes and the liquid cell set-up is outlined, but beam-sample interactions and water radiolysis are addressed as well.

In Chapter 3 we develop a general method to image the wet etching of silicon dioxide (silica, SiO$_2$) nanoparticles in situ via LC-(S)TEM. We have characterized electron-beam effects on silica nanoparticles in water and under etching conditions (sodium hydroxide, NaOH). We identified an expansion and a dissolution beam-induced phases that became more pronounced under etching conditions. Through electron trajectory simulations we related the expansion phase to the cumulative electron dose on the imaging area, while the dissolution phase appeared to be driven by the electron dose rate. We applied our results to determine the best imaging conditions to image silica in water and in a basic aqueous solution, which consisted of minimizing the electron dose rate and the use of blanking windows to minimize the cumulative electron dose. We also showed that these conditions impose the limit of how small the nanoparticles a given TEM can be imaged. We then characterized the etching process in aqueous basic media of Stöber and water-in-oil reverse microemulsion (WORM) silica nanospheres, as well as core-shell Au@Stöber and Fe$_2$O$_3$@WORM silica nanospheres in situ with nanometer resolution.

In Chapter 4 we apply and expand the knowledge gained in the imaging of the wet etching of silica to larger, colloidal rod-like silica particles via LC-(S)TEM. We characterized the progression of the base etching (NaOH) of microscopic silica rods into silica cones at the single particle level and at nanometric resolution. We observed that the larger size of silica rods compared to silica nanospheres imaged in Chapter 3 imposed an additional optimization step, since high flow rates of liquid through the cell resulted in drag forces on the rods sufficient to detach them from the imaging windows. Furthermore, we showed that the closed geometry of the liquid cell can affect the etching process compared with that of ex situ etching of rods adhered to a surface instead of immersed in a bulk etching solution. The preferential etching of less-condensed parts of silica particles was demonstrated by etching segmented silica rods with consisting of segments with different well-defined condensation degrees. This method was then expanded to explore the internal condensation degrees of a recently developed system of bent-core silica micro-rods.

In Chapter 5 we explore the feasibility of LC-(S)TEM to study the thermoresponsive and co-nonsolvency behavior of Poly(N-isopropylacrylamide) (PNIPAM) microgels and shells in situ with nanometer resolution. PNIPAM is a polymeric material known to undergo a phase transition when the temperature of the surrounding water medium is above a particular threshold, known as the lower critical solution temperature (LCST). Furthermore, it is known that PNIPAM has a co-nonsolvent behavior for alcoholic aqueous solutions with certain alcohol molar fractions. We first determined optimal imaging conditions and electron beam induced effects. We showed that PNIPAM microgels irreversibly collapsed under (S)TEM imaging in water which could be partially prevented by the addition of isopopanol as scavenger of radiolysis radicals. We propose that PNIPAM shrinkage under (S)TEM imaging is mediated by an increase of the internal cross-linking of the microgels due to the reaction of the polymeric chains with water radiolysis radicals. Importantly, some parts of PNIPAM microgels were not resolvable due to their low polymer volume fraction. Attempts to resolve such volumes by changing the solvent composition, as well as decorating the surface of the microgels are discussed. The decoration in situ of the microgels with high contrast nanoparticles was the strategy of choice, which allowed us to successfully characterize the thermoresponsive behavior of PNIPAM and hybrid core-shell silica@PNIPAM microgels. Furthermore, the co-nonsolvent behavior of PNIPAM microgels for methanol/water mixtures was also imaged.
In Chapter 6 we switch topic and technique, and we describe the SA of fluorescent bent-core silica rods (which were also studied for other reasons in Chapter 4) into LC phases via confocal scanning laser microscopy. The introduction of bending in the colloidal building blocks of the liquid crystals results in the formation of bend deformations of the LC phase. These bend deformations cannot extend over long distances and are resolved with the formation of twist or splay deformations, resulting in twisted ($N_{TB}$) or splay ($N_{SB}$) bent nematic phases. In this chapter we describe the identification of a $N_{SB}$ phase from such bent-core silica rods in a sediment in a sedimentation-diffusion equilibrium. These phases were found between a smectic ($Sm$) and a nematic ($N$) phase. Interestingly, the characterization of such phases revealed that the pitch of the identified $N_{SB}$ phases was several times the length of the colloidal building blocks. A $N_{SB}$ phase was also identified in a sediment of hematite-functionalized bent silica rods offering the possibility of manipulation by external magnetic fields.
Chapter 2

Liquid Cell Electron Microscopy and Colloidal Self-Assembly

The purpose of this chapter is to develop LC-EM concepts to familiarize the reader with this technique and its main limitations. First, the operation of a conventional Scanning Transmission Electron Microscope ((S)TEM) is described. Then the unique features of liquid phase EM are outlined. Electron radiation-matter interactions are briefly reviewed with a focus on water radiolysis, water being the solvent of choice for most of the LC-EM experiments described in this thesis. Finally, a number of concepts in the theory of self-assembly (SA) are introduced as well.

2.1 The Transmission Electron Microscope

The basic working principle of transmission electron microscopy is simple: A beam of electrons is accelerated to high energies (typically 80 to 300 keV) and focused on a sample. Electrons are then transmitted through the sample, interacting with it and generating various types of signals (e.g. scattered electrons, X-rays).

A TEM consists of two main parts, an electron gun and a column, both working under high vacuum conditions (Figure 2.1). The electron gun, where an electron source is exposed to an accelerating system operating at high voltages, is commonly found at the top of the column. Electrons are extracted from an electron source in the gun by the application of high electric fields (hundreds of kV), which also accelerate them. This generates a beam of high energy electrons commonly referred to as the electron beam. Once electrons are accelerated, they enter the column in which the sample is suspended. Along the TEM column, a number of electromagnetic lenses focus and shape the electron beam on the sample. After the electrons are transmitted through the sample, another set of electromagnetic lenses focuses the outcomming electrons on the detector(s). Sometimes the back focal plane of the electron beam image is focused (for diffraction studies). The energy of the electrons that transmit through the sample is almost entirely determined by the acceleration voltage of the gun, as through the column only electron trajectories are changed while any energy losses are almost negligible in comparison to the total energy of the electrons. Depending on how the electromagnetic lenses are set and tuned, different beams shapes are obtained. Illumination of a sample by a broad, parallel beam is considered regular or bright field TEM, while the scanning of the sample with a focused electron beam is considered Scanning TEM ((S)TEM).
Figure 2.1: **Simplified schematic of the operation of a TEM** A TEM consists of an electron gun operating under high voltages. Electrons then enter the column, in which a first set of electromagnetic lenses, known as condenser lenses, shape and focus the electron beam on the sample. After transmitting through a sample, the electron beam is focused and shaped again on the cameras and detectors by another set of electromagnetic lenses, known as objective lenses.

The way an image is generated and constructed from the signal after electrons have passed through the sample is fundamentally different between TEM and (S)TEM (Figure 2.2). A regular TEM image (also Bright Field TEM, BF-TEM) is typically acquired by wide-beam illumination of an area of the sample and recording the image formed by the transmitted electron beam behind the sample. Contrast differences between the different elements in a sample arise from the different processes taking place between the electrons and the sample, which will be described later in this chapter. However, it is easy to imagine that sample regions that scatter electrons more strongly will have lower intensities on a recorded image, as less electrons from that part of the sample would arrive at the detectors. In most commercial TEMs, TEM signal collection is done with devices like charge-couple devices (CCD) in combination with a scintillator. Scintillators are materials that emit photons when hit by radiation (i.e. high energy electrons in TEM). When scintillators are coupled with sensors that can detect photons pixel-wise (like CCD devices) one can digitalize TEM
images. State-of-the-art detectors, such as direct electron detector devices (DDD), can detect single electrons and thus increase the ability to record high-quality and high-resolution TEM data. DDDs are installed in most state-of-the-art TEM as means to achieve higher resolutions with lower beam intensities. A (S)TEM image, on the other hand, is generated by scanning the sample with a focused electron beam and collecting the transmitted electrons per scanning point to determine the pixel intensity. Importantly, in (S)TEM imaging different signals can be collected at the same time. If the electrons that are detected and integrated per pixel come from the direct beam (beam with almost no changes in trajectory), the recorded image is named a Bright Field image (and the technique is coined BF-(S)TEM). If an annular detector is used to detect electrons scattered through a given range of angles, an Annular Dark Field image is generated (ADF-(S)TEM).

![Imaging modes for TEM](image)

**Figure 2.2: Imaging modes for TEM.** Bright Field TEM (BF-TEM) images are obtained by recording the image of a widely illuminating electron beam after transmittance through a sample. Bright Fields (S)TEM images are obtained by scanning a sample with a very focused electron beam and integrating per pixel the signal detected from the electrons that do not deviate much from their trajectories. Annular Dark Field (S)TEM images are obtained by scanning a sample with a thin electron beam and integrating per pixel the signal detected by an annular detector (ring shaped), that captures only electrons that scatter with higher angles (dashed line) and can reach the detector.

### 2.2 Liquid Cell Electron Microscopy

Imaging liquid samples with nanometer resolution with transmission electron microscopes has been a quest for many decades. Because TEMs operate in high vacuum conditions, low vapour pressure liquids such as water cannot be imaged at room temperature. Some liquids, on the other hand, have high enough a vapour pressure to withstand vacuum conditions, and among them ionic liquids have gained increasing attention in the last years.¹⁻³,²⁰,²¹ But the challenge to image liquids as a general electron microscopy technique should not rely on the nature of the liquid, and should
depend on it as less as possible. A direct approach widely applied to image "liquids" is Cryo-EM, a technique in which a thin liquid sample is snap-frozen and kept at cryogenic temperatures in the electron column, avoiding liquid evaporation. Cryo-EM has found immensely powerful application for instance in the characterization of biological systems (e.g. protein configurations).\textsuperscript{22,23} However, Cryo-EM cannot investigate dynamic processes happening in liquid, nor processes at non-cryogenic temperatures. A partial solution to the limitations imposed by the low column pressure is to build microscopes with differential pressure pumps to increase the pressure at the sample region (Environmental TEM, ETEM).\textsuperscript{4,24,25} ETEM allows for imaging of free-standing liquid samples with less strict limits imposed by the vapour pressure of the liquids. A strategy to prevent liquids to be exposed at all to the vacuum inside the TEM without compromising the ability of electrons to be transmitted is then needed. The encapsulation of liquids in sealed cells started by sandwiching liquid pockets in nitrocellulose, although the resolution was severely affected by these membranes. Since then, two main approaches have been developed where liquid thin layers inside TEMs were encapsulated with great success: chip-based and graphene-based liquid cells (LCs).\textsuperscript{25,26}

![Figure 2.3: Basic concepts of a chip-based liquid cell. A. A thin layer of liquid is sealed between two silicon chips a silicon nitride (Si$_x$N$_y$) imaging windows. B. The chips are loaded, aligned and sealed on a dedicated liquid cell TEM holder. C. Dedicated holders often include various capabilities, such as the ability to flow a solvent through the liquid volume \textit{in situ} and thus to control what solvent is present in the liquid cell.\textsuperscript{9}]

Chip-based LCs benefit from advancements in chip microfabrication, and enclose a liquid layer between two silicon chips sealed with o-rings and containing silicon nitride (Si$_x$N$_y$) imaging windows (Figure 2.3). These windows, one in each chip, consist of a thin layer (less than 100 nm thick) of low density and therefore almost electron transparent material and need to be carefully aligned during sample preparation. Nowadays, commercial LC chip systems, as well as dedicated holders to host such assemblies, are available. The specialization of TEM sample holders to liquid cell holders
(and a variety of other in situ holders like gas cell holders) comes with its own developments. Microfluidic capabilities allow the exchange of the solvent in between the chips in real time in situ. With most modern systems it is also possible to apply electric fields at the sample region. When this is coupled with Micro-Electro-Mechanical Systems (MEMS) technology embedded in the sealing chips, current can be exploited for many applications. For example, if electrodes are printed on a chip and currents or voltages are applied, experiments observing electrochemistry effects due to the electric potentials on electrodes is possible. Or, if resisting circuits are properly designed, local heating of the liquid volume can be achieved and controlled. Graphene LCs are typically made by encapsulating liquid pockets between graphene sheets. Sample preparation and sealing for graphene LCs typically consists of drop-casting of a supported graphene membrane, and the deposition of another graphene layer on top. Graphene layers seal onto each other, and thin pockets can be imaged with relatively optimal resolution, as graphene is also as thin as a membrane can be. In this thesis, chip-based LCs are used. Novel methods combine microfabrication and graphene sheets to achieve better resolutions with specific sample configurations and capabilities.

Along the development of LC-EM, the technique has been applied to a broad range of topics (Figure 2.4). Since the beginning it was observed that the illumination of solvents containing metal ions results in the reduction of such ions into solids, and allows the study in situ of beam-induced particle reduction and growth. Also etching processes can be followed with nanometer resolution for systems such as nanoparticles and nanostructures. The diffusion of nanoparticles in liquid has been a recurrent theme in the history of LC-TEM, from early puzzling observations showing slowed down diffusion, to the determination of 3D diffusion constants of nanoparticles. Bubbles, droplets and wettability phenomena have been investigated as well. The presence of gas bubbles in liquid cells also allows for the study of processes such as particle motion in ultrathin liquid layers. Soft matter has also received increasing LC-EM attention and diverse phenomena have been successfully observed (e.g. micelle formation, fusion and fragmentation). Biological samples, closely related to soft matter, have been explored as well. It is still unclear, however, to what extent living cells withstand the large amounts of radiation received during LC-TEM experiments. The possibility of introducing electrodes and controlled heating at the sample region has also brought many interesting studies to light, such as dendritical growth at electrodes upon the application of voltages, or thermoresponsive polymer assembly.

Properly describing experiments: representative entities for electron radiation

Given the sensitive nature of LC-EM observations to experimental conditions, there exists a need to define as well as possible the imaging conditions during LC-EM experiments. Most experiments can just be completely defined by reporting all parameters used during an experiment. For TEM experiments those would be the diameter of the beam and the current (intensity) of such beam. For (S)TEM experiments the pixel dwell time (time the beam is stopped at each scanning point), the intensity of the beam, the pixel size and the total number of pixels. However, there exist two physical entities whose magnitudes are commonly given in LC-EM experiments that combine some of these parameters and allow quick comparisons between studies: total electron dose (or simply electron dose) and electron flux (or electron dose rate). Electron beam effects are generally related to these two parameters, as described later in this chapter.

Electron flux/dose rate

Electron dose flux refers to the number of electrons that are transmitted through a sample per second per unit area:
Electrochemistry

- Electrode dendrite growth\textsuperscript{50,51}
- Electrode break down
- Corrosion

Materials growth and etching

- \textit{In-situ} reduction of metal ions\textsuperscript{7,30-34}
- Etching of oxides\textsuperscript{36}
- Etching and sintering of nanostructures\textsuperscript{35}

Nanoparticle and fluid dynamics

- Nanoparticle diffusion\textsuperscript{37,38}
- Motion in thin liquid layers\textsuperscript{42}
- Droplet and bubble motion\textsuperscript{39-41}

Biological samples

- Imaging of cells\textsuperscript{46-48}
- Imaging of labelled biostructures

Soft-materials

- Polymer thermo-responsive processes\textsuperscript{52}
- Micellar structures\textsuperscript{43-45}

Future fields

- Electrochemical double layer dynamics
- Ion exchange processes
- Self-assembly

\[ D_r = \frac{I}{A \cdot e^-} \] (2.1)

where \( I \) is the beam current, \( A \) is the area covered by the beam (for TEM) or the area over which the beam is scanned (for STEM) and \( e^- \) is the elementary charge. Importantly, the imaged area does not always match the illuminated area in TEM since sometimes the electron beam covers an area larger than the camera collecting the signal. Therefore, special attention has to be given to electron doses measured in TEM.

The electron flux is typically used to compare the intensity of the beam between experiments, as it is normalized for the observed area. However, the electron flux does not reflect how much time the beam is shone onto a sample (for TEM) or how much scans are done and at what speed or pixel size (for STEM). Therefore, that information has to be explicitly given next to \( D_r \).

(Total/Cumulative) electron dose

The total electron dose refers to the total amount of electrons that have gone through a sample per unit area along the entire period of imaging. It is closely related to the Electron dose flux, and given the intensity of the electron beam it accounts for the duration of experiments. This magnitude is very useful to compare the progression of dynamic processes between experiments.
and it is defined as:

\[ D_c = D_r \cdot t \cdot A \]  \hspace{1cm} (2.2)

where \( t \) is the total imaging time and \( A \) is the imaged area. Total imaging time of course will depend on experimental design and will vary from the total length of an experiment of continuous exposure experiments to shorter times for experiments involving blanking of the electron beam. For STEM experiments, although the beam scans over the imaging area and not all points are illuminated all the time, the total electron dose is still a good representative parameter to compare different observations, as it will be seen in Chapters 3 and 5.

### 2.3 Electron-matter interactions

In transmission electron microscopy electrons interact with the entire volume of the imaged area as they transmit through and reach the detectors. In this section, electron-matter interactions will be reviewed with a focus on beam-induced damage and water radiation chemistry. Extensive reviews on these topics can be found in literature, while this section is a brief distillation of the main concepts.\textsuperscript{13,53,54}

#### Electron-matter interactions in electron microscopy

When a sample is imaged in a TEM either by conventional TEM operation or (S)TEM operation, electrons transmit and scatter through the sample and generate information that is later recorded and interpreted. The electrostatic (Coulombic) nature of the scattering processes determines thus the information that can be obtained with TEM imaging and also defines the different beam-induced effects imaging can have on samples. Such processes are commonly divided between elastic and inelastic scattering events, depending on the amount of energy transferred from the incident electrons to the electrons/atoms of the imaged sample.\textsuperscript{55}

- **Elastic scattering**: During elastic scattering events, incoming electrons retain most of their kinetic energy. These events happen when the trajectory of an electron brings the electron close enough to an atom nucleus rendering attractive electrostatic interactions between the negatively charged electron and the positively charged nucleus relevant. Importantly, direct head-on collisions between the nucleus and the electrons are extremely unlikely due to the small size of the nucleus. Therefore such interactions can be pictured as changes in the trajectories of incoming electrons around the atomic nucleus. For typical electron beam energies used in TEM, most of these changes in direction are small, and the values for energy transfer are almost negligible. These low energy transfer values can not only be inferred from the small scattering angles, but also from the proportionality of the transferred energy to the mass ratios between the incident electron and the atomic nucleus. As such, the interacting nucleus can be assumed to remain still after most elastic scattering events. However, given the stochastic nature of scattering, some elastic events can achieve high scattering angles, even resulting in a full U-turn around the atomic nucleus (a \( 180^\circ \) change in the electron trajectory) if the electron happens to pass close enough to it. This events represent the maximal possible elastic energy transfer. Transferred energies for these extreme elastic events, for typical TEM beam energies, can be as high as hundreds of eV for small atoms to a few eV for large atoms. Sample damage arising from these high scattering angle events is known as knock-on damage. This sub-set of interactions that transfer relevant amounts of energies to the atoms are not considered elastic anymore, which brings us to the next point.
• **Inelastic scattering:** Inelastic scattering events are characterized by the transfer of a significant fraction of energy from the incident electron to the atom from the sample or to the bulk of the sample. We have seen that events between electrons and nuclei are mostly elastic but a small proportion can become inelastic. In general, most inelastic scattering events arise from electron-electron repulsive interactions between the incident electron and the electrons from the atoms of the sample. This follows from the fact that the energy transferred (via transfer of momentum) depends on the mass ratio between the interacting objects, now perfectly similar in mass. With this ratio factor ruled out, energy transfer between electrons can be substantial for high scattering angles up to complete energy transfer for electron-electron head-on collisions. However, the probability of such events is still low, and for TEM energies inelastic scattering events result in energy transfers between a few eV (for electrons in conducting bands) to a few hundred eV (for electrons in the inner electron shells). Inelastic events can cause excitation or even ionization of the affected atoms, which are important drivers for radiolysis damage.

2.4 **Electron beam damage**

The fact that the incident electrons can transfer physically relevant amounts of energy to atoms of a sample implies that beam-induced effects will happen during TEM observation. So far we have discussed two main inelastic processes regarding energy transfer to the atoms: high-scattering electron-nucleus and electron-electron interactions. However, the frequency at which both processes happen is not the same. The probability of a given type of scattering between an incoming radiant excitation (electrons for TEM) and a given atom in a sample is determined by a parameter named the cross-section. Depending on the irradiated material and the energy of the incident radiation, cross-sections for knock-on and radiolysis processes are different. For example, for organic samples the cross-section for knock-on damage is tens to thousands times smaller than the cross-section for radiolysis damage.\(^{53,56}\) Understanding what type of scattering prevails for each material is key to the interpretation of EM results.

One can intuitively understand how direct energy transfer between incident electrons and atomic nuclei can lead to atomic displacement in a sample, but when electron-electron interactions mediate beam damage, particular relaxation processes have to take place in order to transfer the energy from the electron shell to the nucleus. When an electron receives enough energy and an atom is ionized, a vacancy (hole) in the electron configuration is created, the energy of which depends on whether the excited electrons belonged to the inner or outer shells of electrons. In metals, other electrons rapidly re-occupy such vacancies, thus limiting radiolysis damage (so knock-on events will dominate as a cause of damage in conductive, metal structures). In materials in which this cannot happen as fast (i.e. insulators, semiconductors), other relaxation processes can occur. Already in 1973 radiolysis beam damage in alkali halides was described in terms of non-radiative decay of self-trapped beam-induced excitons resulting in momentum transfer to the nucleus.\(^{57}\) Whether affected atoms can change their position in a sample is determined by a threshold displacement energy, which is characteristic per element and also per material, and is defined as the minimum energy an atom needs to acquire to be effectively displaced. Other relaxation mechanisms involve the generation of phonons (oscillatory collective atomic motions) which in turn can be the result of the relaxation of radiation-excited plasmons (oscillatory collective electron motions).\(^{58}\) Of particular interest for this thesis is the radiolysis effects of the electron beam on liquid water, as our LC-(S)TEM observations described in Chapters 3-5 were of nanoparticles and colloids immersed in water.
Water radiation chemistry

Water radiolysis is relatively well known and has been studied in the context of many different fields ranging from medical physics (radiotherapy) to nuclear waste management. Effects from water radiolysis were reported for the first time on aqueous solutions of radioactive salts shortly after the discovery of radioactivity and X-rays at the end of the 19th century, a phenomenon that would be described as “a pseudo-electrolysis without electrodes”. Early observations would comprise the reduction of metal ions in solution into solids, and the formation of hydrogen and oxygen from irradiated water. The development of the understanding of the radiation chemistry of water would develop further during the following decades, as beautifully summarized by Belloni (2011). The basic processes involved in the formation of the main radiolytic species formed after water irradiation by electrons with an energy ranging from a few keV up to the MeV range are summarized in Figure 2.5. The processes happening starting from water irradiation to the final yield of such species can be split in three consecutive stages: physical, physico-chemical and chemical.

The physical stage is defined as the stage spanning the first femtosecond (10^{-15} seconds) after the interaction between ionizing radiation and water molecules. During this brief fraction of a second, energy is deposited to the water molecules, which undergo rapid relaxation processes (ionization, excitation). Also some electrons with velocities not able to ionize water anymore are generated. Immediately after the formation of these species (10^{-15} to 10^{-12} s), chemical reactions between these species and with water happen, resulting in a wider variety of radiolysis products. Concurrently some electrons further slow down, thermalize, and become solvated electrons (e_{aq}^{-}). Finally, there is a chemical stage (10^{-12} to 10^{-6} s) in which reactions between all species of the system happen and the full range of water radiolysis products is achieved. Diffusion also starts to become relevant and reactions between the species formed in the irradiated volume with non-irradiated solvent take place as well.

2.5 Colloidal Self-Assembly (SA)

Colloidal particles (colloids) are particles with sizes in the range of a few nanometers to a few micrometers in at least one of their dimensions. Most systems of colloids are considered soft matter, and are defined for being structured on mesoscopic scales (and thus can be locally inhomogeneous) and for having inter-particle interactions with energies of the order of the thermal energy (∼ k_B T). Systems consisting of colloidal building blocks are thus characterized by thermodynamic behavior similar to that of atoms and molecules (e.g. crystallization, melting), as well as by the possibility to easily manipulate them with external fields. Colloids (and nanoparticles and molecules) can spontaneously assemble into ordered structures under the right circumstances (pressure, particle number density, monodispersity). This process happens at almost all scales of nature and is commonly referred to as Self-Assembly (SA). SA reflects the properties of the building blocks such as shape, charge or surface properties, as well as their interaction potentials. For instance SA crystals of spherical hard particles will show a periodicity matching the size of the colloidal building blocks. Moreover, the possibility to easily obtain monodisperse particle systems from synthesis makes colloidal systems excellent model systems to study processes analogous to those of atoms and molecules. In contrast with their smaller counterparts (atoms/molecules), the size range of colloidal particles implies that their diffusion and behavior are slow enough to be easily followed by conventional light microscopy techniques. Among them, Confocal Laser Scanning Microscopy (CLSM) has been widely used to study dispersions of fluorescently labelled colloidal particles. Furthermore, SA can be facilitated or even directed by the application of external fields to which that generally couple to colloids strongly, such as gravitational, electric or magnetic fields.
Figure 2.5: Cascade of reactions leading to common water radiolysis products from exposure to ionizing radiation. When ionizing radiation (e.g., an electron beam) interacts with water molecules, three stages of processes can be distinguished. The first stage (physical, $10^{-15}$ seconds) is when inelastic scattering events result in the excitation or ionization of water, as well as the slowing down of some of the electrons. The second stage (physico-chemical, $10^{-12}$ to $10^{-15}$ s) includes reactions between the different species and the further development of excited molecules. For instance, excited water molecules can split into radical hydrogen and hydroxide molecules. Also, some electrons slow down further, undergoing thermalization and becoming solvated electrons ($e_{(aq)}^{-}$). The third stage (chemical, $10^{-6}$ to $10^{-12}$ s) includes all other reactions between all chemical species that are present, and the formation of the full range of water radiolysis products.

Another interesting feature of colloidal systems is the wide variety of particle geometries and surface chemistries that can be easily achieved via top-down or bottom-up synthesis methods. The fact that the interactions between colloids can be changed while it cannot be changed for atoms or molecules, makes colloids very interesting for tailored SA processes. For instance, the surface of particles can be modified (e.g., chemistry, ligands) to change their effective interaction from hard-particles type of interactions to softer interactions. Also of particular interest is the possibility of synthesizing anisotropic colloids such as rod-like particles, which are known to assemble in a rich variety of liquid crystal (LC) phases (Figure 2.6). LCs are ordered phases of matter with properties in between those of solids and liquids. When a dispersion of rods is sufficiently dilute, it is known to be in an Isotropic ($I$) phase, in which there is no long-range order. However, as particle number density (or pressure) increases, SA takes place and more free-energetically favorable configurations known as Nematic ($N$) phases start forming. $N$ phases are characterized by their long-range order in the orientation of the rods. If the number density of particles keeps increasing Smectic ($Sm$) phases form, characterized by both positional and orientational order of the rods. If this trend is continued, full 3D crystallization of the system takes place. When additional complexity is added to the rod-like building blocks such as a continuous bending or a bent-core particle geometry, a number
of additional LC phases can form. In order to accommodate bend deformations arising from the assembly of the bent rods over extended regions, long-range modulations in the direction of the orientation of $N$ phases arises, resulting in bend ($N_{SB}$) and twisted ($N_{TB}$) nematic phases.\textsuperscript{74,75}

The first experimental realization of a $N_{SB}$ phase from colloidal banana-shaped particles dates from 2020,\textsuperscript{76} more than 40 years after its first prediction in 1976.\textsuperscript{74} In Chapter 6 we describe via CLSM the assembly of $N_{SB}$ phases from bent-core fluorescent silica rod-like particles in a sedimentation equilibrium profile.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{liquid-crystal-phases.png}
\caption{Liquid Crystal (LC) Phases. Diluted dispersions of rod-like particles show non-ordered orientational and positional distributions, and are considered to be in a Isotropic ($I$) state. When particle number density increases, phase transitions to LC phases happen. First, Nematic ($N$) phases form, characterized by long-range order in particle orientation. If the trend continues, and before the system fully crystallizes, Smectic ($Sm$) phases form, characterized by long-range order in particle orientations and positions. (Phases here denoted at too low densities for clarity)}
\end{figure}
Chapter 3

*In Situ* Study of the Wet Chemical Etching of SiO$_2$ and Metal(Oxide)@SiO$_2$ Core-Shell Nanospheres

Based on: Grau-Carbonell, A. et al. "*In Situ* Study of the Wet Chemical Etching of SiO$_2$ and Metal(Oxide)@SiO$_2$ Core-Shell Nanospheres" *ACS Applied Nanomaterials*, DOI: 10.1021/ac-sanm.0c02771, (2020).
The recent development of Liquid Cell (Scanning) Transmission Electron Microscopy (LC-(S)TEM) has opened the unique possibility of studying the chemical behavior of nanomaterials down to the nanoscale in a liquid environment. Here we show that the chemically induced etching of three different types of silica-based silica nanoparticles can be reliably studied at the single particle level using LC-(S)TEM with a negligible effect of the electron beam, and we demonstrate this method by successfully monitoring the formation of silica-based heterogeneous yolk-shell nanostructures. By scrutinizing the influence of electron beam irradiation we show that the cumulative electron dose on the imaging area plays a crucial role in the observed damage and needs to be considered during the experimental design. Monte-Carlo simulations of the electron trajectories during LC-(S)TEM experiments allowed us to relate the cumulative electron dose with the deposited energy on the particles, which was found to significantly alter the silica network under imaging conditions of nanoparticles. We used this optimized LC-(S)TEM imaging conditions to systematically characterize the wet etching of silica and metal(oxide)-silica core-shell nanoparticles with cores of gold and iron oxide, which are representative of many other core-silica-shell systems. The LC-(S)TEM method reliably reproduced the etching patterns of Stöber, Water-in-Oil Reverse Micro emulsion (WORM) and Amino Acid-Catalyzed (AAC) silica particles that were reported before in literature. Furthermore, we directly visualized the formation of yolk-shell structures from the wet etching of Au@Stöber silica and Fe$_3$O$_4$@WORM silica core-shell nanospheres.

3.1 Introduction

Silica nanoparticles or core-shell silica-shell nanoparticles are of general interest, as they are biocompatible and stable in a large number of solvents, in which they also form stable dispersed colloidal systems with interactions tunable over a large range of distances. Three widely used strategies to synthesize spherical colloidal silica systems, and perform seeded growth of nanoparticles, are the Stöber, WORM and AAC methods (Figure 1A). These approaches all have in common that a basic environment is created in which both the hydrolysis and condensation of tetra- and/or tri-alkoxysilanes are catalyzed by OH$^-$ ions (Figure 1B). The Stöber method relies on the hydrolysis and condensation of the precursor tetraethyl orthosilicate (TEOS) homogeneously dissolved in an alcoholic aqueous solution while ammonia catalyzes the hydrolysis and condensation reactions. The WORM method also exploits ammonia as the catalyzing agent, but TEOS condensation happens inside the water phase of a water-in-oil microemulsion. In the AAC method, TEOS condensation is catalyzed by a basic amino acid (L-lysine, L-arginine) in an aqueous solution. All three synthesis approaches result in spherical, relatively monodisperse particles, but potentially different internal silica structures which have shown to be not homogeneous in all cases.

The growth mechanism of Stöber silica consists of an initial aggregation process of several nm-sized siloxane moieties, followed by their continuous growth by monomer addition into smoother, spherical particles. The overall growth rate is limited by the speed of the hydrolysis of TEOS, which is a first order reaction. Towards the end of the reaction the concentration of hydrolyzed silanol groups goes down, the development of siloxane bonds becomes slower and silicon atoms can form more siloxane bonds (highly condensed silica). This growth mechanism results in an inhomogeneous internal distribution of silica condensation levels. WORM silica nanoparticles are also believed to have a less condensed internal structure, as the growth conditions are similar
Figure 1: **Silica synthesis methods and chemistry.** A. Schematics of the Stöber, AAC and WORM methods to synthesize silica nanospheres. In the Stöber method silica growth happens in a basic alcoholic solution after the injection of tetraethyl orthosilicate (TEOS). In the WORM method silica growth takes place by the diffusion of TEOS from the oil phase into the basic water droplets of a water-in-oil reverse microemulsion. In the AAC method silica growth takes place in an aqueous phase containing a basic amino acid from the slow diffusion of hydrolysed TEOS from an unmixed, oil-like layer of non-hydrolysed TEOS. B. TEOS hydrolysis and condensation are two basic steps in silica growth for the Stöber, AAC and WORM silica nucleation and growth processes. Basic media catalyse both the condensation and hydrolysis steps. In fully condensed silica each TEOS molecule has formed four siloxane bonds, however all these procedures do not produce fully condensed silica.
In the AAC method particles form in an aqueous environment, typically at relatively elevated temperatures, but less is known of their internal structure.

Understanding the internal distribution of condensation levels in silica particles can help to explain the reported differences in properties and behavior, such as the dissolution behaviors of the different types of silica nanospheres. Silica is slightly soluble in room temperature neutral pH water and its solubility is strongly dependent on the pH: increased pH values lead to increased solubility. When exposed to an etching solution, loosely condensed parts will dissolve more easily than highly condensed parts, as fewer siloxane bonds need to be broken. Therefore, the organization of the condensation degrees of the silica network can be revealed by exposing the nanoparticles to a basic aqueous solution and studying the etching process. Previously, monitoring how the morphology of particles changes in etching studies consisted on several time-consuming cleaning steps in which the etching needed to be quenched and the particles had to be dried for regular (S)TEM analysis. If drying is not done by e.g. critical drying the drying forces can significantly alter the structures under observation.

LC-(S)TEM allows in situ monitoring of the morphology evolution of nanostructures in chemically active, wet environments and thus could possibly be used to characterize the evolution of silica-based nanoparticles in basic aqueous solutions. LC-(S)TEM studies rely upon the illumination of a sample in a liquid environment by a highly energetic electron beam. However, beyond particular thresholds of intensity, electron beam irradiation is known to directly generate conditions for which particles are affected by radiolysis of the liquid. Up to now, the role of electron irradiation on chemical reactions has been studied extensively for the stability of precursors in solution but its effects on other chemical processes remain not that well investigated. Wet etching (or dissolution) of nanostructures in LC-(S)TEM has been reported in previous studies, but the effects of electron dosage were only partially evaluated or only evaluated after the sample was exposed to electron beam irradiation. The total dose experienced by a sample is also known to play an important role on the behavior of systems and the interpretation of LC-(S)TEM results, as the effects of the electron beam accumulate the damage done. Therefore, describing and understanding the impact of the electron beam exposure onto nanoparticles dispersed in chemically reacting solvents is important for the future development of in situ LC-(S)TEM methodology with a particular aim on finding the conditions where these effects can be neglected.

In this study we first describe the etching of AAC silica particles dispersed in basic aqueous solutions. AAC silica nanoparticles, contrary to Stöber and WORM type silica nanoparticles, were found to reduce in size continuously from the outside inwards. Next, we made use of this better-defined etching of AAC silica nanoparticles to characterize the effects of the electron beam irradiation on this type of silica particles immersed in water and in basic aqueous solutions. The cumulative electron dose during the LC-(S)TEM imaging of the wet etching of silica nanoparticles was the main parameter regarding electron beam effects. Our findings revealed that the effects of the electron beam on the etching and stability of silica nanoparticles can be reduced and even made negligible by minimizing the cumulative electron dose on the particles, independent of the electron dose rate. Furthermore, we found that the electron irradiation directly affected the wet chemical etching of silica by depositing enough energy in the silica to severely alter the siloxane network. This knowledge was used to characterize the wet etching of Stöber and WORM silica nanoparticles, and Fe₃O₄@WORM silica and Au@Stöber silica core-shell nanoparticles. The inhomogeneous etching patterns of silica-based nanospheres synthesized via different methods were characterized. We report the direct imaging of the void formation step of yolk-shell nanoparticles from metal(oxide)@silica core-shell nanospheres via the inhomogeneous etching of the silica shell. LC-(S)TEM shows that the core became mobile during this void formation. Describing the behavior of the gold and iron oxide core-shell silica particles, which are representative of NP@silica core-shell systems in general.
during the yolk-shell formation can lead to a better understanding of the final product, as well as potentially opening the door to new synthesis procedures.

3.2 Results and Discussion

AAC silica spheres as model system to study electron beam effects on wet silica etching

To study the effects of the electron beam irradiation on the wet etching of silica nanospheres, a system of nanoparticles with a quantifiable etching pattern was required. Comparing the LC-(S)TEM observations of etching processes with their known ex situ counterparts would expose the effects of the electron beam irradiation. Furthermore, a system with low polydispersity would make single particle LC-(S)TEM observations comparable. Also, the nanoparticles should be large enough to allow their study at low magnifications and low electron beam intensities to explore the lower range of electron dose rates. Of all silica systems, AAC silica nanoparticles were chosen because of their low polydispersity and the precise size control from synthesis they offer. We characterized the ex situ etching of AAC silica nanoparticles (d = 113 nm, PD: 3%, silica concentration: 10 mM) dispersed in NaOH aqueous solutions. We monitored the etching process by taking samples in time to compare the bulk etching with the in situ etching inside the liquid cell (Figure 2). AAC silica spheres showed an homogeneous decrease in diameter when exposed to the basic media (Figure 2A). The etching process lasted up to weeks if enough NaOH was present in the system (Figure 2B, Figure S2), and could be used to easily tune the size of a batch of AAC silica spheres to any desired size. The polydispersity of the AAC silica nanoparticles increased as the particle size reduced during etching while the width of the size distribution remained roughly the same (Figure S1). These observations indicate that AAC silica was not found to etch through a more condensed outer shell as Stöber and WORM based silica was found to do. A similar etching behavior was observed when AAC silica nanoparticles were etched in HF aqueous solutions (Figure S4). Furthermore, the etching of AAC silica particles was much slower than the etching of Stöber and WORM based silica, as the dissolution of the later completed overnight. Here AAC silica particles were synthesized at higher temperatures than the Stöber particles. Silica further condenses when temperature is increased, but the final AAC silica particles have similar condensation levels to the other types of silica grown at lower temperatures. As amino acids become incorporated inside the silica particles by adsorbing to the nuclei, these aggregating smaller silica units are probably more highly condensed.

The homogeneous internal structure of AAC silica spheres was further studied by closely following their etching in time. As the diameter of the particles changed gradually, dynamic light scattering (DLS) was a suitable technique to follow their etching in the presence of different NaOH concentrations (Figure 2C). The etching process happened in two different phases. For the first ~300 minutes, the particle size decreased slowly (less than 5 nm in a 100 mM NaOH solution). After this initial regime, the etching rate increased and the particle size showed a more pronounced decrease. This second phase was studied as a linear process to compare the etching rates between various base concentrations. It can be readily observed that as the base concentration increased, the etching rate did increase as well (Figure 2D). However, the positive trend of the etching rate slowed down at higher pH.
Figure 2: Ex situ, bulk etching of AAC silica nanoparticles. (A) TEM imaging of AAC silica particles dried after being dispersed in a NaOH aqueous solution. AAC silica particles etch homogeneously, decreasing in size over time. (B) Time evolution of the etching process of AAC silica spheres in aqueous NaOH solutions. The data was obtained from TEM imaging of dried samples taken at different time points. (C) Time evolution of the etching process of AAC silica spheres in aqueous NaOH solutions followed by dynamic light scattering (DLS) during the first 20 hours of the process. The etching process shows two parts. For the first hours (up to around 300 min), the decrease in diameter was slow. The second part (from 300 min to 1200 min) of the etching process showed a faster decrease of diameter. (D) The slope of the second part of the etching was fitted by linear regression. The speed of the etching increased with the base concentration, but the change in rate was smaller for higher base concentrations. The colour of each data point refers to the base concentration as depicted in C.
Characterization of the electron beam irradiation effects on the stability and the wet etching of AAC silica particles

LC-(S)TEM allowed us to study the wet etching of silica nanoparticles at the single particle level by exposing them to an aqueous NaOH solution during (S)TEM imaging. We showed that AAC silica nanoparticles exhibit two different regimes of etching during the first hours of etching. Here we exploited the mode of etching of the AAC silica colloids during the first hours in a 100 mM NaOH aqueous solution to determine the conditions under which the silica wet etching process became not altered by the electron beam. The expected behavior was a slow etching of the spheres, and any change in such behavior could be ascribed to electron beam effects. The low degree of polydispersity of the initial AAC silica particles (3%) allowed for the precise analysis of the morphological evolution of the particles. The size evolution of the particles was followed by digital analysis via ellipsoid fitting to binarized data sets as it was done before in the literature. We prepared the liquid cell set-up as described in the methods; the top window of the chip was covered by the nanoparticles. A region of particles was used to focus on the window, then the imaging area was shifted with the beam blanked to avoid unnecessary irradiation of any area of interest, after which imaging was performed. If the experiment was conducted under NaOH aqueous solution flow, the imaging was started when the liquid leaving the holder outlet was found to be at the desired pH by probing it with pH paper, which was the case one or two minutes after the flow was started. The constant flow of base ensured that the cell was always under a flow of basic solution and in contact with a constantly renewed reservoir of base. Maintaining a flow of basic solution has been shown to prevent base depletion during wet etching of silica in liquid cell experiments. The use of buffers could also help maintaining the pH inside the cell during etching experiments. However, this function is already fulfilled by the constant flow set-up. Furthermore, buffers have been suggested to partially work as stabilizers of silica during STEM imaging in water, but this role would be mainly a result of their radical scavenging ability. Therefore, to better decouple the beam effects to the radical scavenging effects, we did not use scavengers during our experiments. The influence of radiolysis radicals and radical scavengers will be discussed in later parts of this study.

Continuous imaging was performed with 1024×1024 pixels, 16 µs dwell time, and 1.58 nm or 0.78 nm pixel sizes. The nanoparticles were first imaged while pure water was flowing into the cell (milliQ grade, 5 µL.min⁻¹). Our LC-(S)TEM observations showed that in pure water the particles expanded gradually up to ~10% of their starting size (Figure 3A, Supplementary videos 1-3) and subsequently dissolved, in agreement with previous LC-(S)TEM studies of silica particles in water at high electron dose rates. The initial expansion under a flow of pure water happened at a similar rate for all electron dose rates ranging from 570 e⁻·nm⁻²·s⁻¹ to 2.3×10³ e⁻·nm⁻²·s⁻¹, but the dissolution was faster for higher electron dose rates. When the behaviors were compared for similar accumulated doses they became almost the same (Figure 3B). The electron beam irradiation in pure water is known to change the pH of the solution to more acidic values and to produce a number of reducing and oxidizing agents. Scavenging some of these radicals (e⁻(aq), H⁻) was shown to increase silica stability in water during LC-(S)TEM experiments. AAC silica spheres were remarkably more affected by the electron beam if the particles were imaged under a flow of a basic aqueous solution (100 mM NaOH (aq), 5 µL.min⁻¹). Pronounced expansion was consistently observed if the system was imaged in 100 mM NaOH aqueous media for all of the used electron dose rates (Figure 3C, Supplementary videos 4-7). This expansion was found to amount to up to ~30% of the initial size of the nanoparticles. This process was faster for higher electron dose rates, ranging from a few minutes for dose rates of 2.3×10³ e⁻·nm⁻²·s⁻¹ and 3.4×10³ e⁻·nm⁻²·s⁻¹, to around 20 minutes for a dose rate of 520 e⁻·nm⁻²·s⁻¹ to a number of hours for a dose rate of 31 e⁻·nm⁻²·s⁻¹. The disparity was completely lost when the expansion
Figure 3: In situ etching of AAC silica monitored by Liquid Cell (S)TEM. (A) Time evolution of the size of AAC spheres in pure water under different electron dose rates. The size evolution of the same experiments as a function of the cumulative electron dose is shown in (B). (C) Time evolution of the size of AAC spheres in a 100 mM NaOH aqueous solution under different electron dose rates. The size evolution of the same experiments as a function of the cumulative dose is shown in (D). Every electron dose rate for each panel contains data of 4 to 5 different nanoparticles. The flow rate was 5 µL.min\(^{-1}\) in all experiments.
was considered as a function of the cumulative dose on the frame (Figure 3D). The onset and initial evolution of all the expansions happened at similar cumulative electron doses, regardless of the electron dose rate. On the other hand, the dissolution process remained different for different dose rates even if these were compared via the total accumulated doses. At low dose rates \(31 \text{ e}^{-}\text{nm}^{-2}\text{s}^{-1}\), expansion was slow as few electrons were deposited per frame. This allowed the concurrent dissolution process to take place extensively during the expansion period, and therefore the overall expansion was not as high as in other cases. Even at such low electron dose rates the dissolution phase was accelerated by the electron beam irradiation as compared to the \textit{ex situ} counterpart experiments. At a moderate electron dose rate \(520 \text{ e}^{-}\text{nm}^{-2}\text{s}^{-1}\), the expansion happened faster, and therefore the dissolution could not play a large role during the expansion phase. The dissolution of the particles happened faster for higher dose rates than for lower dose rates as the network was continuously affected by the electron beam irradiation. At a high electron dose rate \(2.3 \times 10^3 \text{ e}^{-}\text{nm}^{-2}\text{s}^{-1}\), the network was so strongly affected by the imaging that the dissolution was faster also during particle expansion. This resulted in a slightly reduced expansion, but also in much accelerated dissolution. Finally, at a high electron dose rate \(3.4 \times 10^3 \text{ e}^{-}\text{nm}^{-2}\text{s}^{-1}\), expansion happened so fast that the dissolution process could not affect the expansion period. At such high electron dose rates the scanning induced the deformation of silica particles in the scanning direction, as was previously reported for silica spheres and colloidal silica rods under intense STEM illumination.\textsuperscript{106,109,110} Although this process is still not well understood, silica plasticity increases under electron irradiation even at room temperature,\textsuperscript{111} and thus we might be observing the effects of the intense electric fields created by secondary electron ejection from the surfaces in the liquid cell.\textsuperscript{112} To summarize, our results indicate that the expansion process of silica particles upon electron beam irradiation in presence of water appears to be completely driven by the cumulative electron dose rate on the particle, and that the extent of such expansion depends on the media in which the particles are immersed in. On the other hand, the dissolution process was not only related to the cumulative electron dose. The different dissolution rates are better understood when considering the direct interaction of the electron beam with the silica network, together with the passive dissolution process taking place between the nanoparticle and the surrounding liquid phase.

Accelerated dissolution, or “superdissolution” of oxides has been reported for other oxides and linked to the generation of an oxygen deficient surface by the electron beam and the consequent reduction of the activation energy at the surface.\textsuperscript{113,114} As higher electron dose rates result in higher concentrations of radiolysis products, the dissolution phase is then also sensitive to this parameter. The accelerated dissolution of silica under electron irradiation in water has indeed been partially slowed down by the use of radical scavengers in solution, further supporting our findings.\textsuperscript{106} We were also able to perform free-hand lithography on our AAC particles immersed in water, similarly to previous results in CeO thin films (Figure S5).\textsuperscript{113} Low electron dose rates are crucial to minimize electron beam effects on the diffusion motion of nanoparticles,\textsuperscript{38} and might become an increasingly important parameter in future experiments. In conclusion, the etching of AAC silica nanoparticles was best imaged by minimizing the electron dose rate and more importantly the cumulative electron dose. Considering this, the relative stability of AAC silica particles during the first hours exposed to a 100 mM NaOH aqueous solution was successfully characterized by using a low electron dose rate \(41 \text{ e}^{-}\text{nm}^{-2}\text{s}^{-1}\) and discontinuous imaging (30 minutes blanks) to reduce the amount of electrons accumulated on the imaging area to approximately \(2751 \text{ e}^{-}\text{nm}^{-2}\) \((1024 \times 1024 \text{ pixels, } 16 \mu\text{s dwell time, } 1.58 \text{ nm pixel size})\) (Figure 3C, yellow triangle data, Supplementary video 8).

Even though the accelerated dissolution phase of oxides has been already reported and partially studied in the literature, the expansion phase reported here has not been explored much. Therefore, we experimentally probed the mechanism behind the expansion of silica in water and 100 mM NaOH under electron irradiation. First, we assessed the role of water radiolysis radicals in this process.
Expansion was more severe for a solution with 100 mM NaOH in comparison to pure water. The concentration of radicals under electron irradiation of water can be approximated for both our solvents with the widely used model of Schneider, N.M. et al. (2014). The results as a function for the electron dose rate can be found in Figure S6. The radical species that becomes more prevalent with increasing pH under our dose rates are hydrated electrons ($e^{-}_{aq}$). We then studied in detail the expansion phase in the absence, or in the presence, of a $e^{-}_{aq}$ scavenger (Potassium Persulfate, K$_2$S$_2$O$_8$, 100 mM) under (S)TEM illumination ($370 e^{-}\text{nm}^{-2}\text{s}^{-1}$). No difference in the velocity or the extent of the expansion was observed between either water or 100 mM NaOH when K$_2$S$_2$O$_8$ was present (Figure S7). In consequence, our results indicate that the initial composition of the solvent was more relevant than the formation of radiolysis radicals by the electron beam, in contrast with the accelerated dissolution phase. We then assessed whether the expansion phase was more likely driven by ionization or direct knock-on events between the electrons and the silica network. AAC silica particles were then imaged in (S)TEM mode with a TEM operated at 120 keV. Lower acceleration voltages of the microscope, and therefore lower energies, result in a lower direct knock-on cross section. For example, the knock-on threshold for silicon is around 140 keV, and no knock-on damage would happen for electrons having an energy below this threshold. Expansion and accelerated dissolution were also observed under 120 keV (S)TEM ($960 e^{-}\text{nm}^{-2}\text{s}^{-1}$) illumination in water (Figure S8, A). The extent of the expansion matched the expansion observed with 200 keV imaging and did not differ much in speed. The expansion phase was also faster and more severe in 100 mM NaOH (Figure S8, B). That the expansion phase persists also under illumination of electrons with significantly less energy suggests that the underlying mechanism is likely based in bond ionization. Although the temperature in the liquid cell during imaging is supposed to increase only marginally, silica solubility in water depends on temperature and thus we also assessed this parameter. To this end, we imaged AAC silica nanoparticles in water by performing in situ heating LC-(S)TEM experiments ($500 e^{-}\text{nm}^{-2}\text{s}^{-1}$) with a range (up to 90 °C) of temperatures greatly exceeding the potential local temperature increase induced by the electron beam (Figure S9). No difference in the expansion and accelerated dissolution were observed between different temperatures, proving the effect of local heating in our experiments negligible.

**Monte-Carlo simulations of the electron trajectories during LC-(S)TEM imaging of silica particles in water**

So far, the effects of electron beam irradiation on silica particles in LC-(S)TEM experiments discussed in literature were in terms of the radiolysis of the solvent. However, this interpretation does not fully explain the expansion/dissolution behavior. Our DLS results show that although a higher pH resulted in increased etching rates, this resulted in a small increase in the etching rate above 100 mM NaOH. Lower pH values resulted in slower etching. Therefore, changes in the concentration of OH$^{-}$ ions cannot fully explain our observations. Additionally, the formation of reducing radicals is also supposed to be lower if the pH of the irradiated solution is high. If the expansion was mediated by such species, it would depend on the electron dose rate and it would be reduced at higher starting pH. It is quite clear that chemical modifications of the siloxane structure as caused by the energy deposited by the electron beam also have to play an important role. Here we propose that the direct interaction between the electron beam and the silica network is a main driver of the expansion/dissolution behavior. To explore this possibility the deposited energy in the particles during our LC-(S)TEM experiments was assessed. The energy deposited per unit volume was compared to the energy needed to affect the siloxane bonds to assess whether this effect is relevant for typical LC-(S)TEM imaging conditions. If the electron beam can affect a significant number of bonds in the structure of the silica network during imaging, silica solubility may increase and
etching may be favoured. The loss of condensation all over the particles due to the beam induced excitation of siloxane bonds could then explain their expansion.

The energy that was deposited per cubic nanometer (nm$^3$) of silica during a LC-(S)TEM experiment was modelled by means of Monte-Carlo simulations with the software CASINO 3.3.$^{118}$ This software simulates the electron paths during (S)TEM imaging through a given sample. The elastic scattering interactions are treated as discrete events, and the inelastic interactions are treated with an energy loss model. Secondary electron generation is also considered. A 3D model of a simplified liquid cell environment was defined to model our experiments. The simulated geometry consisted of two silicon nitride windows of 50 nm thickness that enclosed a 1 µm thick layer of water. In this water volume we positioned a silica sphere with a 113 nm diameter to emulate our AAC silica particles. The position of the center of the particle was chosen such that the particle was attached to the top window (Figure 4A). From the pathways of all the simulated electrons, CASINO calculates the total energy absorbed by every component of the simulated volume. The beam parameters were matched with our experimental conditions: The distance between irradiated points was set to be the experimental pixel size, the number of electrons per scanned point was determined as previously done in literature from the beam current and the dwell time,$^{119,120}$ the beam was focused on the particle and a beam convergence semi-angle of 20 mrad was used.

The energy absorbed per frame by the silica particle for the electrons used to generate a given pixel (1.58 nm pixel size, 1024×1024 pixels, 10 pA beam current and 16 µs dwell time) can be seen in Figure 4B. The binding energy of the Si-O bond is 4.60 eV$^{121}$ and there are approximately 216 Si-O bonds in 1 nm$^3$ (if we assume perfectly condensed silica which is not the case for all kind of silica nanoparticles). The following rule of thumb can be derived from CASINO simulations for small silica particles located on the top window of a liquid cell: around 12 eV of energy is deposited per nm$^3$ of silica, per pA of beam current for a frame acquired with 1 µs dwell time and with 1 nm$^2$ pixel size. However, for our experimental conditions the total absorbed energy per frame per nm$^3$ could be ranged from several keV to several MeV (Figure 4C). Furthermore, we see that the deposited energy on the particle barely depended on the depth of the particles in the liquid cell and on whether the beam was focused on the particles or not (Figure 4D). Beam current, dwell time, and pixel size are therefore the main parameters that need to be optimized to reduce the cumulative dose per frame. This highlights the potential of direct electron detectors, which are nowadays routinely used in cryo-EM experiments because of their ability to gather information from a sample at much lower irradiation levels (∼1 e$^-$/Å$^2$).$^{122,123}$

The main contributions to the absorbed energy are the inelastic interactions between the electrons of the beam and those of the material, as the inelastic scattering cross-section is larger than the elastic scattering cross-section for Si and especially for O for 200 keV electrons.$^{124}$ The inelastic scattering of the electrons can promote radiolytic processes in the irradiated material.$^{125,126}$ The decay of the electronic excitations caused by radiolysis can result in breaking of chemical bonds and may result in atomic displacements in a network as energy can be transmitted to the atom nuclei via phonons and excitons. Importantly, not every excitation decay results in atomic displacements, as a broken chemical bond does not directly imply a gain in energy sufficient to displace an atom over the surrounding energy barriers.$^{125}$ In silicates the cross-section for radiolytic atom displacements is 10 times higher than that for direct elastic events.$^{53}$ In silica, electron irradiation promotes the excitation of siloxane bonds, which in turn can result in the formation of point defects and the loss of connectivity of the silica network.$^{126,127}$

Radiolysis damage is typically assumed to be proportional to the energy deposited by radiation.$^{13}$ Deposited energies on quartz (crystalline silica) of a few hundred eV.nm$^{-3}$ via ion beam bombardment have been shown to cause amorphization.$^{14}$ Ion bombardment has also been shown to induce the deformation of spherical particles,$^{128}$ however, the energies involved are in the MeV
Figure 4: Simulation of pathways of electrons during (S)TEM imaging simulated with the CASINO software package. (A) Schematic of the simulated geometry. A 1 µm water layer was placed between 50 nm silicon nitride spacers. A silica sphere of 113 nm of diameter was placed on the top window. (B) Calculation of the energy deposited to the silica particle via elastic and inelastic interactions of the incident electrons (in keV) for a frame acquired with a (S)TEM operated at 200 keV with 1.58 nm pixel size, 10 pA of beam current and 16 µs dwell time. The pixel size of the energy distribution corresponds to the pixel size used during imaging. The energy values indicate the deposited energy on the silica sphere from the electrons initially incident on that pixel. (C) Absorbed energy per unit volume of a silica particle located at the top Si$_x$N$_y$ window for a number of electron beam currents with the previous imaging conditions. (D) Absorbed energy per unit volume of a silica particle located at different depths from the top Si$_x$N$_y$ window for the case of 1 pA beam current.
range which is quite different from the electron beams used in TEM, and quartz amorphization has been well described for electron irradiation.\textsuperscript{127} The energy absorbed by silica can be compared to the landscape of energies that have to be reached to displace an atom in the silica network. The energy needed to displace an O atom or a Si atom in the silica network is of the order of a few eV,\textsuperscript{129} and electron irradiation of SiO\textsubscript{2} films has been shown to displace O atoms, reducing the SiO\textsubscript{2} film into more pure Si areas through the formation of point defects.\textsuperscript{115,126} Therefore, during LC-(S)TEM experiments the typical amounts of energy deposited into the silica network can be up to orders of magnitude higher than the energy needed to strongly affect the siloxane bonds. Such bond breaking (and atomic displacement) events taking place in a particle dispersed in an aqueous NaOH medium will no doubt lead to the easier dissolution of the silica network. Consequently, our results could be explained by an increase in the susceptibility of the silica network to etching by the aqueous base solution as a result of the direct electron beam effects on the material. Additionally, scavenging some of the reducing radicals that are produced by the electron beam was shown to slightly stabilize silica in liquid cell experiments.\textsuperscript{106} However, it is likely that these effects are small compared to the increased solubility by the cleavage of siloxane bonds.

\textbf{In situ study of the wet etching of SiO\textsubscript{2} and metal (oxide)@SiO\textsubscript{2} nanoparticles}

We first characterized the etching of Stöber and WORM silica nanospheres (Figure 5) by exposing them to a solution of 100 mM NaOH (5 \( \mu \)L.min\(^{-1} \)). Stöber silica nanoparticles showed an inhomogeneous etching pattern, in accordance with their internal distribution of silica condensation levels (Figure 5A, Figure S10).\textsuperscript{92} This process happened at different time scales for different particles, and was in contrast to the etching patterns of Stöber silica during LC-(S)TEM experiments under higher electron dose rates and cumulative electron doses.\textsuperscript{106} We minimized the cumulative electron dose by imaging with an electron dose rate of 61 e\textsuperscript{−} nm\(^{-2}\) s\(^{-1}\), 1.58 nm pixels, 1024\( \times \)1024 pixels frames, 24 \( \mu \)s dwell time and 30 minutes blanks between frames (total accumulated dose of 1.4 \( \times \)10\(^4\) e\textsuperscript{−} nm\(^{-2}\) for Stöber silica nanoparticles and 1.5 \( \times \)10\(^3\) e\textsuperscript{−} nm\(^{-2}\) for WORM silica nanospheres). Once the hollow structures became evident, a final frame was taken with 260 e\textsuperscript{−} nm\(^{-2}\) s\(^{-1}\) to get better detail (Figure 5A, inset). By comparing the intensity profile of a cross section of a single particle it can be readily seen that these imaging conditions did not induce any expansion during the etching process, correctly characterizing the final product of the wet chemical etching of Stöber silica (Figure 5A, right panel).

Studying WORM silica was more difficult than studying AAC and Stöber silica spheres, as the size of the nanoparticles was smaller (40 nm). This made it more challenging to image such nanospheres at low total accumulated electron doses, as it required both the dose rate and the magnification to be higher (1.5 \( \times \)10\(^3\) e\textsuperscript{−} nm\(^{-2}\), 0.55 nm pixel size, 24 \( \mu \)s dwell time). Therefore, for silica particles of this size range, it is advisable to image the same area only once. Making use of this knowledge, we followed the evolution of monodispersed WORM silica spheres under a flow of aqueous 100 mM NaOH (5 \( \mu \)L.min\(^{-1} \)) by imaging different areas over time (Figure 5B). WORM silica spheres became hollow after 390 minutes of exposure to the basic solution, consistent with their behavior under hydrothermal treatments.\textsuperscript{101} It is relevant to mention here that this particle size limit could be lowered by utilizing detectors with higher sensitivities that allow imaging with lower electron dose rates and thus with a lower cumulative electron dose per frame. However, there are three factors to be considered: First, if following a single particle is of paramount importance, the particles could be imaged first in water, in which we have shown that the beam damage is lower. Then, water and the etching media could be alternated by making use of dedicated liquid cell holders with dual flow capabilities. Second, although for this particle size one cannot in principle follow the process in the etching media at the single particle level under our lower beam intensity settings, it
Figure 5: *In situ* etching of silica model nanoparticles by exposure to a 100 mM NaOH aqueous solution with a flow rate of 5 μL.min⁻¹. (A) Stöber silica. Particles became hollow when exposed to a flow of 100 mM NaOH. Right: The intensity profile along the nanoparticle shown in the inset. No expansion was observed. (total accumulated dose of 1.4 × 10⁴ e⁻nm⁻²). Inset scale bar: 50 nm. (B) WORM silica nanospheres under a flow of 100 mM NaOH. Due to the small size of the particles (40 nm), our results indicate that the silica network was strongly affected even in a single frame (total accumulated dose of 1.5 × 10³ e⁻nm⁻²). Therefore, different areas needed to be imaged to study how WORM silica nanospheres etched under basic conditions. Particles showed hollowing after 390 minutes of exposure to the basic solution. Right: Intensity profiles of two selected nanoparticles showing the formation of a hollow volume inside the particle, with minimal change in total size of the nanoparticle. Similarly as was reported before for these particles systems.⁹²
is still possible to characterize the process when the particles are relatively monodisperse, as their behaviour should not differ much from particle to particle. Third, we characterized the final product without going through purification, cleaning or any drying steps, which could potentially change the particle shape and would stop any dynamics going on inside the particles. This last factor in itself is already a powerful reason to aim for routine liquid cell (S)TEM experiments in order to follow the wet etching process of nanoparticles.

The formation of yolk-shell structures from metal(oxide)@silica core-shell nanoparticles could also be studied with the methods described previously. Yolk-shell nanoparticles consisting of a catalytically or surface enhanced Raman scattering active cores surrounded by a void and encapsulated by a porous support are known to show superior catalytic and sensing activity in many scenarios.\textsuperscript{130,131} This increased efficiency comes from the role of the hollow shell as a micro/nanoreactor, offering a number of advantages such as: prevention of particle sintering, controlled diffusion rates through the shell pores and homogeneous environments in the void surrounding the catalyst or sensing particle. The synthesis of such particles usually consists of a hard template-based synthesis of core-shell particles and the subsequent removal of material to form a void between the core template and the shell. Here we investigate the void formation step for Au@Stöber silica and Fe\textsubscript{3}O\textsubscript{4}@WORM silica core-shell particles upon wet etching of the silica shell with a NaOH aqueous solution (Figure 6), both these two systems stand as model for a broad range of nano core-silica-shell particles. We showed that by slightly etching the Stöber (Figure 6A) and the WORM (Figure 6B) silica shells with a basic solution, well-defined yolk-shell particles are obtained. Furthermore, the Au and Fe\textsubscript{3}O\textsubscript{4} cores were found to diffuse in the void once such etching was completed. This opens the door to fully treat the surface of the catalyst once the yolk is formed, and also indicates that the behavior of the catalyst could be manipulated separately from the shell in liquid phase via external fields, as it has been reported for similar structures.\textsuperscript{132}

### 3.3 Conclusions

The wet etching of silica and metal(oxide)@silica nanoparticles in an aqueous NaOH solution was studied by means of in situ LC-(S)TEM. We exploited the slow and gradual etching of AAC silica spheres in basic solutions to investigate and mitigate the electron beam effects during LC-(S)TEM studies of silica in aqueous and basic aqueous solutions. Our results pinpoint the cumulative electron dose on silica particles during in situ LC-(S)TEM experiments as the critical parameter determining whether the observations will diverge from ex situ etching behavior, but show that conditions can be found where the effects can be neglected. Under the conditions where the effects of the electron beam did affect the etching we report that silica first was found to expand and then dissolve when irradiated in an aqueous environment, and that the extent of such expansion was directly correlated to the cumulative electron dose on the imaging area, independent of the used electron dose rate. We showed that this behavior was not the result of changes in the pH of the solution. Furthermore, the expansion and dissolution phenomena were more pronounced under basic conditions. The formation of reducing radicals is assumed to be lower at basic conditions,\textsuperscript{16} and therefore we conclude that the reported expansion cannot be explained by the presence of water radiolysis products. By performing simulations of the electron beam energy loss we conclude that the energy absorbed per unit volume, per frame by a silica nanoparticle of around a hundred nanometers on the top window of a liquid cell, and could be up to orders of magnitude higher than the energy needed to directly break bonds in the silica siloxane network. We propose that the direct effects of the electron beam on the silica network make it more susceptible to etching by the OH\textsuperscript{−} ions in solution and the water radiolysis products. If the energy deposited on the nanoparticles during LC-(S)TEM experiments
alters the rate of chemical processes happening between the nanoparticle and the solvent, then the cumulative electron dose (and the electron dose history prior to data acquisition) must be carefully controlled and reported to achieve reliable \textit{in situ} studies on chemical reactions in LC-(S)TEM experiments. Using this knowledge, we reliably characterized the wet etching behavior of multiple silica-based model systems: Stöber silica, WORM silica, and Au@Stöber silica and Fe$_3$O$_4$@WORM silica core-shell nanoparticles under conditions where the effects of the electron beam could be neglected. We showed that LC-(S)TEM can correctly reproduce the expected etching patterns for Stöber and WORM silica, as well as the formation of yolk-shell structures from Au@Stöber silica and Fe$_3$O$_4$@WORM silica core-shell nanoparticles as were reported before in literature.\textsuperscript{133,134} Furthermore, we showed how the catalyst core became a movable particle inside the shell as the yolk was formed, opening the door to extensive post-treatments of such core-silica-shell systems, as well as to manipulations of the movable core with external fields. Our results might be applicable to similar systems. For example, expansion in water under electron beam irradiation has also been reported for niobic acid nanoparticles.\textsuperscript{114} Accelerated dissolution has also been reported for many other oxides (CeO$_2$, VO$_2$, FeO$_3$, CuO, MgO and Al$_2$O$_3$).\textsuperscript{113,114} Future studies following our methodology are invited to consider whether the approach presented in the current paper also allows the imaging of such materials and their etching processes without influence of the electron beam.
Figure 6: *In situ* etching of core-shell metal(oxide)-silica nanoparticles by exposure to a 100 mM NaOH aqueous solution with a flow rate of 5 µL.min⁻¹. (A) Au-Silica. (B) Wet etching of Fe₃O₄ core/WORM Silica Core/Shell nanospheres into yolk-shell particles under the flow of a basic aqueous solution. At the left, particles at the start of the experiment. At the center, yolk-shell particles formed after 165 minutes and 330 minutes of exposure to the basic solution by the etching of the silica shell. Different areas were imaged in both (A), and (B) due to beam sensitivity (total accumulated dose of 1.5 × 10³ e⁻·nm⁻²). At the right, experimental observation of the ‘free’ diffusion of the catalytic cores. The white arrows highlight single cores rattling though the void of the yolk-shell particle.
3.4 Methods

Materials

Tetraethyl Orthosilicate (TEOS, 99%, Sigma-Aldrich), L-arginine (98%, Sigma-Aldrich), Absolute ethanol (Merk), MilliQ water (Millipore system), Sodium hydroxide (reagent grade, ≥98%, pellets, Sigma-Aldrich), Hydrogen tetrachloroaurate trihydrate (HAuCl₄·3H₂O, ≥99.9%, Sigma-Aldrich), Sodium citrate tribasic dihydrate (≥99.0%, Sigma-Aldrich), Polyvinylpyrrolidone (PVP, 10000 g/mol), Polyoxyethylene (5) Nonylphenyl Ether (IGEPAL CO-520, Sigma-Aldrich), Ammonia (NH₃, 30w%, Sigma-Aldrich), Iron chloride (FeCl₃·6H₂O, 98%, Sigma-Aldrich), Sodium Oleate (95%, TCL), Hexane (98%, Sigma-Aldrich), Oleic acid (90%, Sigma-Aldrich), 1-Octadecene (90%, Sigma-Aldrich), Potassium persulfate (K₂S₂O₈, ≥99%, Sigma-Aldrich).

Synthesis protocols

Stöber Silica Spheres

Stöber Silica particles (120 nm, PD: 11%) were synthesized with a simple one pot synthesis. A 1000 mL round bottom flask was cleaned thoroughly with ethanol and milliQ water. Then 500 mL of absolute ethanol was added. 33.3 mL of ammonia was added and the flask was stirred at 600 rpm. Then 20 mL (90 mmol) of TEOS was injected on the top of the stirring cone. The flask was sealed and stirred overnight. The particles were cleaned by centrifugation steps with ethanol and milliQ water.

Amino Acid-Catalyzed (AAC) Silica Spheres

AAC Silica particles (113 nm diameter, PD: 3%) were synthesized with a three step synthesis based on previous literature. All glasswork was etched of residual silica of former silica synthesis via a base bath (2-3 days in a saturated solution of KOH in ethanol, rinsed with milliQ water). First, 28 nm cores were synthesized as follows: in a 500 mL 1-neck flask 182.5 mg (6 mM) L-arginine was dissolved in 169 mL milliQ water. The mixture was heated to 70°C and stirred slowly (200 rpm). After 1 hour, 11.2 mL (49 mmol) of TEOS was added slowly via the wall. The reaction mixture was stirred for 1 day to complete the synthesis. These cores were overgrown up to a diameter of 55 nm as follows: in a 500 mL 1-neck flask (base bath, KOH in ethanol) 183.5 mg (6 mM) L-arginine was dissolved in 169 mL milliQ water. The mixture was heated to 70°C and stirred slowly (200 rpm). After 1 hour, 11.2 mL (49 mmol) of TEOS was added slowly via the wall. The reaction mixture was stirred for 1 day to complete the synthesis. These nanoparticles were overgrown up to the final 113 nm diameter as follows: in a 500 mL 1-neck flask (base bath, KOH in ethanol) 182 mg (0.006 M) L-arginine was dissolved in 169 mL milliQ water. 19.3 mL of the dispersion of 28 nm silica particles was added. The mixture was heated to 70°C and stirred slowly (200 rpm). After 1 hour, 11.2 mL (49 mmol) of TEOS was added slowly via the wall. The reaction mixture was stirred for 1 day to complete the synthesis. If desired, the final size of AAC spheres could be tuned by tuning the initial volume of TEOS (Figure SS1).

Water-in-Oil Reverse Microemulsion (WORM) Silica Spheres

WORM Silica spheres (30 nm diameter) were synthesized following the water-in-oil reverse microemulsion method. 20 mL of cyclohexane and 1.6 mL of IGPAL CO-520 were mixed and stirred for 15 minutes. To this solution, 120 µL of NH₄OH solution and 30 µL of milliQ water were added.
After 15 minutes, 360 µL (2 mmol) of TEOS was added and the reaction mixture was left under stirring for 12 hours at room temperature. The resultant colloids were centrifuged (7500 g, 15 min) and washed with absolute ethanol. The solid product was then redispersed in ethanol.

**Au@Stöber Silica Core-Shell Spheres**

Citrate stabilized spherical AuNPs (15.5 nm diameter) were synthesized using the sodium citrate reduction method:\textsuperscript{77,79} 200 mL H$_2$O and 2.0 mL of an aqueous solution containing 10 g/L H$\text{AuCl}_4$·3H$_2$O were added to a 500 mL two-neck flask with stirring bar and reflux condenser. The flask was placed in an oil bath and heated until boiling. Then, 6.0 mL of an aqueous solution containing 10 g/L sodium citrate tribasic dihydrate was rapidly added under vigorous stirring (1200 rpm) and the mixture was left refluxing and stirring for 15 min. during which a colour change from yellow to dark blue to pink and finally deep red was observed. The stirring was slowed down to 400 rpm and the mixture was allowed to cool down to room temperature. Au@Stöber NPs were synthesized as follows: 2.4 mL 10 wt.% PVP\textsuperscript{81} in water was added to 100 mL of the as synthesized AuNPs and stirred (300 rpm) for 24 h. The PVP-coated particles were collected by means of centrifugation (15000 g, 1 h.), redispersed in 100 mL ethanol and added to a 300 mL Erlenmeyer flask. Then, 10 mL ammonium hydroxide solution was added, followed by 0.2 mL of a 10% (V:V) TEOS in ethanol solution under vigorous stirring (1200 rpm) and left stirring. After 90 minutes, another 0.4 mL 10% TEOS in ethanol solution was added and left to react for 90 minutes after which the particles were collected using centrifugation (15000 g, 1 h.), redispersed in ethanol, centrifuged and finally redispersed in 10 mL ethanol. The ethanolic particle dispersion was stored at room temperature for at least 1 year without loss of colloidal stability.

**Magnetite (Fe$_3$O$_4$)@WORM Silica Core-Shell Spheres**

Monodisperse magnetite nanoparticles (9 nm diameter) were prepared from an iron oleate precursor by thermal decomposition as described in literature.\textsuperscript{138} The method involves two steps. In the first step, the metal-oleate precursor was synthesized by reacting iron chloride (FeCl$_3$.6H$_2$O) with sodium oleate. In the second step, this metal-oleate precursor was further heated to 320 °C using the high boiling point solvent 1-Octadecene. The solution was kept at this temperature for 30 minutes, which lead to the nucleation and growth of the nanoparticles. The resulting solution was washed with excess acetone and collected by centrifugation and dispersed in hexane or cyclohexane. To synthesize the core-shell magnetite-silica spheres (40 nm diameter) we followed the water-in-oil reverse micro emulsion method.\textsuperscript{87,136,137} In a typical synthesis procedure 2.5 mg of Fe$_3$O$_4$ nanoparticles stabilized by oleic acid as ligands was dispersed in 20 mL of cyclohexane and 1.6 mL of IGPAL CO-520 and were stirred for 15 minutes. To this solution, 120 µL of NH$_4$OH solution and 30 µL of milliQ water were added. After 15 minutes, 360 µL (2 mmol) of TEOS was added and the reaction mixture was left under stirring for 12 hours at room temperature. The resultant colloids were centrifuged and washed with absolute ethanol. The solid product was then dispersed in ethanol.

**Ex situ etching experiments**

Nanoparticles were dispersed in plastic vials with final concentrations of 0.6 mg.mL$^{-1}$ (10 mM) in NaOH aqueous solutions with the desired base concentrations and were left to react for the desired amount of time. Samples were collected by drop casting on Formvar/Carbon Film 200 Mesh Copper TEM grids after one cleaning step consisting of 10 minutes of centrifugation at 20000 g and redispersion in milliQ water.
In situ liquid cell experiments

Experiments were performed using a liquid cell dedicated holder (Hummingbird Scientific, USA). Heating LC-(S)TEM experiments were performed using a liquid cell dedicated holder with heating capabilities (Protochips, USA). Silicon chips with silicon nitride ($\text{Si}_x\text{N}_y$) windows ($50 \times 200 \mu\text{m}$, $50 \text{ nm}$ thickness) were used to encapsulate the liquid volume. In all experiments spacers of $100 \text{ nm}$ or $250 \text{ nm}$ were used. The side of the chips which were in contact with liquid were glow discharged for 90 seconds prior use. Around $2 \mu\text{L}$ of a diluted suspension ($0.06 \text{ mg.mL}^{-1}$) of the desired nanoparticles were drop-casted on the top chip and let to dry, this way a number of particles were found to be attached to the top window during the experiments. Then the bottom chip was placed in a dedicated holder, $2 \mu\text{L}$ of milliQ water was drop-casted on it and then the top chip was placed into position aligned over the bottom window. Excess water was removed with filter paper and the cell was sealed. To flow solutions into the set-up, a Hamilton syringe pump was utilized with a 1 mL glass syringe. In heating experiments, heating ramps did not exceed $1 \degree\text{C/s}$ slopes to avoid bubble formation.

Sample characterization and Imaging

Dynamic light scattering (DLS)

The ex situ etching of AAC silica spheres was followed via DLS with a Zetasizer Nano ZS. A dispersion of AAC silica spheres with a final particle concentration of $0.6 \text{ mg.mL}^{-1}$ was prepared in an aqueous solution with the desired NaOH concentration. The particle size was measured continuously every 10 seconds, and averaged per minute.

(S)TEM

Imaging at 200 keV was carried out with a Tecnai-F20 (200 keV) transmission electron microscope (TEM, Thermo Fisher Scientific) equipped with a field emission gun. Imaging at 120 keV was carried out with a Tecnai 120C transmission electron microscope (Thermo Fisher Scientific). Scanning TEM ((S)TEM) imaging was performed with an annular dark-field detector (ADF, E.A. Fischione Instruments Inc., Model 3000, USA) with a camera length of 120 mm. In order to calculate the electron dose rate the beam current in vacuum was measured for each spot size and the resulting electron dose rate was calculated by dividing the beam current by the total frame size as follows:\(^{37}\):

$$d = \frac{I_e}{eA} \quad (3.1)$$

Here $d$ is the electron dose rate, $I_e$ is the beam current, $e$ is the elementary charge, and $A$ is the total frame size determined by the magnification. Free-hand lithography was performed by manually moving the position of a stationary electron beam with a beam current of 70 pA.

Author contributions

A. Grau-Carbonell and S. Sadighikia performed the LC-(S)TEM experiments, nanoparticle synthesis, data analysis and manuscript preparation. T.W. assisted in the LC-(S)TEM experiments. R. van Dijk-Moes, R. Kotni and M. Bransen performed nanoparticle synthesis.
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3.5 Supplementary information

Videos

Supplementary videos can be downloaded free of charge from:
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Figure S1: Size of AAC particles vs TEOS volume. A. The final size of AAC silica particles could be tuned by choosing the appropriate amount of TEOS per synthesis. B. The relation between the initial amount of TEOS and the final diameter of the AAC silica spheres showed a near linear trend when considering a volumetric correction for TEOS.
Figure S2: A The Ex-situ (bulk) etching of AAC silica spheres spans over a period of days. The initial concentrations of base determine the extent of the etching, and thereby the duration of the process (the initial concentration of silica was 10 mM). The final diameter of the particles shows a strong and clear dependence to the initial NaOH concentration. B Zoomed in section up to 10 mM of etchant concentrations.

Figure S3: The progression of the polydispersity (A), and standard deviation (B) in time during ex-situ etching of AAC silica particles with an initial diameter of 113 nm and polydispersity of 3%.
Figure S4: Etching of AAC silica (diameter: 113 nm, concentration: 10 mM) in HF aqueous solutions (A). B shows diameter of the different batches exposed to different HF concentrations. The scale bars indicate 100 nm.
Figure S5: Free-hand lithography of AAC silica nanoparticles by a 70 pA condensed electron beam in water. SCM stands for Soft Condensed Matter, which is the name of our research group. Scale bar is 100 nm.
Figure S6: Calculations of the steady-state concentrations of radiolysis radicals of water (red line) and 100 mM NaOH (blue line). Hydrated electrons increased more drastically in 100 mM NaOH than in water. The OH\(^-\) concentration in 100mM NaOH was clearly dominated by the initial base concentration.
Figure S7: Expansion phase of AAC silica (370 e⁻/nm²/s⁻) with and without potassium persulfate (K₂S₂O₈) under 200 keV STEM imaging. A. In water. B. In 100 mM NaOH. Potassium persulfate should scavenge hydrated electrons, that in turn should increase in basic media. Scavenging hydrated electrons does not show to be effective in preventing or slowing down the expansion phase of AAC silica nanospheres under 200 keV electron beam irradiation. Each condition contains data of single particles from independent experiments, showing consistent reproducibility.

Figure S8: Behaviour of AAC silica particles in water and 100 mM NaOH under 120 keV STEM imaging (960 e⁻/nm²/s⁻). A. Behaviour in water. B. Expansion phase in 100 mM NaOH. Silica shows the same expansion and then accelerated etching that we observed under 200 keV irradiation. Also, expansion is more severe in basic aqueous media. The threshold for knock-on damage in silicon is 140 keV. Our results suggest that ionization and not knock-on damage is the main driver of the expansion and shrinkage phases.
Figure S9: Behaviour of AAC silica particles in water under 200 keV STEM imaging (500 e⁻·nm⁻²·s⁻¹) at different temperatures. Silica shows a similar behaviour at all temperatures. Since the heating from the electron beam irradiation is supposed to stay at a few degrees, our results suggest that local heating by the beam should not be of importance the expansion and accelerated dissolution of silica particles under STEM imaging in water.
Figure S10: Etching of Stöber silica (diameter: 102 nm, concentration: 10 mM). A Hollowing is observed when Stöber silica spheres are etched both with NaOH or HF aqueous solutions. B and C show the diameter of the different batches exposed to different etchant concentrations. The hollowing of Stöber silica was observed above 8 mM NaOH and 0.16 wt % HF. The scale bars indicate 100 nm.
Chapter 4

Low-dose liquid cell electron microscopy investigation of the complex etching mechanism of rod-shaped silica colloids

Understanding the chemical structure of rod-shaped silica colloidal particles is attainable by investigating their etching mechanism in solution. Liquid Cell (Scanning) Transmission Electron Microscopy (LC-(S)TEM) is a promising technique through which the etching of these particles can be observed in real time, and at the single particle level without possible deformations induced by the surface tension of dried particles. However, the presence of high energy electrons, and the different geometry in LC experiments may alter the conditions of \textit{in situ} experiments compared to their \textit{ex situ} counterparts. Here we present a controlled low-dose LC-STEM study of the basic etching process of micron sized silica rods that are immobilized on the SiN window of a liquid cell. The results show that using low-dose imaging conditions, combined with a low accumulated electron dose, and optimized flow rates of solutions allow for investigation of the chemical etching mechanism of silica colloidal particles using the LC-(S)TEM technique with negligible effects of the electron beam. A comparison of \textit{ex situ} etching experiments with LC-STEM observations show that the LC geometry can play a crucial role in LC-STEM experiments where the diffusion of the etching particles is important, which should be considered during the interpretations of LC-STEM results.

4.1 Introduction

The widespread application of anisotropic colloids in the self-assembly of new materials is driven, amongst others, by the ability of such systems to also form colloidal liquid crystal phases. Due to the great potential of anisotropic particles in chemical, electrical, and optical applications, they have attracted much attention.\textsuperscript{139–147} New complex functional materials are achievable by self-assembly of these colloidal building blocks.\textsuperscript{148–150} Colloidal silica particles are also of interest because of their use in physico-chemical studies of colloidal model systems. The ease of chemical modification of colloidal silica particles’ surfaces by using various types of functional groups, allows for making a vast range of silica particles with different functionalities and interparticle interactions.\textsuperscript{65,98,151} The recently developed model system of (fluorescent) silica rods by Kuijk \textit{et al}.\textsuperscript{152} is a powerful model system to study their self-assembly into various liquid crystalline phases in real space.\textsuperscript{65,152–154} The synthesis procedure of these rod-shaped silica particles is a simple one-pot synthesis in which ethanol, water, sodium citrate, and ammonia, are mixed with a solution of polyvinylpyrrolidone (PVP) in 1-pentanol to arrive at a dispersion of water with dissolved ammonia, PVP and citrate. Silica rods start to grow upon the addition of the strongly apolar tetraethyl orthosilicate (TEOS) to the pentanol oil phase. The growth of these rods takes place from the water-in-oil emulsion droplet which starts deposition of silica on the oil-water interface after which the rod grows from silica deposited from the watery droplet attached to the growing end of the rods resulting in an anisotropic bullet shaped particle with a flat end where the watery droplet was attached and a rounded tip due to the anisotropic supply of hydrolyzed TEOS.\textsuperscript{65,98} Although the synthesis of these rod-shaped particles has been studied extensively, there are few studies on their chemical composition. Recently, it has been revealed that these rod-shaped silica particles can be transformed into a cone-shaped colloidal silica particles upon mild etching by NaOH in water.\textsuperscript{155} Understanding the chemical structure of these particles is key to reveal the mechanism of this transformation, which also opens the way to obtain other novel particle shapes.

In order to obtain a fundamental understanding of the etching process of rod-shaped silica particles at the single particle level, we have taken a direct approach of imaging this process \textit{in situ} using Liquid Cell Scanning Transmission Electron Microscopy (LC-STEM).\textsuperscript{26,156,157} In this research we
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wanted to determine if LC-(S)TEM could be used as a technique to study the chemical composition of these rod-shaped silica particles by monitoring their etching mechanism in a basic environment in real time without affecting the process by the observation. However, the presence of high energy electrons as well as the confining geometry of the liquid cell (LC), combined with the fact that only particles stuck to the cell window can be continuously observed in time, could all significantly modify the etching process as compared to etching which takes place while particles are dispersed and undergoing Brownian motion. Furthermore, it is known that the electron beam can affect the imaging area and its surroundings in both direct and indirect ways and chemical reactions could be significantly altered by the electron beam. In order to validate the results in a LC-(S)TEM experiment, we need to control a large number of variables which affect the ongoing chemical and physical processes inside the cell. Therefore, for in situ monitoring of a chemical process, we need to minimize the influence of the electron beam, optimize the flow rate of solutions, and understand the effect of the liquid cell geometry. A known effect of electron irradiation is the growth or degradation of nanomaterials induced by reducing or oxidizing environments due to the presence of reactive radicals and molecular species formed by electron-solvent interactions. So far, by utilizing different solvents and scavengers, the electron beam induced nucleation, growth and degradation of various types of nanomaterials were studied using the LC-(S)TEM technique. However, there are few studies on direct monitoring of a chemical reaction with LC-(S)TEM. Etching of rod-shaped silica particles is a nice model process for investigating if LC-(S)TEM can be used for in situ monitoring of chemical reactions on colloids. If the effects of the electron beam can be minimized, the etching mechanism and therefore the inhomogeneous chemical composition of these particles can be studied at the single particle level in real time at a high spatial resolution. Importantly, LC-STEM also would get rid of artifacts that can be induced by strong drying forces that occur if particles are dried on TEM grids without lengthy procedures such as supercritical drying.

Rod-shaped silica colloids prepared by ammonia-catalyzed hydrolysis and condensation of tetraethyl orthosilicate in water droplets, containing poly-vinylpyrrolidone cross-linked by citrate ions in pentanol, were found to have an inhomogeneous chemical structure both along the length of the particle as in the perpendicular direction along the diameter of the particle. Here, we show that by tuning the pH of the LC solution while the particles are stuck to a Si$_x$N$_y$ window, while continuously flowing NaOH basic solutions through the cell and optimizing the flow rate, together with using low-dose rate imaging conditions (1-10 e$^-$/nm$^2$/s$^{-1}$), direct observation of the silica rods etching mechanism is achievable. Furthermore, using a discontinuous imaging approach by blanking the beam for a certain amount of time between recording the frames, we minimized the accumulated electron dose (e$^-$/nm$^2$) on the particle. The accumulated electron dose is also known to have a great impact on LC-(S)TEM results in certain cases. Comparison with ex situ etching experiments showed that the low electron dose rate in combination with a low total electron dose, which was at least one order of magnitude lower than previous LC-(S)TEM studies, played a significant role in observing the ‘real’ chemical process with negligible effects of the electron beam. However, the effects of the confining geometry of LC in combination with the fact that only particles immobilized on the window were observed, where the Brownian motion of the particles was completely suppressed, should be taken into account. Altered accessibility and diffusion rates could result in different etching pathways of the particles in comparison to the etching happening to particles that diffuse freely in the reactive solution. Our LC-STEM observations revealed how these rod-shaped silica particles undergo inhomogeneous etching along the length of the rod in an aqueous NaOH solution, and how rods with a certain internal morphology for certain NaOH concentrations finally turned into a cone-shaped silica particle. Further validation of the LC-STEM observations for the etching of rod-shaped silica particles was carried out using even more complex structured segmented silica rods.
with a known inhomogeneous chemical structure. Here, the degree of condensation along the silica rod was tuned in such a way that a desired segment of the rod had a less condensed silica structure so that faster etching of that segment was expected simply due to the smaller number of siloxane bonds that needed to be broken as compared to the rest of the particle.\textsuperscript{99,102} This inhomogeneous silica structure could be obtained during particle synthesis by changing the reaction temperature, precursor concentration, and/or ethanol concentration.\textsuperscript{172} Finally we used the information obtained to explore the chemical composition of newly developed, even more complexly structured silica rod-shaped particles known as crooked silica rods. Such crooked rods have been developed recently in our group\textsuperscript{173} and that of others\textsuperscript{174} as such particles can form interesting new colloidal liquid crystal phases.\textsuperscript{175}

4.2 Results and Discussion

Rod-shaped silica particles were synthesized as described by Kuijk \textit{et al.}\textsuperscript{152}. In this process rods grow from a water-in-oil emulsion droplet which is rich in hydrolyzed TEOS, and because of this anisotropic supply of precursor the particle grows from the droplet only in one direction. Since the precursor concentration is highest at the start of the reaction, growth takes place fastest during that stage.\textsuperscript{155} The high concentration of the silicon hydrolyzed alkoxide at the beginning of the reaction results in a porous structure by the addition of oligomers near the round tip of the rod, whereas the lower concentration near the end of the growth results in a more densely condensed structure by the addition of monomers.\textsuperscript{155,176} Furthermore, in the radial direction the chemical composition is inhomogeneous as well; a low concentration of silicon hydrolyzed alkoxide in the pentanol phase result in the formation of a condensed silica shell around the particle. This shell is thickest for the part that is grown first.\textsuperscript{155} Dissolution of silica in alkaline solutions occurs when the hydroxide ion (OH\textsuperscript{−}) attacks the silicon atom and replaces one of the siloxane bonds in a transition state with coordination number five.\textsuperscript{99} In addition, the free energy gain associated with the weakly acidic silanol groups that strongly increases the solubility of silica at pH above \(\sim 10\). Silica rods are typically stable in water for years. By minimizing the electron beam irradiation effects, we showed that silica rods are stable also during the LC experiments in pure water (Figure S4).

To better understand the influence of the LC-STEM parameters on our \textit{in situ} etching experiments, we initially optimized these parameters to the extent where the effects of the electron beam could be neglected by investigating the effects of varying the flow rate, NaOH concentration, and accumulated electron dose on the resulting LC-STEM observations of the etching mechanism of silica rods. Furthermore, we investigated the effect of the liquid cell geometry on the etching mechanism of these rods by performing \textit{ex situ} counterpart experiments that could be more directly compared to etching experiments inside the LC.

\textbf{Static \textit{in situ} etching experiments}

First, static (without flow through the cell) LC-STEM experiments were performed by dispersing the particles in a 100 mM NaOH aqueous solutions and preparing the liquid cell by drop-casting 2 \(\mu\)L of this solution on the Si\(_x\)N\(_y\) chips. No etching was observed after 60 minutes of continuous imaging at an electron dose rate of 18 e\textsuperscript{−} nm\(^{−2}\) s\(^{−1}\) of one particle at this high NaOH concentration (Figure S1). This is surprising, as silica is expected to dissolve at such a high pH. There are two possible causes for this: The sample volume that we used to prepare the liquid cell was quite small (2 \(\mu\)L). It is likely that the solution became immediately saturated with silicate ions due to the presence of a large number of particles in the small volume of the basic solution, and etching did not take place as the pH decreased by the activity of the resulting silanol groups and the solubility
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limit was already reached. The higher initial concentration of silica particles (350 mM) compared to the NaOH concentration (100 mM) also roughly confirms this assumption. It is noteworthy that decreasing the concentration of the silica rods is only possible to a certain minimum number of particles. Below this concentration we were not able to perform the experiment since in most of the experiments there were no particles on the LC window. By performing the static etching experiment at the lowest possible concentration of particles, we did not observe the etching process. We propose that this minimum concentration of silica particles is still enough to deplete the NaOH ions in the solution since the cell volume is approximately tenth of a micro liter.

An other possible explanation is associated with pH changes upon electron beam irradiation in LC-(S)TEM experiments. Calculations involving many radiolysis products, which are always made for pure water and do not take the presence of the silica rods into account, indicate that the pH of the solution in the liquid cell drops upon electron beam irradiation, where alkaline solutions are most strongly affected by the electron beam irradiation. Therefore, the pH of the solution could have decreased when it was continuously exposed to the electron beam, thereby inhibiting the etching process. However, the irradiated volume was small compared to the total volume of the liquid cell, and this contribution was most likely small.

Unfortunately, measuring the pH of the remaining solution after a static experiment showed to be technically impossible with our current set up. Nevertheless, the LC-STEM observations also showed that some dissolved silica redeposited on the surface of the particle and/or on the Si$_x$N$_y$ window in the field of view. This silica could only have come from the dissolution of some of the rods, which clearly therefore is not always visible by looking at changes of the particle shape. It has been shown that the silica redeposition happens in LC-STEM and that it is strongly related to the electron beam irradiation.

Effect of flow rate on in situ LC-STEM etching experiments

The ability to flow NaOH aqueous solutions through the cell, enabled us to overcome several of the issues associated with the previous LC-STEM results by continuously renewing the basic solution in the imaging area. We used a syringe pump to flow the NaOH solutions through the cell at a controlled flow rate within the range of 0.1 $\mu$L.min$^{-1}$ to 5 $\mu$L.min$^{-1}$. Figure 1 shows a few image series of the etching process of rod-shaped silica particles in time. In a 50 mM NaOH solution the etching process was recorded for 6 hours while the solution was flowing through the cell with a flow rate of 0.3 $\mu$L.min$^{-1}$. The observations showed that some particles were passing through the field of view. These particles must have been diffusing inside the cell from the beginning of the experiment and were dragged along by the flow. However, particles that were initially attached to the Si$_x$N$_y$ window remained in the field of view during the complete etching process (6 hours), which enabled us to record the complete etching mechanism of silica rod-shaped particles. We will explain the etching mechanism in the further sections in detail. In order to investigate the effect of the flow rate in our observations, we performed several LC-STEM experiments using different flow rates. Supporting Movie 1 shows a video of a LC-STEM experiment using a flow rate of 5 $\mu$L.min$^{-1}$. These observations show particles initially attached to the Si$_x$N$_y$ window detaching from the window and moving out of the field of view 10 minutes after the flow was started. This indicates that a high flow rate can detach the silica rods from the Si$_x$N$_y$ window within a short time, and observation of the full etching process would then not be possible. The NaOH solution slightly etched the particles and made them attach too loosely to the Si$_x$N$_y$ window, and the 5 $\mu$L.min$^{-1}$ flow rate was capable of detaching these particles from the window. The role of the base in the detachment was confirmed by a reference experiment, where the same flow rate but with deionized water did not detach particles even after 1 hour of continuous flow. Repeating the experiments with
different flow rates revealed that observation of the etching process was feasible within the range of 0.1 µL.min⁻¹ to 1 µL.min⁻¹.

Figure 1: STEM image series showing optimized flow rate of basic solutions in silica rods etching process in real time. The particles were imaged for 6 hours while a 50 mM NaOH solution was flowing through the cell at 0.3 µL.min⁻¹. A total number of 94 frames were recorded with an electron dose rate of 7 e⁻.nm⁻².s⁻¹ and a total accumulated electron dose of 1.1 × 10⁴ e⁻.nm⁻². Scale bars: 1 µm.

To better understand the effect of the flow rate on the etching process, the drag force on the silica rod-shaped particles was estimated by measuring the flow velocity in the main channel (window) of the liquid cell. To obtain the flow velocity, another LC-STEM experiment was conducted by flowing 400 nm spherical silica particles through the cell. The cell configuration was the same as used for the etching experiments with silica rod-shaped particles. A diluted sample of silica spheres in deionized water was loaded in the syringe and the flow was started with a flow rate of 5 µL.min⁻¹. This experiment was repeated for flow rates of 4, 3, 2, and 1 µL.min⁻¹ with the same LC. To increase the accuracy of the experiment, image recording was started 20 minutes after changing the pump speed to stabilize the new flow rate. Sequences of images were recorded with a scanning time of 0.5 seconds per frame and a total duration of 30 minutes for each flow rate. The image sequences were analyzed by tracking the positions of the particles from image to image (MTrackJ plug-in for Image J (Fiji version), Figure S3). The trajectories recorded at different pump speeds were analyzed to obtain a relationship between the flow velocity \( v \) in the main channel and the flow rate setting on the syringe pump. Results shown in Figure 2A show that the flow velocity changes linearly with the flow speed setting of the pump. Therefore, for this microfluidic system it can be concluded that
the flow velocity was proportional to the pump speed, assuming that the velocity of the particles is a measure of the velocity of the liquid front in the main channel thus neglecting Brownian motion. Knowing the flow velocity, the drag force on the rod-shaped silica particles can be estimated as follows:

\[ \vec{F}_{\text{drag}} = -\xi \vec{v} \]  

(4.1)

where \( \xi \) is the drag coefficient of the rods and \( \vec{v} \) is the velocity of the particle relative to the flow. \( \xi \) can be found from the expression for the translational diffusion coefficient \( D_t \) since the diffusion coefficient is equal to \( k_B T \xi^{-1} \). For dilute suspensions, the expression for the translational diffusion coefficient of finite rods, modeled as cylinders, is:

\[ D_t = \frac{k_B T (\ln(\frac{L}{D}) + \gamma)}{3\pi \eta_0 L} \]  

(4.2)

where \( k_B T \) is the thermal energy, \( L \) the total head-to-tail length of the rod, \( D \) the diameter and \( \eta_0 \) the viscosity of the solvent. The factor \( \gamma \) is the so-called end-effect correction which is a function of the rod dimensions and should be applied when \( 2 < L/D < 30 \):

\[ \gamma = 0.312 + 0.565 \frac{D}{L} - 0.100 (\frac{D}{L})^2 \]  

(4.3)

If we derive the drag coefficient \( \xi \) from equation 4.2 and insert it in equation 4.1, the drag force for rod-shaped particles can be calculated as follows:

\[ \vec{F}_{\text{drag}} = -\frac{3\pi \eta_0 L}{\ln(\frac{L}{D}) + \gamma} \vec{v} \]  

(4.4)

Calculations of drag forces for different flow velocities show that the drag force on the particle increases almost linearly with the flow rate (Figure 2B). Using a 5 \( \mu \text{L.min}^{-1} \) flow rate the drag force is 3 times bigger than when using 1 \( \mu \text{L.min}^{-1} \) which according to the LC-STEM observations was enough to wash all the particles away from the Si\(_x\)N\(_y\) window. The calculations also confirm that the drag force on spherical nanoparticles would be too low to remove them from the Si\(_x\)N\(_y\) window even for the highest flow rate in our system (5 \( \mu \text{L.min}^{-1} \)). It is noteworthy that equation 4.1 does not take into account the hydrodynamic interactions between the particle and the cell wall. However, since the ratio between drag force for different flow rates is of importance in our study, we neglect this interaction, which is assumed to be proportional to the flow rate.

During the LC-STEM experiment with silica spheres to calculate the flow rate another interesting observation was made. Particles which were flowed into the cell tended to go out of the window (Figure S2). This was the case for particles which were coming in the Si\(_x\)N\(_y\) window from both the top and the bottom parts. This also happened regardless of the flow rate. This observation is important for the self-assembly experiments via LC-STEM because for these experiments the maximum number of particles is needed in the field of view.

**Effect of base concentration on in situ etching of silica rods**

In order to analyze the dissolution kinetics of silica rod-shaped particles and also the effect of the base concentration on the final shape of the particle, we performed several LC-STEM experiments using three different NaOH concentrations (10 mM, 50 mM, 100 mM) at a fixed flow rate of 0.3 \( \mu \text{L.min}^{-1} \) and a fixed electron dose rate of 7 \( \text{e}^{-}\text{nm}^{-2}\text{s}^{-1} \) together with a fixed total accumulated electron dose of \( \sim 1000 \text{ e}^{-}\text{nm}^{-2} \). Since the etching kinetics are strongly size dependent, we analysed particles with similar particle sizes in each experiment. Figure 3 shows the effect of the three different NaOH
Figure 2: A. The linear relationship between the flow velocity and the flow rate B. The linear relationship between the drag force on a silica rod-shaped particle with dimensions: \( L = 2.6 \, \mu \text{m} \) \( D = 300 \, \text{nm} \) and the flow rate. The drag force on the particle for 5 \( \mu \text{L.min}^{-1} \) is 3 times bigger than the same for 1 \( \mu \text{L.min}^{-1} \).

concentrations on the etching mechanism and etching kinetics of silica rods. The time at which the etching became visible was different for each of these base concentrations. The first signs of etching, which happened at a region in the middle of the particle but closer to the flat end, was observable after approximately 60 minutes for 100 mM NaOH, observable after about 90 minutes for 50 mM NaOH and after about 120 minutes for 10 mM NaOH. We designate the region of the particle in which the etching started first as the sensitive part of the particle. LC-STEM observations show that initially the etching rate was the highest at this sensitive part of the particle, while it gradually decreased as the etching proceeded in time. This could be due to the presence of a thin silica shell around the particle which has a higher cross-linked SiO\(_2\) structure acting as a protective layer against etching.\(^{155}\) This thin silica layer forms around the particle by condensation of TEOS from the oil-phase during particle synthesis and is more prominent around the rounded tip of the particle since this part is exposed to the growth solution for a longer time and it stayed attached to the SiN membrane during the whole \textit{in situ} etching experiment. Furthermore, the flat end of the particle is the most condensed part of the particle due to the slow condensation of silica during the last stages of particle synthesis. Therefore, the LC-STEM observations also suggested that the region in the middle of the particle closer to the flat end was the part of the particle most sensitive to the etchant. The etching process began from this part of the particle and that was the case for all particles with different sizes and for all base concentrations.

**Etching mechanism of rod-shaped silica particles**

Overall, the etching mechanism of rod-shaped silica particles in a confining LC geometry took place in three main steps (Figure 4 and supporting movie 2). By flowing the NaOH aqueous solution into the cell, the etching process started and became observable after a certain period of time for each base concentration at the sensitive part of the particle where the silica shell is the weakest. As was mentioned before, it is likely that some silica was already dissolved without this being visible as a change in morphology and/or density of the particles. This starting region of dissolution was the same for all particles regardless of the size of the particle and the base concentration. Nevertheless, the time when the dissolution started to become visible did depend on the particle size. Next, the etching continued by transport of silica from the inner core through the thin shell at the same
region for a while, making this part of the rod thinner in time. Slight etching also happened at the rounded tip and the flat end of the particle, however, the etching rate at these regions was found to be significantly lower than the etching rate at the edge of the sensitive part of the particle, due to the stronger shell around the rounded tip of the particle and the more condensed silica at the flat end. Finally, after a longer etching period, necking happened at the sensitive region of the particle, eventually leading to break off. The resulting shape after this step was a cone-shaped silica particle which had a smaller length compared to the initial rod-shaped particle (see also supporting movie 2 for a LC-STEM demonstration of the final stage of the etching mechanism). The necking-and-

Figure 3: Etching mechanism of silica rods for different concentrations of NaOH solutions. The flow rate was 0.3 $\mu$L.min$^{-1}$, the electron dose rate was 7 e$^{-}$nm$^{-2}$s$^{-1}$, and the total accumulated electron dose was $\sim$ 1000 e$^{-}$nm$^{-2}$ for all experiments. The etching process time is different for different base concentrations, however the etching mechanism is the same. Scale bars: 1 $\mu$m
breaking step of the etching process was not previously known from *ex situ* experiments\textsuperscript{155} and it was only revealed during the direct observation of the etching process at a single particle level using LC-STEM.

It is worthwhile to mention that the structural characterization cannot be provided directly as there exists no technique that can probe the degree of condensation on the single particle level. Using NMR it is possible to determine the degree of condensation; however, this will result in an average value over the whole system. As such, we determined the degree of condensation through an indirect method (etching) at the single particle level in real time to reveal the different degree of condensation through the length and the diameter of the rod.

![Figure 4: Three step etching mechanism of rod-shaped silica particles attached to the Si$_x$N$_y$ window in basic solutions revealed by LC-STEM experiments (top), and its schematic representation (bottom). In this experiment the NaOH concentration was 100 mM, the flow rate was 0.3 $\mu$L min$^{-1}$, the electron dose rate was 7 e$^{-}$ nm$^{-2}$ s$^{-1}$, and the total accumulated electron dose was $\sim$ 1000 e$^{-}$ nm$^{-2}$. Scale bars: 1 $\mu$m.](image)

**Effect of accumulated electron dose on the etching of silica rods**

We also addressed the question concerning the extent to which the observed etching process was influenced by the electron beam irradiation. This influence can be revealed from a change in the etching rate and/or the etching mechanism during the LC-STEM observations. Using a discontinuous imaging approach, we modified the blanking time of the electron beam between recordings of the images of the particles to investigate the accumulated electron dose effects on the etching process. To this end, we exposed particles to electron beam irradiation for different periods of time by recording a different total number of frames for each particle while fixing the electron dose rate at 7 e$^{-}$ nm$^{-2}$ s$^{-1}$ with these imaging conditions: 1024 $\times$ 1024 pixels, 24 $\mu$s dwell time, and 6.12 nm pixel size. Figure 5 shows image series for four distinct particles imaged with different blanking times during the same LC-STEM experiment. During this experiment a solution of 10 mM NaOH was flowed through the cell at a rate of 0.3 $\mu$L min$^{-1}$. The observations showed that the rate and the mechanism of etching was the same for all particles when they were imaged with low-dose imaging conditions, regardless of the blanking time duration between the recorded images. Furthermore, an overview of the cell after the etching stopped showed that all the particles with similar sizes etched by the same mechanism and at the same rate. This indicates that imaging the etching process of rod-shaped silica particles with low accumulated electron dose did not alter the rate nor the mechanism of the etching process for total doses lower than $3.4 \times 10^4$ e$^{-}$ nm$^{-2}$, since the accumulated electron dose never reached a threshold with the capability of affecting the chemical process. Using a high electron dose rate (>1000 e$^{-}$ nm$^{-2}$ s$^{-1}$) results in a severe shape deformation of the silica rod in the scanning direction (Figure S5). Therefore with a negligible effect of electron
beam irradiation, investigations of the etching process of this system of particles were successfully achieved.

**Effect of confining geometry and sticking of particle as opposed to Brownian motion**

Finally, *ex situ* experiments with the same particles and the same etchant were performed in order to compare them with the LC-STEM observations. Two main *ex situ* experiments were carried on. First, a sample was prepared exactly like the *in situ* sample preparation: 2 µL of the sample solution was drop-casted on one of the liquid cell chips and let to dry at room temperature to assure that a number of particles were attached to the Si₉N₉ window. Then these chips were each placed in a plastic bottle filled with 40 mL of aqueous NaOH. 100 mM and 10 mM NaOH solutions were used for these experiments. The vials were left to stand without stirring at room temperature. One sample was retrieved from its solution every 15 minutes for 7 hours after placing the chips in the bottle. For each etching time a separate Si₉N₉ chip was used in a separate bottle. Next, samples were inspected with STEM using a normal TEM holder. The STEM images confirmed that the mechanism of *ex situ* etching for the particles that were attached to the Si₉N₉ window was the same as the *in situ* etching mechanism where the rod-shaped silica particles turned into cone-shaped silica particles via necking-and-breaking. Figure 6, A and B, show the end result for this *ex situ* etching experiment (data not shown for each etching time).

A second experiment was performed with freely diffusing silica rods. The particles were redispersed in NaOH aqueous solutions. This *ex situ* etching experiment was performed with 0.5, 3, 10 and 100 mM NaOH concentrations. Samples were collected by dipping a conventional TEM grid in each solution every 15 minutes and were subsequently imaged with STEM. Figure 6, c-f, shows the end product of the etching of rod-shaped silica particles that were freely dispersed during etching, for different base concentrations (data not shown for each etching time). Etching at low base concentrations (0.5 mM) showed the same mechanism as the LC observations for etching of these particles. However, at higher base concentrations such as 10 and 100 mM NaOH the etching mechanism/product was different from the mechanism observed in the LC experiments. The STEM images show that the particles dispersed in higher base concentrations turned into sharper cone-shaped silica particles with a a larger length, indicating that the etching also occurred at the tip of these particles and the necking-and-breaking mechanism did not take place. Moreover, it was also observed that the *ex situ* etching at moderate base concentrations such as 3 mM showed both mechanisms with a dependence on the particle size (Figure 6, d). We propose that this difference in final shape is due the presence of a higher cross-linked SiO₂ shell around the particle along with the absence of diffusion inside the LC. The shell around the particle becomes thinner going from the rounded tip to the flat end because the exposure time to the growth solution decreases in this direction. Therefore, when the base concentration was high enough it completely etched this silica shell around the particle and etching took place at the tip of the particle at higher etching rates, resulting in a sharper, longer cone-shaped particle. On the other hand, at lower base concentrations only the weakest part of the silica shell etched away and etching continued at the same region by transport of silica from the inner core through the thin shell which caused the particle to break off at this point. The difference between LC etching and *ex situ* etching where particles undergo free diffusion can be explained by the fact that the particles experience different local NaOH concentrations when they have Brownian motion in the basic solution. In the LC experiments and *ex situ* control experiments, particles were attached to the Si₉N₉ window and the etching reaction took place in a diffusion-limited regime. Since the highly cross-linked silica shell was always attached to the Si₉N₉ window during the whole etching process NaOH always diffused from the weakest
Figure 5: STEM image series of four distinct particles imaged with different beam blanking time indicating different accumulated electron dose ($e^{-} \text{nm}^{-2}$) on each particle. The corresponding total accumulated electron dose is shown on each frame. Scale bars: 1 $\mu$m.
Figure 6: Ex situ etching of silica rod-shaped particles in a basic solution. Particles were attached to the Si$_x$N$_y$ window and placed in a bottle containing 40 mL of A. 10 mM, and B. 100 mM NaOH aqueous solutions without stirring. Particles not attached but freely dispersed in a bottle containing 20 mL of C. 0.5 mM, D. 3 mM, E. 10 mM, and F. 100 mM NaOH solutions. Scale bars: 1 µm for (A-C,F), 1 µm for D and 500 nm for E.

part of the shell and etching continued in that region with higher etching rates. However in the ex situ etching process where the particles diffused freely inside the basic solution the shell around the particle detached at some point due to the self-stirring effect of colloidal particles and etching continued at the tip of the particle with higher etching rates resulting in sharper cone-shaped silica particles. These results reveal that the final shape of the particles can be also tuned by immobilizing them through attachment to a substrate or having them to move freely in the etching solution. However, the higher yields obtainable through bulk etching makes the approach in which the base concentration is optimized for the desired geometries more appealing.

In situ etching of segmented silica rods

As the last validation for the LC-STEM parameters we have described to perform an in situ silica etching experiments, we analyzed segmented silica rods with a known, even more complex internal inhomogeneous chemical composition by in situ LC etching experiments. Segments of silica rods were grown under different temperature conditions. This adds differences in the internal siloxane condensation degree on top of those already discussed. Varying the temperature influences the degree of condensation of silica. A lower temperature results in a lower degree of condensation with a
faster dissolution rate, whereas an increased temperature results in a higher degree of condensation
and thus a network that dissolves more slowly.\textsuperscript{92,98,176} It is also known that segments silica rods
grown at lower temperatures have an increased diameter as the solubility of water in the pentanol
oil phase is reduced.\textsuperscript{172} Nail-shaped silica rods consisting of a segment grown at 50 °C followed by a
segment grown at 5 °C were etched by flowing 100 mM NaOH solution through the LC with these
experimental conditions: 0.3 \( \mu \text{L}\text{min}^{-1} \) flow rate, 4 \( \text{e}^{-\text{nm}^{-2}\text{s}^{-1}} \) electron dose rate, and \( 1.4 \times 10^4 \)
\( \text{e}^{-\text{nm}^{-2}} \) accumulated electron dose. As expected, etching took place at the less condensed silica
segment grown at low temperature even though it was initially thicker, whereas the segment grown
at high temperature remained apparently untouched (Figure 7, A).\textsuperscript{172} As a further illustration,
using the same experimental conditions another LC experiment was performed with rods grown at
5 °C followed by a segment grown at 25 °C, and the tip of the particle was preferentially etched
as expected (Figure 7, B).\textsuperscript{172} With a three-step growth of silica rods (25 °C, 5 °C, and 25 °C),
silica particles with a thicker but less condensed middle part were synthesized. The \textit{in situ} etching
results for this kind of silica rods are shown in Figure 7, C. The LC-STEM observations with the
same mentioned experimental conditions agree with the previous results shown by our group\textsuperscript{172}
for \textit{ex situ} etching of these particles in bulk, as the middle, less condensed segment etched preferen-
tially. Consequently, the experimental conditions determined in this work for conducting LC-STEM
chemical etching experiments of silica rods are applicable to similar systems with a negligible ef-
fect of the electron beam as well as of the LC geometry. The high spatial resolution provided by
LC-STEM also opens the door to quantifying the etching rates of silica grown at different temper-
atures. However, the silica rods used in this study consist of a complex internal distribution with
different levels of condensation, and thus any measured etching rate would not correspond to that
of a particular degree of condensation. We expect that by applying our methodology to simpler
silica colloidal particles, future studies will be able to precisely quantify the etching rates of silica
of different degrees of condensation.

Chemical structure of crooked rod-shaped silica particles

Altering the reaction temperature of the rod-shaped silica particles and/or changing the hydrolysis
rate of silica precursor by introducing partially hydrolyzed TEOS (pre-TEOS) into the reaction
system results in bending of the rod-shaped silica particles. The synthesis procedure started with
synthesizing normal rod-shaped silica particles using standard existing procedures\textsuperscript{152}. The reaction
was initially carried out above room temperature (35 °C). After one hour of reaction, pre-TEOS was
added and the solution was homogenized by shaking for \( \sim 1 \) min. Five minutes after the addition
of pre-TEOS, the reaction mixture was transferred to room temperature for 6-8 hours. As a result,
the rods grew into two segments connected at an angle. This reaction can also be carried out at
room temperature with lower concentrations of pre-TEOS.

Here, we investigated the chemical structure of these two types of crooked silica rods by \textit{in situ}
etching of these particles in basic solutions via LC-STEM. Our results help to better understand
the effect of the temperature and the pre-TEOS concentration on the chemical structure of these
intriguing particle systems and can be used to tune the bending angle of the two segments, which
is important for self-assembly studies.

We applied optimized LC-STEM conditions to study the \textit{in situ} etching of crooked rod-shaped
particles. We flowed 100 mM NaOH solutions with a flow rate of 0.3 \( \mu\text{L}\text{min}^{-1} \) and imaged the
particles using a 4 \( \text{e}^{-\text{nm}^{-2}\text{s}^{-1}} \) electron dose rate with a total accumulated electron dose below
\( 2.8 \times 10^3 \text{ e}^{-\text{nm}^{-2}} \). Figure 8 shows the etching of crooked silica rods in time. The particle in panel
A consists of two segments; the first segment with a rounded tip was grown at 35 °C while the other
Figure 7: STEM image series of 3 types of silica rods with different chemical composition and their etched shape with 100 mM NaOH solution using low-dose LC-STEM technique. **A.** Silica rods consisting of a segment grown at 50 °C followed by a segment grown at 5 °C. **B.** Silica rods grown at 5 °C followed by a segment grown at 25 °C. **C.** Silica rods grown subsequently at 25 °C, 5 °C and 25 °C. Scale bars: 500 nm.

segment with a flat end was grown at room temperature. The LC-STEM observations show that the etching of the segment with the flat end occurred faster than the other segment with the round tip. This is expected since the silica grown at elevated temperatures is more condensed compared to the silica which was grown at room temperature. Interestingly no etching was observed at the neck of the particle where the two segments were connected to each other. This can be explained by the fact that adding concentrated pre-TEOS at that point of reaction forms a condensed silica shell around the joint part of the particle which protected it from etching. The particle in panel B was grown completely at room temperature using less concentrated pre-TEOS. Although adding pre-TEOS caused the bending of the particle, the two segments were not completely separated. The *in situ* etching of this particle showed that the least condensed part of the particle was the bending point. The etching was observable at the bent region of the particle approximately 15 minutes after starting to flow the 100 mM NaOH solution. Then etching continued in both sides of the particle indicating the same silica structure of the bent rod-shaped particle. Apparently adding less concentrated pre-TEOS was not enough to protect the bent part of the particle from etching. Moreover, growing at room temperature resulted in particles with a less condensed silica structure that could be easily etched with 100 mM NaOH solution in a short time.
Figure 8: STEM image series of 2 types of bent silica rods and their etched shape with 100 mM NaOH solution using low-dose LC-STEM technique (4 e$^{-}$nm$^{-2}$s$^{-1}$ electron dose rate). The corresponding total accumulated electron dose is shown on each frame. **Top row** The bent silica rods that were synthesized at 35 °C with high concentration pre-TEOS. **Bottom row** The bent silica rods that were synthesized at room temperature using low concentration pre-TEOS. Scale bars: 1 μm

### 4.3 Conclusions

We present an optimized LC-STEM method to investigate the etching of micron sized rod-shaped silica particles in real-time and at the single-particle level with high spatial resolution. A model system of rod-shaped silica colloidal particles was used to investigate the etching mechanism of these particles in basic conditions. By utilizing low-dose electron beam conditions and by optimizing the flow-rate of the NaOH solutions during the LC-STEM observations, we showed that investigation of the etching of rod-shaped silica particles is attainable with a negligible effect of the electron beam irradiation if also the total dose is kept below 3.4 × 10$^4$ e$^{-}$nm$^{-2}$ by only taking a limited number of images. LC-STEM observations revealed that the base-induced etching occurs inhomogeneously along the length of the rod due to its inhomogeneous chemical structure. Remarkably, the LC-STEM observations revealed a three-step etching mechanism which transformed the rod-shaped silica particles into cone-shaped silica particles. The mechanism started with etching at a region in the middle of the particle closer to the flat end, which is the most etchant-sensitive part of the particle. The etching then continued at a faster rate at the sensitive part of the particle and finally necking-and-breaking occurred at the same point of the particle which yielded a cone-shaped silica particle. Comparison between the LC and the *ex situ* etching experiments suggests that the geometry of the LC plays a significant role in chemical reactions where diffusion of reactants and/or
particles are important, and that with the current equipment for LC experiments the reactions studied in this paper were in a diffusion-limited regime. The optimized LC-STEM conditions were further tested by utilizing a new class of silica rod-shaped particles where the chemical structure of the rod was tailored deliberately. The etching of segmented silica rods happened as was expected, validating the LC-STEM optimized conditions found in this work. Finally, using optimized LC-STEM conditions we revealed the chemical composition of the recently developed rod-shaped silica particles known as crooked silica rods. We expect that using similar low-dose imaging conditions and optimized flow-rates of solutions as determined in this work, will allow for investigations of chemical reactions with colloids using the LC-STEM technique with negligible effects of the electron beam, and will thus serve as a powerful new technique to probe the evolution of etching at the single particle level. Furthermore, we are confident that, although the imaging conditions w.r.t. dose rates and accumulated doses were only possible with the relatively large particles used, future and ongoing improvements of detectors and smart imaging routines will strongly push down the size and structures that can be imaged without major interference from the imaging itself! Especially, not taking continuous movies, but just a few frames is often enough to still obtain the necessary information.
4.4 Methods

Materials

Polyvinylpyrrolidone (PVP, 40000 g/mol, Sigma-Aldrich), 1-Pentanol (> 99%, Sigma-Aldrich), Ethanol (100%, Interchema), Ultrapure Water (milliQ grade, Millipore system), Tetraethyl Orthosilicate (TEOS, 99%, Sigma-Aldrich), Citrate Dihydrate (99%, Sigma-Aldrich), Ethanol (0.5 mL, Interchema, 100%), Ammonium Hydroxide (0.115 mL, Sigma-Aldrich, 28% w/w), Hydrochloric Acid (HCl, 37% ACS reagent)

Synthesis protocols

Rod-shape silica particles

Figure 9: STEM image of typical, monodisperse, silica rod-shaped particles with a length of $2.6 \pm 0.3 \, \mu m$, directly after the synthesis and before the monodispersity was increased by centrifugation to remove the smallest rods recognizable by their lack of a flat end, meaning they lost the droplet early in the synthesis, and large bullet-shaped particles grown from the larger water droplets. Scale bar: 2 \, \mu m.

Rod-shaped silica particles with a length of $2.6 \pm 0.3 \, \mu m$ and a diameter of 300 nm were prepared as described by Kuijk et al.\textsuperscript{152}. Figure 9 shows a typical STEM image of these rods. In a 1 L glass laboratory bottle, 80 g of PVP was dissolved in 800 mL of 1-pentanol by sonication for 2 hours. After all PVP had been dissolved in 2 hours, 80 mL of ethanol, 22.7 mL of ultrapure water and 5.3 mL of a 0.18 M sodium citrate dihydrate solution in water were added to the mixture of pentanol-PVP. The mixture became turbid upon shaking the flask by hand, indicating that the emulsion was formed. Afterwards, 18 mL of ammonia (25% (w/w)) was added, followed by 6.7
mL TEOS. After mixing by shaking, the mixture was left to rest and the reaction was allowed to proceed overnight. Next, to remove the emulsion droplets from the flat end of the particles, the reaction mixture was centrifuged at 1500 g for 1 hour and after removing the supernatant the particles were redispersed in ethanol. The cleaning procedure was repeated twice with water and ethanol where the reaction mixture was centrifuged at 1500 g for 15 minutes and finally the particles were redispersed in ethanol. To further increase the monodispersity of rods, cleaning steps were carried out. The particles were centrifuged 3 times at 700 g for 15 minutes and the supernatant containing smaller rods was removed. As the last step of the cleaning procedure the particles were centrifuged at 10 g for 1 hour to remove the larger rods and aggregates, by removing the sedimentation from the sample. For more information about rod-shaped silica rods see references\textsuperscript{65,152–155}.

**Segmented silica rods**

Segmented silica rods were prepared using regulation of the growth temperature\textsuperscript{181}, based on the original work of Kuijk \textit{et al.}\textsuperscript{152}. In order to vary the chemical composition along the length of the rods, we changed the temperature during the course of the reaction. To this end we performed the reaction at a scale 40.0 mL of emulsion in a 40 mL vial. The vial was placed in a preheated hot air oven after the addition of TEOS. The bottles were cooled down at a slow rate to avoid strong convection in the bottle. The temperature was changed during the reaction in the following schemes:\textsuperscript{172}

1. Nail shaped: 50 °C (2 h), 5 °C (22 h) (Figure 10A)

2. Less-condensed tip: 5 °C (2 h), 25 °C (22 h) (Figure 10B)

3. Less-condensed middle part: 50 °C (1 h), 5 °C (4 h), 25 °C (22 h) (Figure 10C)

![Figure 10: A. STEM image of nail-shaped silica particles consisting of a segment grown at 50 °C followed by a segment grown at 5 °C. B. STEM image of silica rods grown at 5 °C followed by a segment grown at 25 °C resulted in a thicker tip with a less condensed chemical structure. C. TEM image of silica rods grown successively at 25 °C, 5 °C and 25 °C resulting in a thicker middle part with a less condensed chemical structure. Scale bars: 2 µm in A, and B and 1 µm in C.](image-url)
A B

Figure 11: STEM images of crooked silica particles synthesized with two different experimental parameters. A. Particles were synthesized using 20 vol% pre-TEOS at 35 °C. Particles were synthesized using 10 vol% pre-TEOS at room temperature. Scale bar: 1 µm.

Crooked silica rods

In a typical synthesis, 0.5 g PVP was dissolved in 1-pentanol (5 mL) using sonication or vortex mixing for 1 h. After complete dissolution of PVP in 1-pentanol a clear solution was formed; to this 0.5 mL solution ethanol, 0.14 mL distilled water and 35 µL aqueous solution of sodium citrate (0.18 M) were added in sequence. The reactants were mixed gently. Then, 0.115 mL ammonium hydroxide was added and mixed vigorously to make an emulsion. 0.05 mL TEOS was added and the reaction mixture was kept at 35 °C for the first hour. Then a small amount of partially-hydrolyzed TEOS (10 v%, 5 µL) was added. The mixture was homogenized by shaking by hand and transferred to room temperature for 8 h. Eventually, the obtained rods were centrifuged at 1500 g for 10 minutes and dispersed in absolute ethanol. This washing step was repeated twice. The partially hydrolyzed TEOS was prepared as follows: 5 mL normal TEOS was mixed with 11 µL HCl (37%) and 0.40 mL water and vortex mixed for one minute (Figure 11).

Ex situ etching experiments

Ex situ etching of silica rods was carried out as follows. For the experiment on freely dispersed particles the sample volume fraction was fixed at 21 g.L⁻¹ (350 mmol.L⁻¹) and 575 µL of this sample in ethanol was redispersed in 20 mL of the desired concentration of aqueous NaOH (reagent grade, ≥98%, pellets, Sigma-Aldrich) in plastic vials. For the concentrations 100 and 10 mM NaOH samples were collected every 15 minutes for 8 hours. For the concentration 3 mM NaOH samples were collected every 1 hour for 8 hours and 2 final samples were collected after 20 and 24 hours. For the 0.50 mM NaOH concentration samples were collected every 1 hour for 8 hours and 3 final samples were collected after 24, 48 and 72 hours. All samples were prepared by drop-casting 5 µL of the sample solution on a Formvar/Carbon Film 200 Mesh Copper TEM grid. The grids were
dried under a heating lamp to quickly stop the etching reaction on the grid.

For \textit{ex situ} experiments where the particles were attached to a Si$_x$N$_y$ membrane of the LC chip the sample volume fraction was fixed at 21 g.L$^{-1}$ and 2 $\mu$L of this sample was drop-casted on the LC chip and allowed to dry. The chip was then placed in a plastic vial containing 40 mL of the desired concentration of aqueous NaOH. The vials were left to stand on the table without stirring. The experiments were carried out with 100 mM and 10 mM NaOH concentrations. Samples were collected every 15 minutes for 8 hours and for each time interval a separate Si$_x$N$_y$ chip was utilized.

All LC-STEM experiments and STEM measurements were carried out with a Tecnai-F20 transmission electron microscope (TEM, Thermo Fisher Scientific) equipped with a field emission gun and operating at 200 keV using an annular dark-field detector (ADF, E.A. Fischione Instruments Inc., Model 3000, USA) with a camera length of 120 mm. The dose rate was calculated from the beam current of the empty column (no holder) and the frame size as follows:\textsuperscript{37}

\[ d = \frac{I_e}{eA} \]  

(4.5)

Here $d$ is the electron dose rate, $I_e$ is the beam current, $e$ is the elementary charge, and $A$ is the total frame size determined by the magnification.

\textit{In situ} liquid cell etching experiments

A Hummingbird Scientific, (USA) Liquid Cell dedicated holder was used to perform \textit{in situ} experiments. A Hamilton syringe pump equipped with a 1 mL glass syringe was used to flow solutions through the microfluidic tubing into the cell. To flow the 400 nm spherical particles, for measuring the flow velocity, a diluted suspension of the nanoparticles was loaded into the syringe and flowed into the cell. Silicon chips with Si$_x$N$_x$ windows (50 $\times$ 200 $\mu$m $\times$ 50 nm thickness) were used to encapsulate the liquid volume. In all experiments 2 $\mu$m spacers were used between top and bottom chips. The sample preparation started with glow discharging of the side of the chips which was in contact with liquid for 90 seconds to make them hydrophilic. 2 $\mu$L of diluted sample with a volume fraction of 21 g.L$^{-1}$ was drop-casted on the top chip and allowed to dry. This ensured that a number of particles were attached to the top window, where the STEM resolution for LC experiment is the highest. Next, the bottom chip was placed in the dedicated holder and 2 $\mu$L of deionized water was drop-casted onto it to ensure that the cell contained liquid, after which the top chip was placed in position.

Author contributions

A. Grau-Carbonell and S. Sadighikia performed the LC-(S)TEM experiments, data analysis and manuscript preparation. S.S. performed flow velocity and energy calculations. T.Welling assisted in the LC-(S)TEM experiments and the mapping of liquid thickness. F.H and R.K performed particle synthesis.

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4.5 Supplementary Information

Videos

Supplementary videos can be downloaded free of charge from:

Static in situ etching experiments

![Image of LC-(S)TEM images](image)

Figure S1: LC-(S)TEM images of the static etching experiment performed without flowing base inside the cell. No etching was observed after 60 minutes at 100 mM NaOH concentration. This can be explained from a drop of the pH by action of the resulting silanol groups where large numbers of particles are present in a small volume of basic solution. The second explanation is associated with pH changes upon electron beam irradiation in LC-(S)TEM experiments, as explained in the main text. The pH of the solution in the liquid cell drops upon electron beam irradiation, where the pH of alkaline solutions are most strongly affected by the electron beam irradiation. Scale bars: 1 µm

Flow velocity experiments

![Image of flow velocity graph](image)

Figure S2: The direction of motion of particles flowed into the liquid cell.
Figure S3: Flow velocity histogram for various flow rates: A 1 µL.min⁻¹, B 2 µL.min⁻¹, C 3 µL.min⁻¹, D 4 µL.min⁻¹, E 5 µL.min⁻¹. The frame time is 0.5 seconds.
Comparison LC experiments in pure water

In order to reveal the effect of the basic conditions in the etching mechanism of silica rods we performed comparison LC experiments in pure water (Figure S4). Imaging was done with a comparable electron dose rate of $7 \text{ e}^{-\text{nm}^{-2}\text{s}^{-1}}$ with a flow rate of $5 \mu\text{L.min}^{-1}$. No etching was observed after 3 hours of flowing water through the cell, indicating that etching only occurred in presence of a basic solution. Silica rods are typically stable in the water for years. Furthermore, the etching of silica is pH dependent and it is known that the etching of silica happens at relevant speeds for a pH higher than 9. Whereas the pH of the pure water drops drastically during electron beam irradiation.
in the LC experiments.

**LC experiments at high electron dose rates**

In order to reveal the effect of a high electron dose rate on the *in situ* etching experiment we performed LC experiments using high electron dose rates (>1000 e\(^{-}\) nm\(^{-2}\) s\(^{-1}\), Figure S5). The results showed a severe shape deformation after continuous scanning of the silica rod. This observation is consistent with previous studies on silica spheres found in the literature 8. It has been shown that the silica nanoparticles elongate in the electron beam scanning direction. In the case of silica rods, the high electron dose rate resulted in bending of these particles also towards the scanning direction. The exact mechanism of this shape deformation is not yet understood.

![Figure S5: Shape deformation of a silica rod upon electron beam irradiation in the scanning direction. The scanning direction was rotated by 90° at t = 60 min and it can be seen that the deformed rod bent back to the initial rod shape due to the electron beam irradiation. Electron dose rate = 1000 e\(^{-}\) nm\(^{-2}\) s\(^{-1}\), flow rate = 5 \(\mu\)L min\(^{-1}\). Scale bars: 500 nm.](image-url)
LC window thickness

Figure S6: The thickness map of the LC window with a 2 μm spacer A. bottom right corner B. middle right C. top right corner. The liquid thickness was calculated via the method reported by Verch et al.:\textsuperscript{37} \( \frac{I_{\text{screen}}}{I_0} = \exp\left(- \left( \frac{t_{\text{SiN}}}{t_{\text{SiN}}} + \frac{t_{\text{liquid}}}{t_{\text{liquid}}} \right) \right) \).
Chapter 5

*In situ* single particle characterization of the thermoresponsive and co-nonsolvent behavior of poly(N-isopropylacrylamide) microgels and silica@PNIPAM core-shell colloids
Poly(N-isopropylacrylamide) (PNIPAM) microgels and core-(PNIPAM-shells) attract continuous strong interest due to their thermoresponsive behavior, as their size and properties can be tuned by temperature. The direct single particle observation and characterization of pure, unlabeled PNIPAM microgels in their native aqueous environment relies on imaging techniques that operate either at interfaces or in cryogenic conditions, thus limiting the observation of their dynamic nature. Liquid Cell (Scanning) Transmission Electron Microscopy (LC-(S)TEM) imaging allows the characterization of materials and dynamic processes with nanometric resolution in liquids offering an ideal platform to image microgels and core-shell particles with polymer shells. Previously, due to the PNIPAM microgels’ non-homogeneous internal structure their polymer dense core could be resolved but their corona with low polymer volume fraction was effectively invisible to EM imaging in water. Here we show that via a facile post-synthetic in situ polymer labelling step with high-contrast marker core-shell Au@SiO$_2$ nanoparticles (NPs) it is possible to determine the full volume of PNIPAM microgels in water, regardless of the non-homogeneous polymer composition. The labelling allowed for the successful characterization of the thermoresponsive behavior of PNIPAM microgels, as mainly demonstrated by the polymer corona, as well as the co-nonsolvency of PNIPAM in aqueous alcoholic solutions. We also extended this approach to imaging the thermoresponsive behavior of thin PNIPAM shells ($< 100$ nm) by characterizing a system of core-shell SiO$_2$@PNIPAM NPs. We arrived at our labelling procedure after exploring the interplay between electron beam irradiation and PNIPAM systems in water, which resulted in irreversible shrinkage due to the creation of electron beam induced water radiolysis products, which in turn also affected the thermoresponsive behavior of PNIPAM. We found that the addition of 2-propanol as radical scavenger improved PNIPAM stability in water under electron beam irradiation, and discuss the chemistry underlying our observations.

5.1 Introduction

Thermoresponsive microgels are of great interest because of their widely reported rheological, biomedical, optical, catalytic and chemical sensing applications. These systems undergo an abrupt change in aqueous solubility when heated above a temperature threshold, known as the lower critical solution temperature (LCST). Poly(N-isopropylacrylamide) (PNIPAM) was one of the first reported thermoresponsive polymers and has since then been the base for a vast number of thermoresponsive microgels. PNIPAM, and PNIPAM based microgels, exhibit a characteristic LCST of around $32^\circ$C in water which is easily tunable with the incorporation of different comonomers. Below this LCST, microgels are soluble in water and are found in a swollen state. Upon heating above the LCST the hydrogen bonds that are keeping the polymer chains fully solvated, are disrupted and a sharp but reversible conformational transformation from well-solvated coils to a globular state happens. PNIPAM microgels can be functionalized not only by changing the chemistry of the polymer but by forming more complex hybrid structures. These hybrid structures range for instance from hollow microgels to core@shell systems with nanoparticles (NPs) to complex multi-shelled microgels. These more complex hybrid PNIPAM particles systems have a broad range of uses such as temperature tunable depletants and drug delivery vessels that can release chemicals upon temperature changes. Moreover, PNIPAM shows an interesting and still not fully understood co-nonsolvent behavior in alcoholic aqueous solutions.
are well-solvated in pure water or alcohols (i.e. methanol, propanol) but undergo a coil-to-globe transition for mixtures of both solvents.\textsuperscript{191,192}

Despite decades of advancements in the synthesis and application of microgels, their complete characterization is still lacking. A wide toolkit of characterization techniques has been developed regarding many properties of the microgels, and thus PNIPAM as well.\textsuperscript{193} Scattering techniques have been exploited to study microgels with nanometric resolving power, yielding radially averaged properties of ensembles of particles. Dynamic Light Scattering (DLS) in particular has been most often used to characterize the temperature dependent hydrodynamic radius of microgels and thus the thermoresponsive behavior of PNIPAM microgels.\textsuperscript{194} However, scattering measurements rely on models of the scattering units and these are only straightforward to obtain for spherically symmetric, and homogeneous systems and therefore scattering techniques are limited in analyzing quantitatively the thermoresponsive behavior of anisotropic, deformed, or otherwise more complex microgels. Atomic Force Microscopy (AFM) measurements, although constrained to particles on surfaces or adsorbed to interfaces, have been able to follow thermoresponsive microgel behavior at the single particle level, as well as revealing microgel internal features (i.e. the presence of humps on the surface of the microgels even in their most collapsed state).\textsuperscript{195} Light microscopy and light nanoscopy techniques have also been used to image microgels.\textsuperscript{196,197} Light nanoscopy or super-resolution techniques such as stochastic optical reconstruction microscopy (STORM) have shown great success in imaging single particles and studying their internal structure with sub-diffraction resolutions.\textsuperscript{198} Even higher resolution in the direct imaging of single microgels can be achieved with electron microscopy (EM), which is of particular interest to study hybrid microgels in which the microgel is a thin shell around a nanoparticle (not necessarily with a trivial geometry). Cryo-EM (EM imaging of snap-frozen samples) has been able to determine the thermoresponsive behavior of core-shell polystyrene-PNIPAM particles.\textsuperscript{199,200} However, Cryo-EM in microgel characterization is often somewhat limited by the unknown effects of the rapid cooling and blotting on polymer conformations, as well as the difficult preparation of Cryo-EM specimens.

In this study we report the successful characterization \textit{in situ} of the thermoresponsive and co-nonsolvent behavior of PNIPAM microgels in their native water environment with nanometric resolution via liquid cell (scanning) transmission electron microscopy (LC-(S)TEM). In LC-(S)TEM, a thin volume of liquid (up to a few microns) is encapsulated and sealed, typically between two silicon chips harboring two opposing silicon nitride windows (Si\textsubscript{x}N\textsubscript{y}, 30-50 nm thickness) for imaging. These samples are able to withstand the high vacuum inside a TEM and can be imaged with conventional TEM and (S)TEM techniques. LC samples are loaded in dedicated TEM holders, typically equipped with microfluidic, heating and electrochemistry capabilities.\textsuperscript{9,27,28} The main limitations of LC-(S)TEM are the limits in resolution imposed by the liquid layer thickness, as well as the beam-solvent and particles interactions resulting in the formation of, amongst other chemical effects, reactive radiolysis radicals.\textsuperscript{10,120} Efforts to characterize the thermoresponsive behavior of PNIPAM microgels have been reported via incorporation of nanoparticles in the polymeric matrix by chemical synthesis and subsequent imaging in water, but for such systems many concepts have not yet been explored such as electron-beam-sample interactions, reversibility of the phase transition.\textsuperscript{201} Our approach uses an \textit{in situ} post-synthetic labelling step of the PNIPAM microgels with high contrast marker particles, core@shell Au@silica NPs, that allow for the low dose imaging of even swollen microgels that would otherwise yield hardly detectable signals for doses that do not affect the thermoresponsive behavior. Our methodology allows for the direct imaging of previously unlabelled PNIPAM microgels without the need for additional synthetic steps. In order to come up with a good strategy to be able to reversibly image thermoresponsive behavior of single particles, we extensively studied the effects of LC-(S)TEM to the imaging of PNIPAM microgels by evaluating electron beam effects, by exploring the role of radical scavengers on PNIPAM stability under irradiation in water.
and by testing the behavior of various potential marker NPs. Therefore, as order of topics for this chapter we will first discuss electron beam effects on PNIPAM microgels in water and how they can be minimized, and then the best strategy to image the microgels are explored. The knowledge obtained was then applied to the characterization in situ of PNIPAM microgels and their behavior under changing temperatures and solvent composition.

5.2 Results and Discussion

PNIPAM microgels and characterization

In this study two microgel systems were studied. PNIPAM microgels were synthesized via radical emulsion polymerization in the presence of a cross-linker. Hybrid core-shell silica(SiO$_2$)@PNIPAM microgels, consisting of a SiO$_2$ core and a thin PNIPAM shell, were synthesized via radical emulsion polymerization of PNIPAM onto SiO$_2$ cores whose surface had been modified with a silane coupling agent that had a double bond and thus could be chemically attached to PNIPAM chains.

Dynamic Light Scattering and conventional TEM

The PNIPAM microgels showed, as measured via Dynamic Light Scattering (DLS), a hydrodynamic diameter at room temperature (RT, 21 °C) of $D_{RT} = 650-670$ nm, and a hydrodynamic diameter at temperatures above the LCST of around $D_{40°C} = 350-400$ nm (Figure 1A). Under our synthesis conditions, PNIPAM particles are known to consist of a core-shell structure with a more dense core with high a cross-linker density and a less dense corona. Therefore, a structure having a core with a diameter corresponding to $D_{40°C}$ and a total size of core/corona diameter of $D_{RT}$ is expected. PNIPAM particles dried on a silicon nitride window were imaged via (S)TEM and the size of the dry, collapsed microgels was $D_{Dry} = 350-400$ nm (Figure 1B).
Figure 1: Characterization of PNIPAM microgels and SiO$_2$@PNIPAM nanoparticles (NPs). A. Hydrodynamic radius as determined with Dynamic Light Scattering (DLS) of PNIPAM microgels. The thermoresponsive behavior of PNIPAM is clearly visible as function of temperature, with the swollen size of $D_{RT} = 650-670$ nm when the system temperature is below the Lower Critical Solution Temperature (LCTST) and $D_{40^\circ C} = 350-400$ nm when above the LCTST. B. Scanning Transmission Electron Microscopy (STEM) imaging of dry PNIPAM microgels. Scale bars: 5 µm. $D_{Dry} = 350-400$ nm. Scale bar: 500 nm. C. Cryo-Electron Microscopy (Cryo-EM) imaging of PNIPAM microgels snap-frozen in water below the LCTST. The measured size of the PNIPAM via Cryo-EM was $D_{RT} = 650-670$ nm. Scale bar: 2 µm. D. Cryo-EM imaging of SiO$_2$@PNIPAM NPs snap-frozen in water below the LCTST. The measured size of the SiO$_2$@PNIPAM NPs was $D_{RT} = 480 \pm 20$ nm. The size of the SiO$_2$ cores was $D_{Core} = 260 \pm 10$ nm and the thickness of the PNIPAM shell in swollen state was approximately $T_{PNIPAM} = 100 - 120$ nm. Scale bar: 1 µm.

Cryo-EM: PNIPAM microgels and Silica-PNIPAM core-shell Particles

Cryo-EM is a powerful and well established method to image snap-frozen thin liquid layers inside an electron microscope. Here we describe our cryo-EM measurements for our system of PNIPAM microgels (Figure 1C) and as well a system of core-shell SiO$_2$@PNIPAM hybrid microgels (Figure 1D). The measured size of the PNIPAM microgels in water below their LCST was $D_{RT} = 650-670$
nm in accordance with the DLS measurements. Cryo-EM measurements of the SiO$_2$@PNIPAM NPs yielded a swollen total diameter for this system of $D_{RT} = 460 - 500$ nm. The size of the SiO$_2$ cores was $D_{Core} = 260 \pm 10$ nm and therefore the thickness of the PNIPAM shell was in the range of $T_{PNIPAM} = 100 - 120$ nm. We report that attempts to snap-freeze PNIPAM microgels whilst in their collapsed state by starting from a dispersion at a temperature above the LCST were not successful, and their sizes below the LCST were observed.

**LC-STEM imaging of PNIPAM: Radiolysis damage**

LC-(S)TEM offers an excellent platform to study thin (up to a few microns) liquid samples with nanometric resolution. Furthermore, the contents of these thin liquid layers can be precisely heated, thus showing great potential to directly characterize *in situ* the thermoresponsive behavior of PNIPAM microgels and PNIPAM nanometric thin layers. However, LC-(S)TEM relies on the irradiation of a sample with an 80 - 300 keV electron beam, which can affect a sample directly and indirectly though the interaction of both the particles and solvent with beam-induced radiolysis products. Here we characterize such electron beam induced effects on PNIPAM microgels in water and discuss the underlying chemistry.

PNIPAM microgels in water were imaged by means of Bright Field (BF) and High-Angle Annular Dark Field (HAADF) (S)TEM. A small volume of the suspension was drop-casted onto a glow discharged chip (60 seconds). Another glow discharged chip was placed on top of the solution and the cell was closed to create the liquid cell environment. Samples were loaded into a dedicated *in situ* liquid cell TEM holder (Posseidon Select, Protochips). Upon imaging, PNIPAM particles gradually decreased in size while their contrast increased even at low electron doses of 4-20 e$^{-}$nm$^{-2}$s$^{-1}$ (Figure 2A, B) which are already quite low as compared to ‘ordinary’ TEM imaging at these magnifications. This decrease in size was correlated with the intensity of the electron beam (Figure 2C), with higher electron beam dose rates resulting in a faster collapse. The initial size of the visible PNIPAM part of the PNIPAM microgels visible in water corresponded to the previously defined $D_{40^\circ C} (350 - 450$ nm) of this system. Furthermore, the distance between the centers of the adjacent particles corresponded to values close to $D_{RT} (650 - 700$ nm), confirming that a corona with contrast so weak it did not give a detectable signal, is present around the visible cores. This low contrast is consistent with the difficulties to detect the corona with other scattering techniques (e.g.light scattering). Contrast in transmission electron microscopy depends critically on the elemental composition and density of a sample and increases with increasing electron dose rates and signal integration times. The volume occupied by the corona of PNIPAM microgels in water consists of a low volume fraction of polymer ($< 10$ vol. %) and thus it consists mostly of water. Therefore, the generation of contrast for such structures in a swollen state in water relies on the use of relatively intense beams and sensitive detectors. However, our results show that PNIPAM microgels are additionally sensitive to radiolysis damage and therefore more intense beams are not a viable option, so that other strategies needed to be assessed. Use of low electron dose rates has been widely reported by our group and by others as critical to observe dynamic systems and chemically sensitive organic and inorganic particles. By performing "post-mortem” analysis of the sample after opening the liquid cell (Figure 2D) we confirmed that the particles that resulted from electron irradiation in water showed a smaller diameter and an enhanced contrast corresponding to higher density particles. We then confirmed that this high contrast portion of PNIPAM microgels in water corresponded to the relatively thermally unresponsive cores. By means of *in situ* heating LC-(S)TEM we checked that the observable size of the PNIPAM microgels did not depend on whether the temperature of the system was below or above the LCST (Figure 2E). Furthermore, the shrinkage behavior was reproducible and the same for all temperatures. The extent of local beam-induced temperature changes in liquid cell experi-
ments has been recently debated in literature, although it appears to be limited to a few degrees after some minutes of continuous irradiation for low electron dose rates. As the beam effects that we observed did not differ between absolute changes of temperature above tens of degrees, we believe that electron beam damage in water on polymers is not a result of, nor is dependant on beam induced local temperature changes.

In order to understand the reported shrinkage behavior of our PNIPAM microgels, the chemical and physical processes that happen during (S)TEM imaging of a polymeric chain in water have to be considered. The irradiation of water by a (S)TEM electron beam is known to generate a number of radiolysis products. Of such species, two are of interest in this study: hydroxyl radicals (OH) and hydrogen radicals (H). These radicals react strongly with polymeric chains via hydrogen atom abstraction, which results in the transformation of the polymer chains into macroradicals. Concurrently, the electron beam can also directly generate polymer macroradicals. Such macroradicals can react with each other forming crosslinked structures, in a process named inter-molecular recombination (crosslinking between different polymeric chains) or intra-molecular recombination (crosslinking between parts of the same polymeric chain), among other reactions. A schematic of the proposed mechanism behind the shrinkage of PNIPAM microgels upon electron beam illumination in a liquid cell can be found in Figure 2F. Intramolecular crosslinking has been shown to reduce the radius of gyration of a number of polymers (e.g. poly(vinyl alcohol), poly(vinyl pyrrolidone) or poly(acrylic acid)). This process would result in more condensed and crosslinked, smaller PNIPAM particles.
Figure 2: (See next page.)
Figure 2: (Previous page.) Characterization of electron beam effects on PNIPAM microgels in water. A. HAADF-STEM imaging of PNIPAM microgels dispersed in water. Spheres of around 400 - 450 nm in diameter where observed with inter-particle spacings of 650-700 nm. The size of these spheres corresponded to that of the unresponsive PNIPAM cores, and the inter-particle spacing corresponds to the total size of the microgels (core and corona). The observed particle size decreased in time under electron beam illumination, as shown in B. Scale bars: 1 µm. C. Decrease of measured PNIPAM size in time for a number of electron dose rates. Low electron dose rates (4-20 e\textsuperscript{−}·nm\textsuperscript{−2}·s\textsuperscript{−1}) resulted in slower shrinkage compared to a medium electron dose rate (314 e\textsuperscript{−}·nm\textsuperscript{−2}·s\textsuperscript{−1}).

D. Interplay between chemistry of water radiolysis due to electron beam illumination and chemistry of polymers and water radicals.\textsuperscript{10,216} Scale bar: 700 nm. E. Post-mortem LC-(S)TEM sample of an irradiated area showing both irradiated and unirradiated particles. Small, high-contrast particles correspond to the irradiated microgels. Big, low-contrast particles correspond to PNIPAM microgels that diffused and dried on this region during the disassembly of the liquid cell. F. Decrease of measured PNIPAM size in time for an electron dose rate of 314 e\textsuperscript{−}·nm\textsuperscript{−2}·s\textsuperscript{−1} at higher temperatures (around and above the LCST). The initial observed size of the microgels did not change, confirming the unresponsive nature of the polymer dense PNIPAM core.

Increasing microgel stability with radical scavengers

The impact of radiolysis products in LC-(S)TEM experiments has been addressed with varying success by scavenging such species. This is achieved by changing the chemical composition of the liquid media introducing radical scavengers, i.e. molecules that react with radicals more efficiently than the system of interest. This results in a less harsh environment for the polymer, as the main radiolytic species are partially removed from the system by the scavengers. This strategy has been widely used in LC-EM studies, and can be readily adapted to our system.\textsuperscript{10} Hydroxyl radicals (OH\textsuperscript{·}) and hydrogen radicals (H\textsuperscript{·}) can be scavenged by the presence of alcohols in solution, with isopropanol (ISO) being an effective radical scavenger. Hydrated electrons (e\textsuperscript{−}(aq)) can also be scavenged by adding acetones in solution. The reaction of such scavengers with these relevant water radiolysis radicals results in products with lower redox potential and therefore reduces the harshness of the aqueous media to the PNIPAM microgels (Figure 3A).\textsuperscript{10,217,218} Poly(diethylene glycol methyl ether methacrylate) (PDEGMA)-based block copolymers dispersed in aqueous medium have been shown to survive higher electron dose rates in liquid phase EM experiments in the presence of isopropanol.\textsuperscript{52}

We imaged PNIPAM microgels in the presence of various radical scavengers to assess their impact on PNIPAM stability during liquid phase EM experiments (Figure 3B) as compared to pure water. PNIPAM stability in water under electron irradiation increased for concentrations of ISO above 2.5 mM for doses as high as 203 e\textsuperscript{−}·nm\textsuperscript{−2}·s\textsuperscript{−1}. The presence of acetone (1 - 5 mM), scavenging e\textsuperscript{−}(aq), did not result in stability improvements. Although an initial shrinkage phase of around 20% of the particle diameter still happened for all media, the shrinkage stopped and did not proceed as was observed for PNIPAM colloids in pure water. Interestingly, the ability to image PNIPAM colloids at higher electron dose rates (> 500 e\textsuperscript{−}·nm\textsuperscript{−2}·s\textsuperscript{−1}) resulted in the observation of an anisotropic shrinkage/dissolution of the colloids, with higher shrinkage/dissolution rates in the direction perpendicular to the scanning direction of the electron beam and/or deformation of the particle towards an ellipsoidal shape with the long axis in the scanning direction (Supplementary Figure S4). Similar anisotropic and/or deformation of the particle towards an ellipsoidal shape with the long axis in the scanning direction have been previously reported for silica particles dispersed in water and water/ethanol/ammonia mixtures in LC-EM experiments and it is interesting for
Achieving in situ imaging of water-swollen PNIPAM microgels in water

We have shown that volumes of a sample with low polymer volume fractions (<10 vol. %) are not easily resolvable in water via LC-EM at present state of the art electron dose rate conditions. We therefore explored multiple strategies to generate contrast that allows the determination of the entire volume of the microgels, in particular two different approaches to achieve this end: altering the contrast of the solvent and labeling the microgel.

Altering the composition of the water solvent

The contrast contribution of the solvent to a (S)TEM signal depends on the elemental composition and density of the liquid, as well as the thickness of the liquid layer. Thicker liquid layers will result in a higher scattering contribution of the solvent to the overall signal. The cell thickness is determined by the cell assembly conditions and optimized to fit the microgels, changing the elemental composition of the solution appears to be the most straightforward route. Importantly, the liquid medium should stay as chemically close as possible to pure water as the thermoresponsive behavior is known to sensitively depend on the solvent conditions. Here we report the results of dissolving salts consisting of medium-sized metal ions (Cu, from Tetraakis(acetonitrile)copper(I) hexafluorophosphate, 50 mM) to heavy metal ions (U, from Uranyl acetate, 5 mM) in water media in an attempt to increase the contrast of the solvent compared to that of the polymer. Our results show that following the known behavior of ions in water under electron beam illumination, solid particles

future studies to investigate if these electron beam-induced deformations for totally different type of particles are related. 

Figure 3: Increasing PNIPAM microgel stability under electron beam illumination in water with radical scavengers

A. Chemistry describing the scavenging of H, OH, $e_{\text{aq}}^-$ by isopropanol (ISO) and acetone. The resulting product of the reaction between the radicals and the scavengers have less reactive redox potentials. B. The addition of ISO partially stabilized the shrinking of PNIPAM microgels after an initial shrinkage of around 20% of the particle size. A minimal concentration of 5 mM ISO was needed to scavenge enough radicals for this effect to be substantial at a low electron dose of 28 e$^{-}$·nm$^{-2}$·s$^{-1}$. This relative stability was maintained even at medium electron dose rates of 203 e$^{-}$·nm$^{-2}$·s$^{-1}$. The addition of small amounts of acetone to scavenge hydrated electrons did not result in further stability improvements.
from Cu (Figure 4A, SI Video 1) and a solid coating from U happened during LC-EM imaging (Figure 4B, SI Video 2). Interestingly, a strong oxygen signal was detected in such structures. U coating was more prevalent at the microgel parts further away from the windows (Supplementary Figure S2). PNIPAM microgels appeared to be a preferred substrate for the nucleation of these structures. Interestingly, nucleation happened at the outside of the more dense part of the microgels, pointing towards an increase in the concentration of nucleation points. Another possibility is that the shrinkage of the corona happened rapidly, before any observable metal deposition could happen. The chemistry in this process can be understood by combining the current knowledge of the reduction of metal ions and the formation of polymer macro-radicals by electron beam induced radiolysis species. Metal ions are reduced to metal NPs by $H^+$ and $e_{(aq)}^-$, which in turn are created in the radiolysis/interactions of the beam with the water. Metal particles with strong attractive van der Waals forces can have an affinity for the polymer chains and even charged particles can also follow the same fate, especially if polymers also harbor dangling electrons and/or if the radiolysis increases the ionic strength locally. At the same time metal ions can react with the charged chains and act as nuclei for the deposition of more metal. Therefore, because the extensive nature of the interaction between water radiolysis products and metal ions in solution, the dissolution of salts to increase solvent contrast does not appear to be suitable to study PNIPAM microgels in LC-EM studies. Furthermore, although some radical scavengers have shown the ability to minimize or even stop the reduction of metal ions from metal salts under electron beam illumination, we were not able to determine the position of the microgels before metal reduction started and therefore we believe that this strategy does not work for reasonable concentrations of salts.

Therefore, a simple and straightforward way to disperse metal atoms in media avoiding the reduction of new metal NPs in a liquid sample is to use already reduced metals. We approached this route by dispersing a number of NPs in our system. Silica, Au and Au@silica NPs are some of the most readily available and easy to synthesize colloidal systems, and we have shown in previous studies that they are stable under electron beam illumination in water for the low electron dose rates used in this study. Gold was chosen because of its high atomic number and the ease to synthesize monodisperse NPs, being ligand-exchanged and/or coated by silica to disperse the Au NPs in water. Unfortunately, electron beam-sample interactions hindered the applicability of this approach, since particle-particle, particle-window and particle-microgel attachments took place. These attachments were sometimes spontaneous and sometimes beam induced, depending on the dispersed NPs. These findings were however exploited into a successful procedure to image the PNIPAM by decorating the microgels as described in the next section.

Decoration of microgels with high contrast marker NPs to resolve the low-contrast swollen PNIPAM microgels in water

Post-synthetic in situ labelling of PNIPAM microgels was the strategy that worked best to add contrast markers to the outer parts of the microgels. When high contrast NPs were attached to the outer parts of the low volume fraction corona, low electron dose rates could be used without the necessity to resolve the polymer itself. Various types of potential decorative NPs dispersed in water were flown into the liquid cell using the microfluidic capacity of our dedicated liquid cell holder and in situ measurements were subsequently performed.

All measurements of the total size of PNIPAM microgels made at RT (21 °C) with Au and SiO$_2$ NPs yielded particle sizes exceeding the previously measured size of 400 nm from bare PNIPAM microgels dispersed in water (Table 5.1). Silica particles showed efficient spontaneous attachment to PNIPAM but their contrast was low and bigger particles had to be used (113 ± 3 nm) which limited the accuracy of our measurements (Figure 5A). Citrate-stabilized gold NPs (D = 17 ± 1
nm) allowed easier measurements of the total particle size given their small size and high contrast, but their spontaneous attachment was not prevalent enough even at high particle concentrations, and had to be mediated by electron beam illumination (Figure 5B). We tailored the chemistry of the ligands on the citrate-stabilized gold NPs to try and change their interaction with the PNIPAM chains by covalently binding poly-ethylene glycol molecules to the gold surface (Au-PEG NPs), with the PEG molecules functionalized in some cases with either amino (Au-PEG-NH$_2$ NPs) or acid (Au-PEG-COOH NPs) terminations. The Au-PEG NPs attached to PNIPAM NPs upon electron beam illumination (Figure 5C). The attachment took place only in the imaging area. If a particle fell partially inside the imaging area and partially outside, an uneven attachment of NPs happened. The Au-PEG-COOH NPs also attached to PNIPAM microgels upon electron beam illumination but started forming branched structures of NPs (Figure 5D, SI Video 3). The microgel size could only be measured after at least 1 frame and the measured particle size was smaller than $D_{RT}$ ($D_{RT} = 650-670$ nm, RT = $21^\circ$C). The Au-PEG-COOH NPs permanently attached to each other with small (1-2 nm) interparticle distances (Supplementary Figure S3), pointing to possible ligand-ligand crosslinking mediated by radiolysis radicals. Interestingly, Au-PEG-NH$_2$ showed a tendency

Figure 4: Beam induced reduction of metal ions in solution onto PNIPAM microgels
A. Post-mortem HAADF-STEM imaging of the resulting PNIPAM covered by Cu NPs after the electron beam irradiation of microgels in water in the presence of Tetrakis(acetonitrile)copper(I) hexafluorophosphate (50 mM). Scale bars: 200 nm. B. Post-mortem HAADF-STEM imaging of the resulting PNIPAM covered by U NPs after the electron beam irradiation of microgels in water in the presence of Uranyl acetate (5 mM). Scale bars: 5 µm.
towards spontaneously attaching to the cell windows but not to the PNIPAM microgels (Figure 5E). Upon imaging the Au-PEG-NH$_2$ the NPs slowly escaped the imaging area, most likely due to beam-induced charging of the NPs and the silicon nitride window (SI Video 4).

Table 5.1: Overview of sizes and results obtained for PNIPAM microgels at room temperature (21 °C) when decorated with marker NPs.

<table>
<thead>
<tr>
<th>NP type</th>
<th>NP diameter (nm)</th>
<th>Type of attachment</th>
<th>Microgel diameter (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SiO$_2$</td>
<td>113 ± 3</td>
<td>Spontaneous</td>
<td>500-550</td>
</tr>
<tr>
<td>Au-Citrate</td>
<td>17 ± 1</td>
<td>Beam Induced</td>
<td>500-550</td>
</tr>
<tr>
<td>Au-PEG</td>
<td>17 ± 1</td>
<td>Beam Induced</td>
<td>500-550</td>
</tr>
<tr>
<td>AuNP-PEG-COOH</td>
<td>17 ± 1</td>
<td>Beam Induced</td>
<td>500-550</td>
</tr>
<tr>
<td>AuNP-PEG-NH$_2$</td>
<td>17 ± 1</td>
<td>Spontaneous</td>
<td>Attachment to Si$_2$N$_x$ window</td>
</tr>
<tr>
<td>AuNP@SiO$_2$</td>
<td>17@39 ± 1@6</td>
<td>Spontaneous</td>
<td>670 ± 40</td>
</tr>
</tbody>
</table>

Because silica’s high spontaneous attachment efficiency and small Au NP’s ability to allow better measurements at low dose, we predicted that core-shell Au@SiO$_2$ NPs would combine the best of both systems. A combination of small Au cores with a thin silica coating with particle diameters of 17@39 ± 1@6 nm resulted in spontaneous decoration of the PNIPAM microgels and showed to be good marker particles to label PNIPAM swollen microgels including the low density outer corona’s (Figure 5F) nm). The full size of the microgel corona decorated with Au@SiO$_2$ NPs could be precisely resolved despite the low polymer volume fraction (Figure 5D).

**In situ** characterization of the thermoresponsive behavior of PNIPAM microgels and thin layers

The ability to resolve the volume of the complete highly thermoresponsive PNIPAM coronas opens the door to directly observe PNIPAM’s characteristic temperature induced phase transitions. To do so, microgel samples were prepared on liquid cell chips equipped with heating MEMS technology which enables increasing the temperature locally in the liquid cell volume (Variable Temperature LC-(S)TEM, or VT LC-(S)TEM). This allowed the in situ heating of the liquid cell volume, thereby inducing the microgel size transition when the LCST was crossed. Figure 6A shows a swollen microgel below the LCST and a collapsed microgel above the LCST. Multiple thermoresponsive cycles where quantified by imaging at 20 °C and at 40 °C different areas of the liquid cell to avoid beam induced artifacts (Figure 6B). Spectacularly, a large number of particles could be imaged per temperature set above or below the transition temperature, at nanometer resolution. As expected, this process was fully reversible. The observed swollen and collapsed state of the PNIPAM micogels matched with that measured via DLS and Cryo-EM (blue (RT) and red (40 °C) dashed lines). Interestingly, the measured size for the swollen state was slightly below the size measured by DLS and Cryo-EM, and the collapsed size was slightly above that for DLS. This could be an indication of the mechanism with which the Au@SiO$_2$ attached to the PNIPAM corona, with a given depth in the attachment to the swollen corona but with a hard limit when PNIPAM collapses and the SiO$_2$ acts as a spacer between the harder collapsed polymer shell and the high contrast Au core. An image of a dry sample of hard spherical NPs surrounded by Au@SiO$_2$ marker NPs where their silica shell can be seen acting as spacer between the high contrast cores and the ”labelled” particle can be found
in SI Figure S1. A heating cycle was closely followed by increasing and decreasing in gradual steps the temperature of the cell around the LCST (Figure 6C). The decrease in size of the microgels did not happen exactly around the *ex situ* LCST (shifted by around 2.5 °C to 30 °C), reflecting the errors emerging from *in situ* temperature control, since the equilibrium temperature of the liquid volume depends on parameters such as sample thickness or flow speed. For completeness, we next evaluated the impact of electron beam irradiation of the microgels on their thermoresponsiveness (Figure 6D). If cross-linking would be concurrently happening during our imaging, then a decrease in the ability to change in size upon heating would be expected. We found that 1 frame of very low dose electron beam (\(<5 \text{e}^{-}\text{nm}^{-2}\text{s}^{-1}\)) irradiation affects the thermoresponsive capacity of PNIPAM microgels even in the presence of 2-propanol as radical scavenger. More precisely, the recovery of $D_{RT}$ after heating above the LCST and imaging was hindered. A decrease of 100-150 nm of the total swollen size was observed across all samples after a single frame. This was independent on whether particles were first imaged before (swollen) or after (collapsed) heating. If imaging was started at temperatures above the LCST and then the system was cooled down, the real $D_{RT}$ of the
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microgel system could not be recovered even after hours after electron irradiation, confirming the permanent nature of the electron beam induced chemical changes in their polymeric structure. This follows also from our observations that a similar degree of shrinkage was observed for all electron doses even in the presence of radical scavengers reported in the previous sections.

We then extended the same procedure to SiO$_2$@PNIPAM NPs (Figure 6E). The thin PNIPAM shell was indeed clearly observed from the positions of the marker NPs with respect to the core surface. Furthermore, above the LCST we also observed the reversible shrinkage of the thin PNIPAM layers to thicknesses down to 50-60 nm (Figure 6F). Again the measured size for the most swollen state was slightly lower than the size measured via Cryo-EM. In summary, we demonstrate that the thermoresponsive behavior of PNIPAM microgels and PNIPAM shell layers grafted onto hard core-particles can be easily studied in the VT LC-(S)TEM set-up with the use of high contrast marker NPs with nanometric resolution. This possibility opens the door to directly characterize the temperature evolution of PNIPAM systems with thicknesses and geometries not measurable with DLS and other conventional techniques.\(^{222}\)

**In situ** characterization of the PNIPAM co-nonsolvency in aqueous alcoholic solvents

The microfluidic capabilities of dedicated liquid cell holders allow the change in situ of the solvent present in the liquid cell. We exploited this to directly characterize solvent-induced phase transitions. All samples were prepared as described previously by loading them with ultrapure water. Assembling the cell with alcoholic solutions is difficult due to the fast evaporation rates of alcohols, as microliter droplets are dropcasted and the evaporation time can be close to the cell assembly time. Therefore, loading the cells with water and introducing the alcoholic solutions via microfluidics was the most reliable method. We characterized PNIPAM's co-nonsolvency in methanol/water mixtures (Figure 7A). The co-nonsolvency effect is the collapse of PNIPAM microgels in this mixture, while in pure methanol and water the microgel is in a swollen state. A phase transition from the regular swollen state in water was observed in a mixture of 20% molar ratio of methanol comparable to that observed upon heating above the LCST. When the solvent was replaced with just methanol, we observed swollen microgels with slightly bigger particle sizes than that in water. This process was then reversed by going back to the methanol/water mixture and then pure water, for which similar size distributions were measured. These results are in agreement with measurements of this phenomenon using other techniques.\(^{191}\)

5.3 Conclusions

In this study we report the successful single particle characterization of thermally and co-nonsolvency or solvent composition induced phase transitions of PNIPAM microgels and PNIPAM shells in situ in water via liquid phase electron microscopy. We show that the ability to resolve a solvated polymeric volume from its surrounding media in EM depends critically on the polymer volume fraction, which makes imaging at realistic present state of the art doses impossible. We demonstrate that this limitation can be overcome by labelling the PNIPAM structures via an in situ post-synthetical labelling step with high contrast core-shell silica@Au NPs. PNIPAM and silica-based NPs spontaneously attach predominantly on the outside of PNIPAM corona’s, simplifying the labelling step. The ease to resolve these NPs even when immersed in liquid allows us to image and measure the size of PNIPAM microgels and shells at different temperatures and in different solvents with nanometer resolution. Temperature and solvent composition were controlled and changed in situ.
The implications of using electron irradiation to image PNIPAM in water have also been explored experimentally. We show that PNIPAM microgels shrink irreversibly upon electron beam illumination and that this process is mediated via water radiolysis radicals. Scavenging such species with isopropanol partially protect the microgels but not completely, and some degree of shrinkage was unavoidable, while in addition alcohols have a co-nonsolvency effect on PNIPAM in water and thus modify the thermoresponsive behavior. We found as well that PNIPAM microgels is a good substrate for the reduction of metal ions into NPs and coatings. Electron beam induced incorporation of ligand-capped Au NPs onto the microgels was observed for citrate and PEG (with and without chemical functionalizations) capped Au NPs. Au NPs capped with acid-functionalized PEG molecules formed branched structures under electron beam illumination, both on the imaging windows and onto the surface of the microgels.
Figure 6: (See next page)
Figure 6: \textit{(Previous page.)} \textit{In situ} characterization of the thermoresponsive behavior of PNIPAM microgels and thin coatings with nanometric resolution. \textbf{A.} BF-(S)TEM imaging of two different PNIPAM micogels below (20 °C) and above (40 °C) the LCST. Scale bars: 400 nm. \textbf{B.} Measurements for the sizes of multiple microgels during multiple heating-cooling cycles. For each temperature different regions were imaged to avoid beam induced artifacts. Dashed lines indicate the sizes measured via DLS for our system below (blue) and above (red) the LCST. The blue region indicates the size as measured via Cryo-EM. \textbf{C.} Detailed size evolution of PNIPAM microgels during a heating cycle. Particle size started decreasing prior arriving to the LCST, potentially reflecting slight deviations between the expected and the real temperature of the liquid volume. \textbf{D.} Size evolution of the a set of PNIPAM microgels over two heating-cooling cycles imaged at low electron dose rates ($\leq 5 \text{ e}^{-}\text{nm}^{-2}\text{s}^{-1}$). The same microgels were imaged at each temperature. The full size of the particles below the LCST could not be recovered and the thermoresponsive capacity was damped. \textbf{E.} BF-(S)TEM imaging of two different SiO$_2$@PNIPAM microgels below (20 °C) and above (40 °C) the LCST. Scale bars: 750 nm. \textbf{F.} Measurements for the sizes of multiple core-shell SiO$_2$@PNIPAM NPs during multiple heating-cooling cycles. For each temperature different regions were imaged to avoid beam induced artifacts. The blue and red regions indicate the swollen size measured via Cryo-EM for our system below the LCST (blue) and the size of the bare SiO$_2$ core measured via TEM.

Figure 7: \textit{In situ} characterization of the co-nonsolvency in aqueous alcoholic solvents of PNIPAM microgels with nanometric resolution. \textbf{A.} Measured microgel sizes in pure water, a methanol/water mixture (20% molar ratio of methanol) and in methanol. In the methanol/water solvent e articles underwent a phase transition to a collapsed state comparable to that for heating in water above the LCST. Microgels were swollen in water and in methanol, and particle sizes measured in methanol were slightly larger than in water.
5.4 Methods

Materials

N-isopropylacrylamide (NIPAM, 99 %, Sigma-Aldrich), N,N'-methylenebis- acrylamide (BIS, 99 %, Sigma-Aldrich), Ultrapure water (milliQ grade, Millipore system), Potassium Persulfate (KPS, 99%, Sigma-Aldrich), Tetraethyl Orthosilicate (TEOS, 99%, Sigma-Aldrich), 3-(Trimethoxysilyl)propyl meth- acrylate (TPM, 98%, Merck), L-arginine (98%, Sigma-Aldrich), Potassium Hydroxide (KOH, reagent grade, ≥98%, pellets, Sigma-Aldrich), Hydrogen Tetrachloroaurate Trihydrate (HAuCl₄ · 3H₂O, ≥99.9%, Sigma-Aldrich), Sodium Citrate Tribasic Dihydrate (≥99.0%, Sigma-Aldrich), Sodium Hydroxide (NaOH, ≥97.0 %), α-Mercapto-ω-Mercapto Polyethylene Glycol (MW 5 kDa, H₃CO-PEG-SH, Rapp Polymere), α-Mercapto-ω-Amino Polyethylene Glycol Hydrochloride (MW 5 kDa, HS-PEG-NH₂·HCl, Rapp Polymere) and α-Mercapto-ω-Carboxy Polyethylene Glycol (MW 5 kDa, HS-PEG-COOH, Rapp Polymere).

Synthesis of PNIPAM microgels and SiO₂@PNIPAM NPs

PNIPAM microgels were synthesized by radical emulsion polymerization. 10 mg of NIPAM and 1.0 mg of BIS were dissolved in 710.0 g of water. This solution was then sealed and bubbled with nitrogen to remove dissolved oxygen. Bubbling was then stopped and the flask was placed in an oil bath at 70 °C. When the solution reached 70 °C a solution of 10 mg of KPS in 10.0 mL of water was quickly injected to start the radical polymerization process. This reaction was left to continue at 70 °C for 4 hours while stirring. The final product was cleaned by letting the solution to cool down, centrifugation and redispersion in water. SiO₂@PNIPAM NPs were synthesized via the same radical emulsion polymerization method but in the presence of TPM coated Stöber silica.

Synthesis of Amino Acid Catalyzed (AAC) silica NPs

AAC Silica particles (113 nm diameter, polydispersity: 3%, from TEM imaging) were synthesized with a three step synthesis based on previous literature. All glass work was etched of residual silica of former silica synthesis via a base bath (2-3 days in a saturated solution of KOH in ethanol, rinsed with milliQ water). First, 28 nm cores were synthesized as follows: in a 500 mL 1-neck flask 182.5 mg (6 mM) L-arginine was dissolved in 169 mL milliQ water. The mixture was heated to 70 °C and stirred slowly (200 rpm). After 1 hour, 11.2 mL (49 mmol) of TEOS was added slowly via the wall. The reaction mixture was stirred for 1 day to complete the synthesis. These cores were overgrown up to a diameter of 55 nm as follows: in a 500 mL 1-neck flask (base bath, KOH in ethanol) 183.5 mg (6 mM) L-arginine was dissolved in 169 mL milliQ water. 19.3 mL of the dispersion of 28 nm silica particles was added. The mixture was heated to 70 °C and stirred slowly (200 rpm). After 1 hour, 11.2 mL (49 mmol) of TEOS was added slowly via the wall. The reaction mixture was stirred for 1 day to complete the synthesis. These nanoparticles were overgrown up to the final 113 nm diameter as follows: in a 500 mL 1-neck flask (base bath, KOH in ethanol) 182 mg (0.006 M) L-arginine was dissolved in 169 mL milliQ water. 19.3 mL of the dispersion of 55 nm silica particles was added. The mixture was heated to 70 °C and stirred slowly (200 rpm). After 1 hour, 11.2 mL (49 mmol) of TEOS was added slowly via the wall. The reaction mixture was stirred for 1 day to complete the synthesis.
Synthesis of citrate stabilized and PEG-capped gold nanoparticles (AuNPs)

Synthesis of AuNPs

Citrate stabilized spherical AuNPs were synthesised using the sodium citrate reduction method:\textsuperscript{1,2} 100 mL water and 1.00 mL of an aqueous solution containing 10.0 g/L \( \text{HAuCl}_4 \cdot 3 \text{H}_2\text{O} \) were added to a 250 mL two-neck flask with stirring bar and reflux condenser. The flask was placed in an oil bath and heated until boiling. Then, 3.0 mL of an aqueous solution containing 10.0 g/L sodium citrate tribasic dihydrate was rapidly added under vigorous stirring (1200 rpm) and the mixture was left refluxing and stirring for 15 minutes during which a colour change from yellow to dark blue to pink and finally deep red was observed. Stirring was slowed down to 400 rpm, the oil bath was removed and the mixture was allowed to cool down to room temperature. The resulting AuNPs had a mean diameter of 16.7 nm with a polydispersity of 1.1 nm (6.3%) based on TEM imaging.

PEGylation of AuNPs

The AuNPs were functionalized by covalently binding thiolated polyethylene glycol (PEG) ligands to the AuNP surface. To 10.0 mL of the as synthesized AuNP dispersion 0.50 mL of an aqueous 10.0 g/L solution (2 mM) of one of the PEG ligands (\( \text{H}_3\text{CO-PEG-SH} \), \( \text{HS-PEG-NH}_2\cdot\text{HCl} \) or \( \text{HS-PEG-COOH} \)) was added, followed by 0.25 mL of a 0.10 M aqueous NaOH solution. The ligand concentration corresponded to \( 50 \text{ PEG molecules per nm}^2 \text{ of gold surface}. \) The reaction was left to proceed for at least 1 hour, but the solution could be stored without further purification for \( \geq 2 \) years. To concentrate the particles and remove unreacted ligands prior to further use, the particles were washed using centrifugation (15000 RCF for 30 minutes in 5 mL Eppendorf centrifugation tubes) and redispersion of the sediment in 0.50 mL of water.

Coating of Au core NPs with a SiO\textsubscript{2} shell

A silica shell was grown around the AuNP seeds using a PVP-mediated Stöber shell growth:\textsuperscript{81,221} 720 \( \mu \text{L} \) of a 0.10 g/mL solution of PVP in water was added to 30 mL of the AuNPs and stirred (300 rpm) for 24 h. The PVP-coated particles were collected by means of centrifugation (15000 g, 20 min. in 5 mL Eppendorf tubes), redispersed in 30 mL ethanol and added to a 40 mL glass vial, and stirred at 1200 rpm. Then, 3.00 mL ammonium hydroxide solution was added, followed by 50.0 \( \mu \text{L} \) of a freshly prepared \( 10\% \) vol. TEOS in ethanol. After 5 minutes, the stirring speed was reduced to 400 rpm and the mixture was left to react for 4 h. The resulting AuNP@SiO\textsubscript{2} particles had a total diameter of 39 nm with a polydispersity of 6 nm. The particle dispersion could be stored at room temperature without further purification for at least 1 year prior to use.

Cryo- Electron Microscopy (Cryo-EM)

Samples were prepared in cryo-EM dedicated grids with a Thermo-Fischer Scientific Vitrobot. Blotting time was 1 second and snap dipped in liquid ethane. Samples were kept in liquid nitrogen at all times after preparation. Samples were loaded in a Gatan cryo holder and images by a Thermo-Fisher Scientific Tecnai 20 transmission electron microscope equipped with a field emission gun operating at 200 kV.
Liquid Cell Transmission Electron Microscopy

Liquid Cell Scanning EM samples were loaded in a dedicated liquid cell holder (Protochips, Poseidon Select) equipped with flow and in situ heating capabilities. The spacer size was of 500 or 1000 nm in all experiments with PNIPAM microgels and 150 nm for all experiments with hybrid silica@PNIPAM microgels. To make sure that the particles were in pure water, samples were subjected to a flow of ultrapure water (1 µl/min) for at least 3 hours. Frames were acquired at 1024x1024 pixels, 3 nm pixel size, 28 µs dwell time.

Sample preparation

The top and bottom silicon chips were glow discharged for 1 minute to ensure the wettability of the chips. Then, 2 µl dilute dispersion of ~ 10 mg/L of the desired microgels in water was drop-casted on the top chip and was allowed to dry almost completely. When the sample was almost dry, the bottom chip was loaded on the holder tip and 2 µl of water or a aqueous dispersion of NPs were drop-casted on it and the sample was sealed with the top chip (containing the microgels). Bottom chips with gold spacers of 150 for SiO$_2$@PNIPAM NPs and of 500 and 1000 nm for PNIPAM microgels. This sample preparation procedure ensured high microgel coverage of the liquid cell imaging window and as well a minimal liquid thickness.

Imaging

TEM and (S)TEM imaging were performed with a Thermo-Fisher Scientific Talos F200X transmission electron microscope equipped with a field emission gun operated at 200 kV. High angle annular dark field (HAADF) STEM imaging was performed with camera lengths of 120 mm for dry samples and from 120 to 220 mm for liquid samples, as depending on the liquid layer thickness resulting from sample preparation different camera lengths gave the best contrast. Beam currents were measured though the fluorescent screen current in vacuum and are reported in the main text.

The electron dose rate was calculated as follows:

$$d = \frac{I_e}{eA} \quad (5.1)$$

Where $d$ is the electron dose rate, $I_e$ is the beam current, $e$ is the elementary charge, and $A$ is the total frame size determined by the magnification.

Dynamic Light Scattering

Particle sizes were measured by a home build dynamic light scattering (DLS) setup. A glass cuvette filled with a dispersion of the particles was suspended in a toluene bath. The toluene bath was thermostated using a Haake DC50 water bath. The sample was illuminated with a 632.8 nm He laser (30 mW) and the scattered light was collected by two optical fibers. Light was detected with a single photon detector (PMT-120-OP, S/N: 14962). A digital correlator (Flex01-12D) was used to cross-correlate the detected signals from both both collected signals. The data was analyzed using the cumulant method and particle diameter was calculated using the Stokes-Einstein relation (which should be considered an approximation for microgels, given the diffused nature of the microgel surface).
Author contributions

A. Grau-Carbonell performed Cryo-EM and LC-(S)TEM experiments. F. Hagemans synthesized PNIPAM microgels and performed DLS measurements. N. Elbers synthesized the silica-PNIPAM hybrid microgels. M. Bransen synthesized Au based NPs. R.J.A. van Dijk-Moes synthesized silica NPs. S. Sadighikia and T.A.J. Welling assisted in LC-(S)TEM experiments.

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5.5 Supplementary Information

Figure S1: Sample of Au@SiO$_2$ marker NP dried together with SiO$_2$. As marker NP rest on the SiO$_2$ it is readily seen that for hard particles the distance measured between the gold cores is slightly above the real size of the NP due to the presence of the SiO$_2$ shells. SiO$_2$ shells seen in this image are effectively invisible in water for all our imaging conditions. Scale bar: 500 nm.
Figure S2: A. Post-mortem (dry) (S)TEM image of an area covered with several PNIPAM microgels imaged in an uranyl acetate aqueous solution (0.2 w/w %) and its surroundings. A solid layer reduced from the metal ions in solution. The microgels in this layer appear with a lower contrast due to the lower electron density of their volume due to the polymer compared to the purely precipitated surrounding. B. Post-mortem (dry) top view of a PNIPAM microgel imaged in the same uranyl acetate aqueous solution. Metal ions reduced preferentially in the particle regions directly exposed to the solvent as can be observed if the sample is tilted, as seen in C. and D..
Figure S3: Au-PEG-COOH gold NPs irreversibly attached due to electron beam illumination of a dispersion of such NPs in water. Assembly in this case happened on the silicon nitride window of a liquid cell. Between some particles a minimal spacing can be seen, indicating that their surfaces are not necessarily attached. A possible interpretation for this is that the attachment is mediated by the ligands on the NP surface. Scale bar: 100 nm.

Figure S4: PNIPAM microgel imaged at a high electron dose rate (920 e$^{-}$ nm$^{-2}$ s$^{-1}$). The microgel became elongated in the scanning direction of the electron beam. Scale bars: 150 nm.

Videos

Supplementary videos can be downloaded free of charge from:
https://drive.google.com/drive/folders/1ZnDsAiwgq5yOy3ReZG0dfuSRlX-JbKXm?usp=sharing
Chapter 6

Splay-bend nematic phases of bent colloidal silica rods induced by polydispersity
Liquid crystal (LC) phases are in between solids and liquids with properties of both. Nematic LCs composed of rod-like molecules or particles exhibit long-range orientational order, yielding characteristic birefringence, but they lack positional order, allowing them to flow like a liquid. This combination of properties as well as their sensitivity to external fields make nematic LCs fundamental for optical applications e.g. liquid crystal displays (LCDs). When rod-like particles become bent, spontaneous bend deformations arise in the LC, leading to geometric frustration which can be resolved by complementary twist or splay deformations forming intriguing twist-bend ($N_{TB}$) and splay-bend ($N_{SB}$) nematic phases. Here, we show experimentally that the elusive $N_{SB}$ phases can be stabilised in systems of polydisperse micron-sized bent silica rods. Our results open avenues for the realization of $N_{TB}$ and $N_{SB}$ phases of colloidal and molecular LCs.

6.1 Introduction

In 1949 Onsager predicted that, at sufficiently high densities, fluids of hard rod-like particles align along a common nematic director $\hat{n}$, thereby yielding an entropy-driven phase transition from an isotropic ($I$) to a nematic ($N$) phase characterized by long-range orientational order but bereft of any positional order. Although being derived for infinitely long rods, Onsager’s predictions found confirmation in computer simulations on rods of large but finite length. Abundant experimental evidence has since then followed. The entropy-driven nature of the isotropic-to-liquid crystal phase transitions of hard particles makes it especially susceptible to subtle changes in the particle shape and symmetry that are hard to predict based on intuition. For instance, over 3 decades ago, it was shown by computer simulations that hard rods with a spherocylindrical shape do form smectic phases which are 1-dimensional (1D) long-range ordered liquid crystal phases composed of parallel layers of orientationally aligned rods forming 2D liquids, while hard rods with an ellipsoidal shape do not. A more recent example can be found for systems of hard brick-like particles, for which it has been theoretically predicted to promote a so-called biaxial nematic ($N_B$) phase in which both particle axes are simultaneously aligned along two orthogonal nematic directors pointing in the direction of long-range orientational order. However, the stabilization of this biaxial nematic phase turned out to be hindered by a competition with a positionally one-dimensionally (1D) ordered smectic ($Sm$) phase. Biaxial nematic phases can indeed be stabilized in colloidal LCs by destabilizing the smectic phase using polydispersity. However, the first experimental observation of stable biaxial nematic phases of molecular LCs has been historically challenging and was not reported until 2004 for systems of so-called bent core mesogens which are, because of the similarity in shape, also referred to as banana-shaped LCs.

It was once believed that banana- or boomerang-shaped molecules would not be compatible with the formation of liquid crystal phases. However, studies that started almost a century ago showed this not to be the case and the LC phase behavior of molecules or particles with bent-core shapes is now known to be truly rich, although these first studies hardly had any impact for over 60 years. It was realized about 50 years later that the frustration brought about by rods with a bend could bring about interesting new nematic liquid crystal phases in which the fact that spontaneous bend deformations in the nematic director field cannot extend over long distances is reconciled with complementary splay or twist deformations, yielding $N_{SB}$ or $N_{TB}$ nematic phases, respectively. An $N_{TB}$ nematic phase is characterized by spontaneous breaking of chiral symmetry (even for achiral molecules) and an $N_{SB}$ nematic phase by alternating splay and bend domains, making these ‘frustrated’ nematic phases of interest to arrive at fundamental studies, but also for
applications as the different length scales associated with these phases allow for many new possible applications\textsuperscript{252,253}. Schematics of the $N_{SB}$ and $N_{TB}$ phases, in which the average particle orientations vary in space yielding a continuum nematic director field $\mathbf{n}(\mathbf{r})$ with head to tail invariance, are depicted in Figure 1a. Despite numerous experimental realizations of stable $N_{TB}$ phases in systems of bent-core mesogens\textsuperscript{254}, an experimental observation of an $N_{SB}$ phase was still lacking more than 40 years after its first theoretical prediction in 1976\textsuperscript{74}. After finishing this work we became aware of a recent paper in which $N_{SB}$ phases of molecular LCs are reported, but they were induced by electric fields\textsuperscript{255} in agreement with theoretical predictions\textsuperscript{256}. Very recently, however, computer simulations showed that the stability of the $N_{SB}$ phase is hindered by an overly stable positionally ordered $Sm$ phase, and that smooth curvature in the particle shape and polydispersity in the length distribution of bent particles could be two experimentally realizable approaches to lift the "smectic blanket" and reveal stable $N_{SB}$ phases\textsuperscript{257}. Interestingly, a colloidal model system from an organic material with a smooth particle curvature was recently developed, and quasi-2D samples of these smoothly curved particles display indeed an $N_{SB}$ phase\textsuperscript{76}. In this paper, we consider bent silica rods that strongly favor smectic phases because of the interlocking of the sharp particle kinks. Here, we follow the approach of destabilizing the smectic phase by introducing polydispersity to stabilize the $N_{SB}$ phase. We employ polydisperse bent silica rods, which is based on a relatively recently developed new colloidal silica rod-shaped model system\textsuperscript{258,259} that can be imaged quantitatively on the single-particle level. Already it has been shown that by fluorescently labeling these silica rods it becomes possible to image not only their position, but also the orientation of these anisotropic rods even in nematic and smectic LC phases\textsuperscript{64,232,260}. Moreover, when these rods are sterically stabilized with short linear alkane ligands these systems can be refractive-index matched and the interactions between the charged and sterically stabilized systems can be tuned from almost hard-rod-like to long-range repulsive to an extent where the silica rods form a plastic crystal which is the ‘opposite’ phase from a liquid crystal in the sense that for these solids positional order is long-range but the rotational degrees of freedom are disordered\textsuperscript{261,262}. Both the plastic crystals and LC phases could be manipulated and ordered over large distances using electric fields\textsuperscript{261,263}, which is interesting for applications similarly as it is for their molecular counterparts. Additionally, even more recently an other group\textsuperscript{264,265} and our group\textsuperscript{266} were able to modify the synthesis of the rods so that also bent-core silica particles can be made. All these recent advances allowed us to realize a colloidal model system for which a splay-bend $N_{SB}$ phase was realized and that can now be studied on a single-particle level.
Figure 1: Schematics of LC phases and characterization of a system of bent silica rods (BSRs). a Side and top schematic views of a uniaxial N, splay-bend N<sub>SB</sub>, and twist-bend N<sub>TB</sub> nematic liquid crystal phase of bent-core rod-like particles. The darker particles highlight the change in orientation of the nematic director along the sample, which extends to a 3D modulation in the N<sub>SB</sub> and N<sub>TB</sub> phases. The xy and xz planes are indicated as will be defined further for confocal microscopy. b Synthesis process of the BSRs, consisting of a first growth step at 35 °C followed by an injection of pre-hydrolized TEOS and 7 more hours of growth to finish the reaction. c Scanning transmission electron microscopy of BSRs (scale bar 3 µm). d Schematic of the density distribution of the fluorophores along the BSRs and characterization of the length of the short (L<sub>s</sub>) and long (L<sub>l</sub>) segments of the BSRs. e Distribution of bend angles (α = 154 ± 8°) of the BSRs.

### 6.2 Results and Discussion

In this study, it was found that when the previous approaches to arrive at bent silica rods (BSRs)<sup>264,266</sup> were combined (Figure 1b) a system of smooth, rigid core bent-shaped fluorescent silica colloids...
could be synthesized (Figure 1c). See SI for more details on the procedure used. As a result of the synthesis method the BSRs incorporated more dye molecules at the start of the reaction, resulting in a non-homogeneous distribution of fluorescence levels (Figure 1d). We were able to realize and observe an \( N_{SB} \) phase by studying a sedimentation-diffusion profile of fluorescent BSRs with sufficient intrinsic polydispersity in diameter (\( D = 490 \pm 100 \text{ nm (20\%)} \)), length (\( L = 2,110 \pm 270 \text{ nm (13\%)} \) and \( L_{I} = 3,190 \pm 410 \text{ nm (13\%)} \)), and angle (\( \alpha = 154 \pm 8^\circ \) (5\%)), see Figure 1e). Confocal microscopy was used to characterize the self-assembly of fluorescent BSRs as a function of height for differences in the LC phases formed along the sedimentation-diffusion profile. We characterized the \( N_{SB} \) phase by identifying its unique structure at the single-particle level and obtaining its characteristic "wavy" nematic director field. We utilized computationally generated fluorescence confocal-like data stacks of \( N_{SB} \) and \( N_{TB} \) phases to confirm the qualitative and quantitative accuracy of our analysis methodology and results. Furthermore, we extended the synthesis of our bent silica rods to design bent rods with single crystalline corundum hematite ellipsoidally shaped seeds, which also showed a \( N_{SB} \) phase thereby opening the door to the assembly of \( N_{SB} \) phases with functional colloidal building blocks and additionally manipulate their self-assembly both by external electric and magnetic fields.

Suspensions of BSRs were left to sediment and equilibrate a sedimentation-diffusion profile for several days in DMSO/water (78 \% DMSO) index matched (\( n = 1.45, T = 20{}^\circ\text{C, WAVELENGTH} \)) solvent in which silica colloids are stable due to their surface charge. Salt (0.64 mM LiCl) was used to set a well-defined electric double-layer thickness (\( \kappa^{-1} = 10 \text{ nm} \))\textsuperscript{267}. Then, the samples were mounted on a confocal microscope and imaging of the phase behavior was performed as a function of height. When a sedimentation-diffusion equilibrium is reached in a colloidal dispersion of hard rods, a number of phases establish as a function of height in the sample, corresponding to the local osmotic pressure and density\textsuperscript{268}. Here, at the uppermost parts of a sedimentation-diffusion profile of BSRs, around \( 14 \pm 1 \mu\text{m} \) above the bottom surface, an isotropic (\( I \)) phase was identified (Figure 2a). A nematic (\( N \)) phase was found between \( 8 \pm 1 \mu\text{m} \) and \( 14 \pm 1 \mu\text{m} \) above the bottom surface (Figure 2B), whereas an \( Sm \) phase was found between the bottom surface and the \( N \) phase (Figure 2c). Confocal microscopy images were analyzed for their local structure at the single-particle level by investigating the structure tensor of every pixel. This local orientation was visualized as a color map, as seen for the \( I \) phase (Figure 2D), \( N \) phase (Figure 2e) and \( Sm \) phase (Figure 2f). We next studied the interface between the \( N \) and \( Sm \) phases as \( N_{SB} \) phases are expected around this pressure regime.

In polydisperse systems of crooked rod-like particles, \( N_{SB} \) phases are expected to be found in a pressure range in between that of a \( N \) and \( Sm \) phase\textsuperscript{257}. In our experimental system, we indeed found an \( N-N_{SB} \) transition above the \( Sm \) phase, between \( 4 \pm 1 \mu\text{m} \) and \( 8 \pm 1 \mu\text{m} \) above the bottom surface, with the \( N_{SB} \) displaying periodic modulations in the long-range orientational order of the bent silica rods (Figure 3a). Such \( N_{SB} \) domains were found across multiple samples (Figures S1 - S4). Because our \( N_{SB} \) phase was formed in a gravitational field, the modulations of the long-range order of the nematic director field was found to be in planes perpendicular to that of gravity and thus in planes parallel to the \( xy \) imaging plane of our confocal set up. We were thus interested if the modulation identification and discrimination between an \( N_{SB} \) and related \( N_{TB} \) phase could be accomplished by analyzing 2D planes only. To investigate this, proxy ad-hoc \( N_{SB} \) (Figure 3b) and \( N_{TB} \) (Figure S5) phases were computationally generated and a confocal 3D stacks were simulated using a theoretical point spread function for a confocal microscope as a means to confirm our phase identification and analysis from 2D confocal images only. The details about the generation and analysis of these proxy phases can be found in the Supplementary Information. It can be readily seen that the \( N_{SB} \) and the \( N_{TB} \) phases were fundamentally different under simple visual inspection as well, the latter presenting periodic regions with particles oriented perpendicularly to the imaging
Figure 2: **Liquid crystal phases of fluorescently labelled bent silica rods (BSRs) from a sedimentation-diffusion equilibrium profile**, as imaged with fluorescence confocal microscopy. **a** Isotropic (I) phase (above 14 ± 1 µm from the bottom surface), **b** Nematic (N) phase (between 8 ± 1 µm and 14 ± 1 µm) and **c** Smectic (Sm) phase (from the bottom surface to the N phase). On the right, color code for image generation, brighter parts indicate higher signal and thus fluorophore density. **d,e,f** Orientational analysis of the I, N and Sm phases, respectively. The color code indicates the local orientation as obtained from an analysis of the local pixels. Scale bars: 10 µm.

plane independently of the orientation of the phase. The 2D cuts of the experimental $N_{SB}$ phase reported here match the structure of the proxy $N_{SB}$ phase. Additionally, we checked that the modulation pattern as abstracted from the generated images were close to those as used to generate the structures (SI). Thus we analyzed the modulation pattern of our experimental confocal images in the same way.

To confirm the identification of the elusive $N_{SB}$ phase beyond any reasonable doubt, we performed an orientational analysis of Figure 3a, resulting in a vector field which maps the local orientation of the rods in the images and reflects the 2D nematic director field of the phase. The resulting nematic director field of the experimental phase of Figure 3a was well fit by the theoretical nematic director field of a $N_{SB}$ phase $\hat{n}(y) = (\sin(\theta_0 \cdot \sin(qy)), \cos(\theta_0 \cdot \sin(qy)), 0)$, the $y$-axis corresponding to the global nematic director, i.e. the average particle orientation of the whole phase, and $\theta_0$ being the maximum angle of oscillation of the particle orientation, $q = 2\pi/p$ the wave number of these periodic oscillations, and $p$ the pitch length. This good description confirmed the experimental determination of the $N_{SB}$ phase (Figure 3c). This analysis was repeated for all $N_{SB}$ phases (shown in Figures S1 - S4), and yielded an average measure of the amplitude and periodicity of the splay-bend modulations $\theta_0 = 0.55 \pm 0.11$ rad and $p = 86.2 \pm 11.7$ µm. Interestingly, the pitch
of the $N_{SB}$ phase was several times larger than the total (end-to-end) particle size, showcasing the presence of multiple length scales in the self-assembled system and thereby making it a promising system for optical applications as well. To confirm that the pitch and amplitude of the phase could be reliably characterized in this way, the same analysis was also performed on the proxy $N_{SB}$ phase (Figure 3d). We found that the fitting parameters extracted from the analysis of the proxy $N_{SB}$ phase $\theta_0 = 0.64 \pm 0.01$ rad and $p = 89.9 \pm 1.1 \mu m$ were consistent with the known values $\theta_0 = 0.59$ rad and $p = 84.2 \mu m$, thereby confirming that $N_{SB}$ phases could indeed be reliably characterized with our methodology.

As already discussed, the orientational order of nematic phases can be characterized by continuum director fields with head to tail invariance $\hat{n}(r)^{269}$. At places where $\hat{n}$ is not uniquely defined, like in a point or line in an $N$ phase, a nematic defect is present. At finite temperatures entropy dictates that defects are always present in condensed phases of matter in equilibrium, but they are often also out-of-equilibrium manifestations of the formation of the phase that did not have enough time to anneal out. For instance for LCs they may stem for instance from symmetry breakings in different directions and different regions remaining from an I-$N$ transition$^{270}$. It is not surprising therefore that we also observed many defect structures for which we now have the opportunity to study these on the single-particle level, which is not trivial for ordinary LCs$^{271}$. Line defects can be categorized by their disclination strength ($S$), which is defined as the number of times the nematic director rotates by $2\pi$ for a close path in the plane perpendicular to the line defect. Examples of line defects with disclination strength $S = \pm 1/2$ are presented in Figure 4$^{271}$. Such defects were commonly found in multiple samples (Figure S6).

After the successful creation of an $N_{SB}$ phase from BSRs we realized that even more interesting $N_{SB}$ phases from both a fundamental and application point of view might be realized if the BSR synthesis could be combined with a seeded growth procedure that was already developed for the kind of silica rods we used. As proof of principle we combined the BSRs with single crystalline hematite ellipsoids which in a magnetic field have a magnetic dipole moment in the direction of the long axis of the ellipse$^{273}$ and combined these seeds with non-fluorescent BSRs (BSRs, Fig. 5a). As mentioned, this particle system was synthesized via a seeded growth method, followed by the previously described BSRs synthesis procedure, and consisted of particles with ($D = 281 \pm 33$ nm (12%), $L_s = 1,315 \pm 84$ nm (6%), $L_l = 2,570 \pm 390$ nm (15%)) and $\alpha = 151 \pm 9^\circ$ (6%). This hematite-BSR system is special in several ways: The hematite seed particles used were single crystalline corundum seeds with an ellipsoidal shape. The seeded growth of the bent silica rods was initiated by droplet attachment to only one side of the ellipsoid which means that the magnetic dipole is induced in a direction perpendicular to the long axis of the ellipsoid, is directed parallel with the bent rod director. This opens up the possibility to modify the self-assembly of these colloidal BSRs not only with electric fields but independently also with magnetic fields$^{274,275}$, which is of interest both for fundamental studies, but also for photonic applications as the hematite ellipsoids are strongly scattering particles. At the bottom of the sedimentation-diffusion profile, we were able to identify a $Sm$ via confocal microscopy, by collecting the reflected laser signal at 495 nm (signal mainly from silica) and 670 nm (signal mainly from the hematite ellipsoids) (Figure 5b). However, the particles were not fluorescently labelled and imaging at higher positions in the sediment was therefore not possible. We thus allowed our samples to slowly dry and analyzed the resulting pellets via scanning electron microscopy (SEM). Figures 5c and 5d show SEM images of the dried pellets. It can be observed that the internal structures seen are compatible with an $N_{SB}$ phase, as revealed by the wavy modulation pattern, along which some of the structure also cracked because of drying induced stresses. The pitch of the modulation was again 10-15 times larger than the particle size. Preliminary experiments showed that dispersions of BSRs could be manipulated with external electric fields (Figure S7).
Using polydispersity to realize $N_{SB}$ and/or $N_{TB}$ phases is a general strategy that can be applied to other systems as well. A preliminary promising example is shown in Figure 5e where a wavy pattern pointing towards a tendency to form a $N_{SB}$ phase can be recognized in this dried self-assembled thin phase of boomerang shaped brookite TiO$_2$ nanorods ($D = 7.1 \pm 0.8$ nm (12.6%), $L$

![Figure 3](See next page.)
Characterization of the $N_{SB}$ phase. a Fluorescence confocal microscopy image of a $N_{SB}$ domain located between the previously identified $N$ and $Sm$ phases (between 4 ± 1 µm and 8 ± 1 µm from the bottom surface). b Proxy, computationally generated fluorescence confocal microscopy image of a dispersion of rods in a $N_{SB}$ configuration. c Characterization of the nematic director field of the experimental $N_{SB}$ phase. The extracted values were $p = 86.2 \pm 11.7$ µm and $\theta_0 = 0.46 \pm 0.01$ rad. Spatial coordinates are given in terms of the average diameter of the particles ($D_{avg}$). Images were binned to make $D_{avg}$ as close as possible to 6 pixels to match the experimental data to the computationally generated data. d Characterization of the nematic field of the proxy $N_{SB}$ phase. The extracted values were $p = 89.9 \pm 1.1$ µm and $\theta_0 = 0.64 \pm 0.01$ rad, which are representative of the parameters used to generate the phase: $p = 84.2$ µm and $\theta_0 = 0.59$ rad. Spatial coordinates are given in terms of the average diameter of the particles ($D_{avg}$). Scale bars: 5 µm.

= 82.9 ± 10.4 nm (12.6%) simply by drying the nanorods on a TEM grid. We finally, expect and suggest that $N_{SB}$ phases could be realized for LCs of bent-core mesogens by combining molecules with different lengths to obtain polydisperse systems. Mixtures of bent-core molecular liquid crystals and mixtures of straight and bent-core LCs were already studied to extend and/or shift transition temperatures, but as far as we know not with the intention to realize $N_{SB}$ phases. Thus, we believe our approach is easily applicable to systems over many length scales. Additionally, it has recently been shown that LC phases with regions with more bent and splay are interesting to spatially separate differently shaped colloids, which would also be interesting to investigate on the single-particle level.

Figure 4: Fluorescence confocal microscopy images of defects in nematic phases of BSR. a Line defect with disclination strength $S = -1/2$. b Pair of line defects meeting each other with disclination strengths $S = \pm 1/2$. Scale bars: 10 µm.
6.3 Conclusions

To conclude, we reported on the experimental realization and identification of an $N_{SB}$ phase from polydisperse bent-core fluorescently-labelled colloidal building blocks that could be analyzed on a single-particle level. The bent silica rods were made using a modification of an existing general emulsion based growth method that is also compatible with seeded growth. The $N_{SB}$ was characterized via the analysis of its nematic director field from an analysis of fluorescence confocal microscopy data sets, and yielded a pitch of $p = 86.2 \pm 11.7 \, \mu m$, i.e. about 16 times the particle length. The $N_{SB}$ phase was found to form in between a $Sm$ and an uniaxial $N$ phase, as expected from theory and simulations\textsuperscript{250,251,257,279,280}. $N_{SB}$ LC phases were also observed for a system of hematite functionalized bent silica rods oriented perpendicular to the single crystalline hematite
ellipsoids that can be magnetized in magnetic fields with a dipole moment in the direction of the ellipsoids. Our results together with those on polymer based smoother bent rods developed independently from us, suggest that using polydispersity is indeed a viable and generally applicable methodology to create these frustrated $N_{SB}$ liquid crystal phases. Additionally, the feasibility of creating such interesting 'frustrated' nematic liquid crystal phases also from more complex shaped bent silica rods like the bent silica rods with well oriented hematite cores in addition to preliminary results on bent titania nanorods make it likely in our opinion that $N_{SB}$ phases might also be formed from mixtures of bent molecular mesogens even without external electric fields. Further work has to demonstrate if also twist-bend nematic phases can be realized in these systems. As this will allow for single-particle based studies, it may resolve for instance recent controversies around these experimentally more abundant phases.
6.4 Methods

Materials
Polyvinylpyrrolidone (PVP, Mn = 40 kg/mol), 1-pentanol (>99%, Sigma-Aldrich), ammonia solution (NH₃, 25 wt% in water, Sigma Aldrich), anhydrous ethanol (>100%, Interchema), sodium citrate tribasic dihydrate (>99%, Sigma Aldrich), tetraethyorthosilicate (TEOS, >99%, Sigma-Aldrich), (3-aminopropyl)-triethoxysilane (APTES, MERCK, >98%), 7-nitrobenzo-2-oxa-1,3-diazol (NBD, 98%, Across organics), FeCl₃.6H₂O (97%, Sigma-Aldrich), sodium hydroxide (NaOH) (pellets, Merck), hydrogen chloride (HCl, 37 wt.%, ACS reagent), dimethylsulfoxide (DMSO, >99.8%, Sigma Aldrich).

Synthesis of bent silica rods (BSRs)
In a typical synthesis, 1.0 g of PVP (25 µmol) was dissolved in 10.0 mL 1-pentanol (92 mmol) using sonication (Branson Bransonic CPXH Digital Bath 8800, Emerson) for 1 h. After the complete dissolution of PVP in 1-pentanol, a clear solution was formed. To this solution, ethanol (1.0 mL, 17.4 mmol), distilled water (0.280 mL, 15 mmol), and an aqueous solution of sodium citrate (67.0 µL, 0.18 M) were added in sequence. The reactants were homogenized by shaking the flask with hand for 3-4 times. Then, 0.225 mL of ammonium hydroxide (1.7 mmol ammonia, 9.4 mmol water) was added and the reactant mixture was mixed by continuous shaking for one minute to create an emulsion. To this emulsion, TEOS (0.10 mL, 0.45 mmol) was added and the reaction mixture was kept at 35 °C for one hour. Subsequently, 15.0 µL (0.06 mmol) of partially-hydrolyzed TEOS was added. All the ingredients were homogenized by carefully tilting the bottle side to side for 3-4 times and the vial was transferred to room temperature and left to react for 7 h. Eventually, the obtained rods were centrifuged at 3000 rpm for 10 minutes and dispersed in absolute ethanol. This washing step was repeated two times. The partially hydrolyzed TEOS was prepared as follows; 5.0 mL TEOS (22.3 mmol) was mixed with 11.0 µL HCl (0.44 mmol) and 0.4 mL distilled water (22.2 mmol) and vortexed (MS2 Minishaker, IKA) for one minute.

Particle labeling with NBD for fluorescence confocal microscopy imaging
For fluorescent labeling, we first reacted a silane coupling agent (APTES) covalently with the dye (NBD) in ethanol which we refer to as NBD-APTES solution. In a typical procedure, 18.0 µL (0.06 mmol) of NBD was dissolved in 5.0 mL absolute ethanol (85.6 mmol) in a 30 mL laboratory vial and sonicated for 5 minutes. To this NBD ethanol solution, 35.0 µL (0.15 mmol) of APTES was added and the solution was stirred slowly at room temperature overnight. The NBD-APTES solution was added to the above reactant mixture. In a typical synthesis of BSRs, 20.0 µL of NBD-APTES was added to the reactant mixture just before the addition of TEOS (0.10 mL, 0.45 mmol).

Synthesis of hematite ellipsoids
Monodisperse hematite ellipsoids were synthesized by using the hydrothermal reduction method. The typical synthesis procedure is as follows; In a 250.0 mL of a volumetric flask, (2.0 M) FeCl₃.6H₂O solution was prepared by dissolving 54.0 grams of iron (III) chloride hexahydrate in 100 mL of water. To this, 100.0 mL (5.40 M) of NaOH solution was slowly added under continuous stirring till the solution reached to pH 7. As a result, a brownish solution was formed and this solution was stirred at high speed (1100 rpm) for 30 minutes to ensure the homogeneous distribution of reactants. After stirring, a highly viscous gel was formed. Further, this gel was tightly sealed in an autoclave and
immediately transferred to a preheated oven at 100 °C and kept for 3 days of aging, the solution was washed two times with water and finally disperse the obtained hematite ellipsoids in water.

**Synthesis of hematite core-shell BSRs (CS-BSRs)**

To synthesize hematite CS-BSRs, 1.0 g PVP was dissolved in 10.0 mL of 1-pentanol using sonication or vortex for 1 h. To this solution, 6.0 mg of hematite ellipsoids (Long axis: 198 ± 24 (12.0 %) nm, short axis: 143 ± 18 (12.5 %) nm) was added, and the solution was sonicated for another one hour. As a result, a clear red colored suspension was formed. Subsequently, the remaining reactants (i.e. 1.0 mL anhydrous ethanol, 0.280 mL water, and 67.0 µL of sodium citrate) were added in sequence. The reactants were mixed by carefully tilting the bottle side to side for 3-4 times to achieve an homogeneous distribution of the reactants. Then, 0.225 mL of ammonium hydroxide (1.7 mmol ammonia, 9.4 mmol water) was added and the solution was mixed by shaking the flask with hand for one minute to form the emulsion. To start the growth of the silica rods, 0.10 mL of TEOS (0.45 mmol) was added. All the ingredients were homogenized by carefully tilting the bottle side to side for 3-4 times, and the reaction mixture was kept at 35 °C for the first one hour. To this 10.0 µL partially-hydrolyzed TEOS was added and allowed the reaction to continue for 7 hours at room temperature. The particles were collected by 3 consecutive centrifugation (3000 rpm for 10 minutes) and re-dispersion steps in ethanol. After final washing, particles were stored in ethanol.

**Sample preparation**

BSR with a final concentration of 0.4 vol% were dispersed in a DMSO/water mixture (78 % w/w) adjusted to a final refractive index n = 1.45 with a refractometer (Abbe refractometer, NAR-3T, Atago). 400 µL of such dispersion were placed in a home-made cell suitable for confocal microscopy. This cell was built from an end of a Pasteur pipette glued to a #0 Glass-Menzel coverslip supported on a tailored made microscopy glass slide.

**Scanning Confocal Fluorescence microscopy**

Confocal microscopy was performed with a Leica SP8 confocal microscope equipped with a white light laser and a 90x oil confocal immersion lens. The excitation wavelength was 495 nm, and the fluorescence signal was collected for the range between 500 and 650 nm. If the microscope was operated in reflection mode the wavelengths are indicated in the corresponding section.

**Electron microscopy**

For TEM analysis a Tecnai 20 electron microscopy operating at 200 kV, and for STEM analysis a Tecnai 20F electron microscope operating at 200 kV in scanning transmission electron microscopy (STEM) mode were used. Scanning electron microscopy measurements were performed by Phenom ProX.

**Proxy phases preparation**

Proxy $N_{SB}$ and $N_{TB}$ with given amplitude and periodicity were realised by inserting in a simulation box non-overlapping crooked rods of shape randomly sampled from the BSR experimental shape distribution, each with a random position $\mathbf{r}$ and an orientation corresponding to the phase nematic director field $\hat{n}(\mathbf{r})$. The resulting configurations were then compressed via a rapid quench at high
pressures using an NPT Monte Carlo simulation method. The nematic director field of the resulting proxy phase was then obtained from the particle orientations, and fitted with the theoretical expression of the nematic director field (see Figure 3d, dashed lines) to measure $p$ and $\theta_0$.

3D confocal data sets where generated from the above described $N_{SB}$ configurations. To obtain data sets representative of our experimental system we first generated ground truth stacks consisting of rods whose fluorescent signal was located at their surface. This was achieved by defining a backbone for the bent rods from the coordinates and particle orientations obtained from the NPT Monte Carlo compression step and then assigning values to the voxels found at a distance of a particle radius from such backbone. The rest of the voxels remained thus as voxels with no signal. The ground truth stacks where then convolved with a theoretical point spread function for a confocal microscope with the Huygens Professional software. Then photon noise was added to achieve realistic images with a signal-to-noise ratio of 10. Final simulated 3D confocal datasets were analyzed for structure exactly as experimental confocal datasets.

Analysis orientation of the confocal microscopy images

Particle orientations as the color maps in Figure 2d-f and Figure 3a-b, and the 2D nematic director field as in Figure 3c,d where obtained via a local gradient structure tensor analysis using the OrientationJ software, with Cubic Spline gradient and a local window of 30 pixels ($\sim$ 5 particle diameters)

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Author contributions

R. Kotni performed synthesis and characterization of the colloidal systems. A. Grau-Carbonell and R. Kotni performed self-assembly experiments. A. Grau-Carbonell performed confocal microscopy. A. Grau-Carbonell and M. Chiappini generated simulated 3D confocal data sets. M. Chiappini performed analysis of the data sets. M. Dijkstra also designed and supervised this research.
6.5 Supplementary Information

Figure S1: \( N_{SB} \) phases of BSR with particle dimensions of \( D = 490 \pm 100 \text{ (20 \%)} \) nm, \( L_s = 2.110 \pm 270 \text{ (13 \%)} \) nm, \( L_l = 3.190 \pm 410 \text{ (13 \%)} \) nm) and \( \alpha = 154 \pm 8 \text{ (5 \%)} \) \( ^\circ \). Scale bars: 10 \( \mu \text{m} \).
Figure S2: $N_{SB}$ phases of BSR with particle dimensions of $D = 490 \pm 100$ (20 \%) nm, $L_s = 2.110 \pm 270$ (13 \%) nm, $L_t = 3.190 \pm 410$ (13 \%) nm) and $\alpha = 154 \pm 8$ (5 \%) $^\circ$. Scale bars: 10 $\mu$m.
Figure S3: $N_{SB}$ phases of BSR with particle dimensions of $D = 490 \pm 100 \,(20\%) \,\text{nm}$, $L_s = 2.110 \pm 270 \,(13\%) \,\text{nm}$, $L_l = 3.190 \pm 410 \,(13\%) \,\text{nm}$) and $\alpha = 154 \pm 8 \,(5\%) \,^\circ$. Scale bars: 10 $\mu$m.
Figure S4: $N_{SB}$ phases of BSR with particle dimensions of $D = 490 \pm 100$ (20\%) nm, $L_s = 2.110 \pm 270$ (13\%) nm, $L_l = 3,190 \pm 410$ (13\%) nm) and $\alpha = 154 \pm 8$ (5\%) $^\circ$. Scale bars: 5 $\mu$m.

Figure S5: Proxy, computationally generated fluorescence confocal microscopy image of a dispersion of rods with a $N_{SB}$ configuration. The dashed lines indicate the regions where the nematic director becomes perpendicular to the imaging plane. Scale bars: 10 $\mu$m.
Figure S6: Fluorescence confocal microscopy imaging of line defects in nematic phases of BSR with disclination strengths $S = \pm 1/2$. Scale bars 10 $\mu$m.

Figure S7: Fluorescence confocal microscopy imaging of very diluted suspensions of BSR. When an electric field ($E = 0.045$ V/$\mu$m, 1 MHz) was applied, the orientation of the particles aligned with the external field. Scale bars: 10 $\mu$m.
**Calculation of Debye length ($\kappa^{-1}$)** This characteristic double-layer parameter was calculated for a monovalent electrolite in liquid medium following:

$$
\kappa^{-1} = \sqrt{\frac{\epsilon_r \epsilon_0 k_B T}{2 N_A e^2 I}}
$$

(6.1)

Where $\epsilon_r$ is the dielectric constant the solvent, $\epsilon_0$ is the electrical permitivity of vacuum, $k_B$ is Boltzmann’s constant, $N_A$ is Avogadros number and $e$ is the electron charge.
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Summary


Summary

The aim of the study described in this Thesis is the imaging of sensitive processes at the nanoscale, which is achieved by performing specialized experiments using either electron microscopy or light microscopy. The first set of studies (Chapters 3-5) focuses on the use of liquid cell transmission electron microscopy (LC-TEM). LC-TEM is a technique in the realm of in situ transmission electron microscopy (TEM) specialized in imaging liquid samples. TEM typically images samples suspended in high vacuum, which makes the imaging of colloidal and nanometric samples dispersed in their native state impossible. LC-TEM prevents the exposure of the liquid to the high vacuum of the microscope by encapsulating and sealing a thin liquid layer between two membranes. State-of-the-art LC-TEM is performed with the use of dedicated sample holders, which not only seal the liquid layer but also allow flowing liquid through the sample and apply electric currents to the sample region. The development of MEMS-based chip technology as part of the encapsulation method of LC-TEM has allowed in recent years to perform in situ heating and electrochemistry experiments inside a liquid cell in real time. This research is focused at the study of processes taking place in colloidal systems happening in liquid. In the second part of this thesis described in Chapter 6, the focus of our research is switched to light microscopy. Confocal Scanning Light Microscopy (CSLM) is a technique that allows imaging of fluorescent samples by scanning a sample with a laser and rejecting out-of-focus light with a pinhole in the back-focal plane of the imaging lens to increase resolution. If fluorescent colloidal particles are dispersed in a solvent with a refractive index close to the refractive index of the colloids, CSLM can be used to image deep into samples and determine the mesoscopic order of colloidal systems at the single particle level.

Chapter 2 summarizes some of the key concepts of LC-TEM. The development and principles of LC-TEM are outlined, and we define the two main physical entities, the electron beam dose rate and the total electron dose, that are typically used to describe the imaging parameters during LC-TEM experiments. As LC-TEM is characterized by the prevalence of electron beam-induced effects, we summarize the main scattering events between incident electrons and a sample during EM imaging. During LC-TEM experiments described along this thesis (Chapters 3-5) a number of nanoparticle and colloidal systems were imaged in aqueous media. The radiolysis of water upon exposure to ionizing electron radiation, which leads to the formation of reactive radiolytic species, is also reviewed. A few words are devoted to colloidal self-assembly, the spontaneous ordering of colloidal matter, as that is the research subject of Chapter 6.

In Chapter 3 we develop a general method to image the wet etching of silicon dioxide (silica, SiO$_2$) nanoparticles in situ via liquid cell scanning transmission electron microscopy (LC-(S)TEM). First, the ex situ etching of a model system of amino-acid catalyzed silica nanospheres was fully characterized. This model system was chosen because of its well defined, slow and gradual etching process in basic solutions, which we also followed with dynamic light scattering (DLS) measurements. This allowed us to compare our in situ LC-(S)TEM observations with the ex situ etching counterpart and determine any electron-beam effects induced by imaging with an electron beam. We identified that silica nanoparticles in water under electron beam illumination undergo an expansion phase and a dissolution beam-induced phases which became more pronounced under etching in basic medium (100 mM NaOH) (see Chapter 3, Figure 3.3). Through electron trajectory simulations for 200 keV electrons we determined that enough energy was deposited in the nanoparticles per acquired frame to strongly affect the siloxane network (even at low electron doses). We showed that the expansion phase was directly correlated to the cumulative (total) electron dose on the imaging area, while the dissolution phase appeared to be driven by the electron dose rate. We propose that the direct interaction of the beam with the silica network drives the expansion
phase of the nanoparticles, while the dissolution phase is also strongly linked to the chemistry of the solvent (etchant and water radiolysis products). We used these outcomes to determine the best EM imaging conditions to study silica in water and in basic aqueous solutions. Such conditions include minimizing the electron dose rate and the use of blanking windows to minimize the cumulative electron dose. We also showed that these conditions impose the limit of how small silica nanoparticles a given TEM can image, as smaller sizes require higher magnifications which would render excessive electron dose rates unavoidable. We then characterized the etching process in aqueous basic media of Stöber and water-in-oil reverse microemulsion (WORM) silica nanospheres. Both these systems are known to have an inhomogeneous internal distribution of silica condensation: the silica condensation increases closer to the particle surface. The *ex situ*, bulk etching of these particles has been widely reported to proceed through first a hollowing step followed by complete dissolution. We were able to follow the formation of hollow silica Stöber and WORM nanospheres *in situ* at the single particle level (see Chapter 3, Figure 3.5). Therefore, LC-(S)TEM is a tool that can be used to probe the internal distribution of condensation degrees for silica particles and, potentially, for other oxide materials. Silica core-shell particles, such as metal@silica or metal oxide@silica are of interest for many applications, and some of such applications (e.g. catalysis) rely on the hollowing of the silica shell to create a cavity for the core. For this reason, we also characterized the etching process of core-shell Au@Stöber and Fe$_2$O$_3$@WORM silica nanospheres *in situ* at the single particle level. Interestingly, we could show that after the etching process of the interior of the shells had taken place, the metal and metal-oxide cores were free to diffuse inside the hollow shell. (see Chapter 3, Figure 3.6) This observation highlights the potential of LC-(S)TEM in studying this type of etching-based-synthesis procedures, since regular TEM would image dry samples and thus would not be able to determine the behavior of the core.

In Chapter 4 the etching process of colloidal, micron-sized rod-like silica rods into silica cones was studied via LC-(S)TEM. This process had been described previously by our group, but it had never been followed at the single particle level. Silica rods, similarly to the Stöber and WORM silica nanospheres studied in the previous chapter, have an inhomogeneous internal distribution of silica condensation degrees: There is a gradient of condensation along the length of the particle, and a highly condensed shell surrounds the entire rod. This internal structure results in an etching mechanism that leads to conical particles. In this chapter we describe how we extend the study of the internal condensation degrees of silica particles to these micron sized silica colloids. The knowledge gained in Chapter 3 on how to image silica-based particles (low dose, sparse imaging) was applied to characterize such etching processes with no influence of electron beam effects. We successfully followed the progression from silica rods to silica cones upon etching in basic aqueous solutions at the single particle level with nanometric resolution. We were able to describe a previously unknown step of this etching process in which a part of the rod breaks from the tip of the forming cone. (see Chapter 4, Figure 4.4). Interestingly, the bigger size of silica rods compared with the nanoparticles used in Chapter 3 result in drag forces from the continuous flow of liquid in the cell sufficient to detach them from the imaging window. This imposed the additional optimization step of choosing an adequate flow rate. Comparison of calculated drag forces on small nanospheres (100 nm) with calculated drag forces on micro rods supported our observations. Furthermore, we show that the closed geometry of the liquid cell can affect the etching process compared with that of the etching of rods adhered to a surface immersed in a bulk etchant solution. To do so, *ex situ* etching experiments of silica rods attached to a silicon nitride surface were performed. Striking differences were observed between the etching process inside a liquid cell and upon exposure to bulk etchant, where in particular the size distribution of the resulting etched particles was different, possibly pointing towards slightly different etching mechanisms. This result is important for every other LC-EM study of chemical processes, as virtually any chemical reaction taking place on particles on the
imaging windows can to a certain degree be affected by these confinement effects. We then extended our results to show that the preferential etching of less condensed parts of other silica colloids could also be followed via LC-TEM. This was done by etching well known silica rods systems consisting of well defined segments with different condensation degrees (see Chapter 4, Figure 4.7). We then demonstrated the potential of this approach by probing the internal structure of a novel system of bent-core silica rods. Two systems of particles with similar final geometries and sizes but with different synthesis conditions (temperature, precursor) were studied. Our results revealed that these particles show different degrees of silica condensation in their bent regions (see Chapter 4, Figure 4.8).

In Chapter 5 we characterized the thermoresponsive and co-nonsolvency behavior of Poly(N-isopropylacrylamide) (PNIPAM) microgels with nanometer resolution. PNIPAM microgels are colloidal polymeric particles that experience a phase transition triggered by heating above a certain temperature in water. This temperature is known as the lower critical solution temperature (LCST) and this phase transition leads to a decrease in microgel size. Our LC-(S)TEM observations of PNIPAM microgels in water show that their inhomogeneous internal structure (consisting of a dense core and a corona with a low polymer volume fraction) implies that not all parts of the microgels can be resolved. In other words the low density corona, responsible for most of the phase transition, is effectively invisible in water for reasonable electron beam intensities. In order to progress towards developing strategies for imaging of PNIPAM, the stability of the microgels upon electron beam illumination was assessed. Strong beam effects took place for PNIPAM microgels in water during LC-EM imaging, as microgels irreversibly shrunk after a few frames even for very low electron dose rates (< 5 e⁻⁸nm⁻²s⁻¹, see Chapter 5, Figure 2). This could be partially prevented by the addition of isopopanol (2.5 mM) acting as radical scavenger. Isopropanol can react with some water radiolysis products into less reactive species, thereby reducing the harshness of the irradiated solvent with the polymer. We propose that PNIPAM shrinkage under (S)TEM imaging is mediated by an increase of the internal cross-linking of the microgels due to the reaction of the polymeric chains with water radiolysis radicals, which was supported by our findings showing positive effects of scavenging radiolysis radicals. Next, different strategies to resolve the low contrast particle parts were tested. We report that adding metal salts in solution to increase the solvent contrast results in the beam-induced reduction and deposition of these ions into solids that grow preferentially on the PNIPAM microgels. We then explored the possibility to label in situ PNIPAM volumes with marker nanoparticles to determine the full extent of the microgels. We showed that Au nanoparticles attached to PNIPAM upon beam illumination, while particles with silica surfaces attached spontaneously to the microgels. Given the beam-effects particles underwent after just one frame, core-shell Au@silica were chosen as in situ markers of choice, because of the high contrast of gold and the spontaneous stickiness of silica. PNIPAM microgels were successfully labelled in situ via a post-synthetic labelling step with such marker particles. By means of in situ heating in the liquid cell we successfully characterized the reversible phase transition of PNIPAM microgels upon heating above the LCST and subsequent cooling down (see Chapter 5, Figure 5). Also, the microfluidic capabilities of our set-up were utilized to exchange the solvent present in the cell and the reversible size change due to the co-nonsolvency of PNIPAM microgels in the presence of alcoholic/water mixtures, which was successfully followed as well (see Chapter 5, Figure 6). We showed that electron beam illumination hinders the thermoresponsiveness of the irradiated particles in particular for multiple cycles. Finally, by using the same Au@silica marker nanoparticles we characterized the thermoresponsive behavior of hybrid PNIPAM microgels consisting of a silica core and a thin (around 100 nm) shell of PNIPAM.

In Chapter 6 we used confocal laser scanning microscopy (CSLM) to image the self-assembly (SA) of fluorescent bent-core silica rods (BSRs). A dispersion of fluorescent BSRs in index-matched
solvent (DMSO/water (78 % w/w)) was let to sediment and equilibrate. Fluorescent BSRs silica rods were synthesized by modifying an already existing emulsion based growth method. Particles in a sedimentation-diffusion equilibrium profile experience a range of pressures and number densities, and for rods this implies the SA into a variety of liquid crystal phases. Of such liquid crystal phases, we were interested in nematic (N) phases that are characterized by long-range order in particle orientation. When a liquid crystal is composed of building blocks incorporating bending, long-range bend deformations arise which cannot extend over indefinitely long distances and need to be resolved. In practice, the physical consequence is the formation of splay and twist deformations of the N phase, resulting in the formation of two characteristic phases, namely twisted (N$_{TB}$) and splay bent (N$_{SB}$) nematic phases (see Chapter 6, Figure 1). Although N$_{SB}$ phases were already predicted more than 40 years ago, their first experimental realization and observation was reported only very recently for banana-shaped colloids. Here we report that BSRs assemble into N$_{SB}$ liquid crystal phases in sedimentation-profiles (see Chapter 6, Figure 3). Our observations reveal that the pitch of such N$_{SB}$ was around 16 times larger than the size of the colloidal building blocks. A N$_{SB}$ phase was also identified in a sediment of BSR functionalized by an hematite ellipsoid synthesized via seeded-growth approach (see Chapter 6, Figure 5). The magnetic properties of such hematite particles, together with the possibility to align silica rods with an electric field, make this system promising for advanced manipulation of the SA process and inducing particular phases via combinations of external fields.
Samenvatting

Het doel van de studie, die in dit proefschrift beschreven is, is het in beeld brengen van gevoelige processen op de nanoschaal, hetgeen bereikt wordt door gespecialiseerde experimenten uit te voeren door middel van elektronenmicroscopie ofwel lichtmicroscopie. De eerste reeks van studies (Hoofdstukken 3-5) houdt zich voornamelijk bezig met vloeistofcel transmissie-elektronenmicroscopie (TEM). Vloeistofcel TEM, een techniek binnen het bredere kader van in situ TEM, is gespecialiseerd in het in beeld brengen van vloeistofmonsters. TEM wordt normaalgesproken gebruikt om monsters in hoog vacuum in beeld te brengen, wat het in beeld brengen van colloïdale en nanometrische monsters in hun oorspronkelijke staat onmogelijk maakt. Vloeistofcel TEM zorgt ervoor dat de vloeistof niet in contact komt met het vacuum van de microscoop door een dunne vloeistoflaag tussen twee membranen af te sluiten. State-of-the-art vloeistofcel TEM wordt uitgevoerd met behulp van toegewijde monsterhouders. Deze houders sluiten de vloeistof niet alleen af van het vacuum, maar laten het ook toe om vloeistof door de houder te laten stromen en elektrische elektrische velden aan te leggen. De ontwikkeling van op MEMS gebaseerde chip technologie als onderdeel van het afsluiten van de dunne vloeistoflaag in vloeistofcel TEM laat het in recente jaren toe om live in situ verwarming en electrochemische experimenten te doen in een vloeistofcel. In dit onderzoek bestuderen we processen die plaatsvinden in colloïdale systemen in vloeistoffen. In het tweede deel van dit proefschrift, hetgeen beschreven is in Hoofdstuk 6, verleggen we de focus van ons onderzoek naar lichtmicroscopie. Confocale scanning-lichtmicroscopie (CSLM) is een techniek waarmee fluorescerende monsters in beeld gebracht kunnen worden door een monster te scannen met een laser en het licht dat niet in focus is uit te sluiten door middel van een gaatje in het back-focale vlak van de beeldens om de resolutie te vergroten. Als fluorescerende colloïdale deeltjes in een vloeistof met een vergelijkbare brekingsindex zitten kan CSLM gebruikt worden om gebieden diep in het monster in beeld te brengen. We kunnen dan de mesoscopische orde van colloïdale systemen bepalen op het niveau van een enkel deeltje.

Hoofdstuk 2 vat belangrijke begrippen in vloeistofcel TEM samen. De ontwikkelingen en principes van vloeistofcel TEM worden geschetst en we definiëren de twee belangrijkste fysische parameters in vloeistofcel TEM: de elektronenflux en de totale elektronendosis. Omdat vloeistofcel TEM gekarakteriseerd wordt door de invloed van effecten die veroorzaakt worden door de elektronenbundel, vatten we de belangrijkste interacties tussen inkomende elektronen en een monster samen tijdens het in beeld brengen van het monster. Tijdens vloeistofcel TEM experimenten, die in dit proefschrift beschreven zijn (Hoofdstukken 3-5), worden verschillende nanodeeltjes en colloïdale systemen in beeld gebracht in waterige oplossingen. Radiolyse van water vindt plaats wanneer het blootgesteld wordt aan ioniserende elektronenstraling, hetgeen ook leidt tot de vorming van reactieve, radiolytische producten, wordt ook beschreven. Een paar woorden zijn gewijd aan colloïdale zelfassamblage, wat het spontane ordenen van colloïdale materie is, omdat dit het onderwerp is van het onderzoek beschreven in Hoofdstuk 6.

In Hoofdstuk 3 ontwikkelen we een algemene methode om het etsen van silicium dioxide (silica, SiO₂) nanodeeltjes in situ in beeld te brengen door middel van vloeistofcel scanning transmissie-elektronenmicroscopie (STEM). Eerst werd het ex situ etsen van een modelsysteem van aminozuur gekatalyseerde silica nanobollen volledig gekarakteriseerd. Dit modelsysteem was gekozen vanwege het goed gedefinieerde, langzame en geleidelijke etsproces in basische oplossingen. Dit proces hebben we ook gevolgd met dynamische lichtverstrooing. Dit maakte het mogelijk om onze in situ vloeistofcel STEM observaties te vergelijken met ex situ etsexperimenten. Daardoor konden we bepalen wat de invloed van de elektronenbundel was. We vonden dat silica nanodeeltjes in water, als ze met de elektronenbundel beschoten werden, twee verschillende effecten ondervonden: een fase
waarin ze uitdijden en een fase waarin ze oplosten. Deze laatste fase uitte zich meer wanneer de deeltjes zich in basische oplossing (100 mM NaOH) bevonden (zie Hoofdstuk 3, Figuur 3.3). Door het simuleren van de banen die elektronen (200 keV) afleggen door het monster bepaalden we dat genoeg energie in de nanodeeltjes afgegeven werd per plaatje om het siloxaanennetwerk sterk te bevrijden (zelfs bij lage elektronendosis). We lieten zien dat de uitdijfase direct gecorreleerd kon worden aan de cumulatieve (totale) elektronendosis op het belichte oppervlak, terwijl de oplosfase door de elektronenflux aangedreven leek te worden. We stellen voor dat de directe interactie van de bundel met het silicaneutwerk de uitdijfase aandrijft, terwijl de oplosfase ook sterk afhankt van de chemie in het oplosmiddel (etsmiddel en radiolyse producten vanuit het water). We gebruikten deze uitkomst om te bepalen wat de beste condities zijn om silica in water en basische oplossingen te bestuderen in een elektronenmicroscoop. Met deze condities bedoelen we bijvoorbeeld het minimaliseren van de elektronenflux en het uitzetten van de elektronenbundel tijdens bepaalde periodes om de cumulatieve elektronendosis te beperken. We lieten ook zien dat deze condities beperkten hoe klein de silica nanodeeltjes kunnen zijn die een bepaalde TEM in beeld kan brengen, omdat voor kleinere deeltjes hogere vergrotingen nodig zijn waardoor hogere elektronenfluxen onvermijdelijk zijn. We karakteriseerden toen het etsproces in waterige, basische oplossingen van Stöber en water-in-olie omgekeerde micro-emulsie (WORM) silica nanobollen. Beide systemen staan erom bekend dat ze een inhomogene interne distributie van silicacoondensatie hebben: de mate van silicacoondensatie stijgt dichterbij het deeltjesoppervlak. Het \textit{ex situ} bulk etsen van deze deeltjes wordt vaak gerapporteerd als een proces dat begint door het uithollen van het deeltje, waarna het deeltje vervolgens compleet oplost. We konden de formatie van hol silica Stöber en WORM nanobollen \textit{in situ} op het niveau van één deeltje volgen (zie Hoofdstuk 3, Figuur 3.5). Daarom is vloeistofcel (S)TEM een manier om de interne distributie van de condensatiegraad van silica deeltjes en wellicht andere oxides te bekijken. Silica kern-schilddeeltjes, zoals metaal@silica of metaaloxide@silica zijn interessant voor veel toepassingen en veel van deze toepassingen (zoals katalyse) zijn afhankelijk van het uithollen van een silica schil om een holte te maken voor het kerndeeltje. Daarom karakteriseerden we ook het etsproces van kern-schil Au@Stöber en Fe$_2$O$_3$@WORM silica nanobollen \textit{in situ} op het niveau van één deeltje. Interessant genoeg konden we laten zien dat, na het etsproces van het binnenste van de schillen plaatsgevonden had, de metaal en metaaloxide kernen vrij waren om te diffunderen binnenin de holle schil (zie Hoofdstuk 3, Figuur 3.5). Deze observatie laat het potentieel zien van vloeistofcel (S)TEM in het bestuderen van dit soort synthese procedures gebaseerd op etsen, omdat normale TEM alleen monsters in vacuum in beeld kan brengen en dus het diffuse gedrag van de kern niet kon vaststellen. In Hoofdstuk 4 werd het etsproces van colloïdale silica staafjes (grootte van rond de micrometer) naar silica kegels bestudeerd met vloeistofcel (S)TEM. Dit proces was voorheen al beschreven door onze groep, maar het is nog nooit gevolgd op het niveau van één deeltje. Silica staafjes, vergelijkbaar met de Stöber en WORM silica nanobollen zoals bestudeerd in het vorige hoofdstuk, hebben een inhomogene, interne distributie van silica condensatiegraden: er is een graden van condensatie langs de lengte van het deeltje en een hoog-gecondenseerde schil rondom het volledige staafje. Deze interne structuur resulteert in een etsmechanisme dat leidt tot koolstofvormige deeltjes. In dit hoofdstuk beschrijven we hoe we de studie van de interne condensatiegraad van silica deeltjes uitbreidden naar deze micrometer silica deeltjes. De kennis over het in beeld brengen van silica deeltjes, die vergaard was in Hoofdstuk 3 (lage dosis, klein aantal afbeeldingen), werd gebruikt om zulke etsprocessen te karakteriseren zonder invloed van elektronenbundeleffecten. We volgden met succes de progressie van silica staafjes naar silica kegeltjes tijdens het etsen in basische, waterige oplossingen op het niveau van één deeltje met nanometer resolutie. We konden een voorheen onbekende stap van dit etsproces beschrijven waarin een deel van het staafje afbreekt van de punt van het vormende kegelte (zie Hoofdstuk 4, Figuur 4.4). Omdat de silica staafjes groter zijn dan de
nanodeeltjes, die in Hoofdstuk 3 gebruikt werden, konden de wrijvingskrachten door de continue stroming van vloeistof in de vloeistofcel genoeg zijn om de silica staafjes van de plek waar ze vast zaten los te maken. Dit maakte het nodig om ook de stroomsnelheid te optimaliseren. De vergelijking van de berekende wrijvingskrachten die op kleine nanobollen (100 nm) en op microstaafjes werken ondersteunde onze waarnemingen. Verder laten we zien dat de gesloten geometrie van de vloeistofcel het etsproces kan beïnvloeden vergeleken met het etsen van staafjes die aan een oppervlak vastzaten dat ondergedompeld was in een bulk etsoplossing. Om dit te doen werden ex situ etsexperimenten van silicastaafjes die vastzaten aan een siliciumnitride membraan uitgevoerd. Opvallende verschillen werden waargenomen tussen het etsproces in de vloeistofcel en bij blootstelling aan bulk etsmiddel, waar vooral de distributie in grootte van de resulterende, geëtste deeltjes anders was, hetgeen misschien kan betekenen dat er ietwat verschillende etsmechanismen zijn. Dit resultaat is belangrijk voor elke andere vloeistofcel EM studie van chemische processen, omdat zo ongeveer elke chemische reactie die plaatsvindt op deeltjes die vastzitten aan het venster van de vloeistofcel tot op zekere hoogte beïnvloed kan worden met deze begrenzingeffecten. We breidden toen onze resultaten uit om te laten zien dat het voorkeursetsen van minder gecondenseerde delen van andere silica colloïdale ook gevolgd kon worden door vloeistofcel TEM. Dit werd gedaan door het etsen van bekende silica staafjes model systemen, die bestaan uit segmenten met verschillende condensatiegraden (zie Hoofdstuk 4, Figuur 4.7). We demonstreerden toen het potentieel van deze benadering door de interne structuur van een nieuw systeem van silica staafjes met een geboogd kern te onderzoeken. Twee systemen van deeltjes met vergelijkbare uiteindelijke geometrie en groottes, maar met verschillende synthesecondities (temperatuur, precursor), werden bestudeerd. Onze resultaten lieten zien dat deze deeltjes verschillende graden van silica condensatie hadden in hun het buigingsgebied tussen de twee rechte segmenten (zie Hoofdstuk 4, Figuur 4.8).

In Hoofdstuk 5 karakteriseerden we de thermostressiviteit en dubbel-niet-oplosbaarheid van Poly(N-isopropylacrylamide) (PNIPAM) microgels met nanometer resolutie. PNIPAM microgels zijn colloïdale polymeerdeeltjes die een faseovergang ondergaan als ze opgewarmd worden tot boven een bepaalde temperatuur in water. Deze temperatuur staat bekend als de lagere, kritieke oplossingsstemperatuur en deze faseovergang leidt tot inkrimping van de microgel. Onze vloeistofcel (S)TEM observaties van PNIPAM microgels in water laten zien dat hun inhongene, interne structuur (die bestaat uit een dichtere kern en een corona met een lage polymeervolumefractie) impliceert dat niet alle delen van de microgels kunnen worden bekeken met elektronen. In andere woorden afgebeeld dit dat de corona met een lage dichtheid, die voornamelijk verantwoordelijk is voor de faseovergang, eigenlijk niet te zien is in water met redelijke elektronenbundelintensiteiten. Om strategieën te ontwikkelen voor het in beeld brengen van PNIPAM, moest de stabilititeit van de microgels onder bestraling met een elektronenbundel onderzocht worden. Sterke bundeleffecten vonden plaats bij PNIPAM microgels in water tijdens het in beeld brengen in vloeistofcel EM. De microgels krompen namelijk na een paar opgenomen beelden, zelfs voor heel lage elektronenflux ($< 5 \text{e}^{-}\text{nm}^{-2}\text{s}^{-1}$, zie Hoofdstuk 5, Figuur 5.2). Dit effect was onomkeerbaar. Het kon echter deels voorkomen worden door het toevoegen van isopropanol (2.5 mM), dat als radicaalverwijderaar kon fungeren. Isopropanol kan reageren met sommige waterradiolyseproducten naar minder reactieve verbindingen, waardoor het effect van de bestaande oplossing op het polymeer minder werd. We stellen voor dat het krimpen van PNIPAM tijdens het in beeld brengen met (S)TEM komt door een verhoging in de interne verknoping van de microgels door de reactie van de polymeerkettingen met waterradiolyseradicalen, wat ondersteund werd door onze bevinding waarin positieve effecten optraden door het gebruik van radicaleneters. Vervolgens testten we verschillende strategieën om de deeltjesonderdelen met een laag contrast in beeld te brengen. We rapporterden dat het toevoegen van metaalzouten aan de oplossing om het contrast van het oplosmiddel te verhogen resulteerde in bundel-geïnduceerde reductie en depositie van deze ionen in vaste materialen die het fijst op
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de PNIPAM microgels groeiden. We onderzochten verder de mogelijkheid om in situ de PNIPAM volumes te markeren met nanodeeltjes om de volledige grootte van de microgels te bepalen. We lieten zien dat Au nanodeeltje vast gingen zitten aan PNIPAM als we het monster beschenen met de elektronenbundel, terwijl deeltjes met silica aan het oppervlak spontaan vast gingen zitten aan de microgels. Door de bundeleffecten die de deeltjes ondergingen na zelfs één beeldopname, kozen we ervoor om kern-schil Au@silica deeltjes te gebruiken als in situ markeringen, vanwege het hoge contrast van het goud en het spontaan blijven vastzitten van silica. PNIPAM microgels werden in situ met succes gemarkeerd na de synthese met dit soort markeerdeeltjes. Door middel van in situ opwarmingsexperimenten in de vloeistofcel karakteriseerden we met succes de omkeerbare faseovergang van PNIPAM microgels, wanneer deze opgewarmd werden tot boven de lage, kritieke oplossingstemperatuur en de daaropvolgende afkoeling (zie Hoofdstuk 5, Figuur 5.5). Bovendien werden de microfluidische mogelijkheden van onze setup gebruikt om de aanwezige vloeistof in de vloeistofcel uit te wisselen. Zodoende kon de omkeerbare grootteverandering van PNIPAM microgels in de aanwezigheid van alcohol/watermengsels succesvol gevolgd worden (zie Hoofdstuk 5, Figuur 5.6). We lieten zien dat elektronenbundelbeschijning de thermoresponsiviteit van de beschenen deeltjes belemmert, vooral bij meerdere cycli. Ten slotte karakteriseerden we de thermoresponsiviteit van hybride PNIPAM microgels, die bestonden uit een silica kern en een dunne (rond de 100 nm) schil van PNIPAM, door gebruik te maken van dezelfde Au@silica markeerdeeltjes.

In Hoofdstuk 6 gebruikten we confocale laserscanmicroscopie (CSLM) om de zelfassemblage van fluorescerende silica staafjes met een gebogen vorm in beeld te brengen. Een dispersie van fluorescerende silica staafjes met een gebogen vorm in een oplossing met dezelfde brekingsindex (DMSO/water (78 % w/w)) werd de mogelijkheid gegeven om naar beneden te zakken en in evenwicht te geraken. Fluorescerende silica staafjes werden gesynthetiseerd door een reeds bestaande groeimethode gebaseerd op emulsies. Deeltjes in een bezinking-diffusie evenwichtsprofiel ervaren een reeks van drukken en nummerdichtheden. Voor staafjes betekent dit zelfassemblage in verschillende vloeibare kristallijne fasen. Van zulke vloeibare kristallijne fasen, waren wij vooral geïnteresseerd in de nematische (N) fasen die gekenmerkt worden door langeafstandsvolgorde in de oriëntatie van de deeltjes. Wanneer een vloeibaar kristal gemaakt is van bouwstenen die gebogen zijn, verschijnen er lange afstands buigingsvervormingen, die zich niet uit kunnen strekken over oneindig lange afstanden. In praktijk leidt dit tot de vorming van spreidings- en draaiings-vervormingen van de nematische fase, die kunnen resulteren in twee karakteristieke fasen, namelijk een twisted (N_{TB}) of splay-bend (N_{SB}) nematische fase (zie Hoofdstuk 6, Figuur 6.1). Alhoewel N_{SB} fasen reeds 40 jaar geleden voorspeld waren, werd hun eerste experimentele realisatie en observatie pas heel recent gerapporteerd voor banaanvormige deeltjes. Hier rapporteren we dat gebogen fluorescerende silica staafjes samenkomen in N_{SB} vloeibare kristallijne fasen in bezinking-profielen (zie Hoofdstuk 6, Figuur 6.3). Onze observaties onthullen dat de pitch van zulke N_{SB} ongeveer 16 keer groter was dan de colloidale bouwstenen zelf. Een N_{SB} fase werd ook waargenomen in een sediment van silica staafjes met een gebogen kern die gefunctionaliseerd waren met een hematietellips die gesynthetiseerd en door een seeded-growth benadering (zie Hoofdstuk 6, Figuur 6.5). De magnetische eigenschappen van zulke hematietdeeltjes, samen met de mogelijkheid om silica staafjes met een elektrische veld allemaal in één bepaalde richting te laten wijzen, zorgen ervoor dat dit systeem veel potentie heeft voor gevanceerde manipulatie van het zelfassemblageproces en het indienen van bepaalde nieuwe fasen via combinaties van externe velden.
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I first stepped in the SCM group as a master student, so there have been many people along this way. I will dash though this time a bit, starting from my very first day in the group. That day, I was assigned a desk in the student room (felt very exciting) and I met my daily supervisor, Fabian. I remember very vividly that Fabian asked me if I had done any synthesis before. I said no, and he immediately kicked me in the wet lab to train me. I am pretty sure he felt like giving up that very day when my first action was to pop my head inside the fume hood to open a bottle. However, he was very patient and I actually learned to do chemistry stuff! Along the way, I also made a very good friend. Dank je wel, let’s not stop having burgers out! Meanwhile, squeezed in the student room, me and the other students spent our days. I think we were a very fun and easygoing group and I had a very good time. Also the old guard of PhDs was very welcoming to us. Thanks to all of you! Little I knew that many of them will go on to be colleagues, collaborators, coauthors and, above all, friends.

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List of Publications

This thesis is composed of:

A. Grau-Carbonell/S. Sadighikia, T.A.J. Welling, R.J.A. van Dijk-Moes, R. Kotni, M. Bransen, A. van Blaaderen and M.A. van Huis "In Situ Study of the Wet Chemical Etching of SiO$_2$ and Metal(Oxide)@SiO$_2$ Core-Shell Nanospheres" ACS Applied Nanomaterials, 4(2), 1136-1148, DOI: 10.1021/acsanm.0c02771, (2020). Chapter 3


A. Grau-Carbonell, F. Hagemans, M. Bransen, N.A. Elbers, R.J.A. van Dijk-Moes, S. Sadighikia, T.A.J. Welling, A. van Blaaderen and M.A. van Huis "In situ single particle characterization of the themoresponsive and co-nonsolvent behavior of poly(N-isopropylacrylamide) microgels and silica@PNIPAM core-shell colloids" To be submitted. Chapter 5

R. Kotni/A. Grau-Carbonell/M. Chiappini, M. Dijkstra and A. van Blaaderen "Splay-bend nematic phases of bent colloidal silica rods induced by polydispersity" Submitted. Chapter 6

Other publications by the author during the work on this thesis:


About the Author

Albert Grau Carbonell was born in 1992 in Puigcerdà, Catalonia, Spain. After finishing high school in his hometown he completed a Physics Degree at Universitat Autònoma de Barcelona (UAB), during which he followed a track in applied physics. Next, he moved to Utrecht (The Netherlands) to study the masters degree "Nanomaterials: Physics and Chemistry", that he completed cum laude. His master thesis, at the Soft Condensed Matter group (SCM, Utrecht University), already focused on colloidal matter and confocal microscopy with the title "Depletion induced self-assembly of silica rods" under the supervision of Arnout Imhof and Alfons van Blaaderen. Albert completed his PhD at the SCM group under the supervision of Marijn A. van Huis and Alfons van Blaaderen imaging sensitive processes at the nanoscale via liquid cell transmission electron microscopy.