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Synthesis and Surface Modification of Birefringent Vaterite Microspheres

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This paper reports on the synthesis of birefringent vaterite microspheres with narrow size distribution using a seeded growth method. In a post-treatment the microspheres were stabilized and functionalized through coating with a combination of organosilica and silica. The coating vastly enhanced the stability of the vaterite microspheres in biological buffers and allowed the attachment of biomolecules such as DNA or proteins. As an example, streptavidin was attached to the surface of the functionalized microspheres. These results pave the way for the use of birefringent vaterite particles for the micromanipulation of single biological molecules such as DNA or specific proteins in an optical trap capable of exerting and measuring torques. The stabilized birefringent microspheres may also find use for biosensor and biological screening applications.

Introduction

Vaterite, previously referred to as vaterite B or μ-$CaCO_3$,[1] is one of three crystalline polymorphs of calcium carbonate, the other two being calcite and aragonite. It is the least stable phase and rarely appears in nature since it slowly dissolves and recrystallizes as calcite in contact with water.[2] Under certain conditions, however, the metastable vaterite phase is stabilized and can therefore be found in gallstones, fish otoliths, mollusc shells, sediments, and metamorphic rocks.[3]

Vaterite can also be manufactured in the laboratory, either by mixing concentrated solutions of calcium and carbonate containing salts[4,5] or by bubbling carbon dioxide through a calcium salt solution.[6,7] When the solution becomes supersaturated, amorphous calcium carbonate (ACC) forms rapidly. The ACC transforms to crystalline vaterite within a few minutes. The result is usually 1−10 μm spherulitic crystals composed of fibrous aggregates radiating from the center.[8] The aggregates are in turn composed of 25−35 nm sized crystallites.[9]

Vaterite is positively uniaxial with indices of refraction of n_e = 1.650 and n_o = 1.550.[4] The spherical aggregates described above maintain remarkably strong birefringence.[10] These properties make them perfect for torque measurements in an angular optical trap.[11,12]The spherical shape also allows simple formulas to be used for viscous drag calculations, enabling the measurement of the viscosity in specific microenvironments.[13]

A novel method for the fabrication of spherical birefringent vaterite particles was previously developed in our lab by Bishop et al.,[12] using a combination of Ca, CO_3, and Mg containing salts followed by surfactant stabilization. In the work presented here, this procedure has been modified in our lab using a seeded growth method, resulting in higher yield and increased size uniformity than the previously published method. The synthesis method is detailed in this paper.

Vaterite microspheres developed in our lab have been shown to be useful in various fields, such as microrheology[13] and microfluidics.[14,15] Central to the success of microfluidic systems has been the development of innovative methods for the manipulation of fluids within microchannels.[15] Leach et al. applied rotating vaterite spheres to generate flow within microfluidic channels and hence created an optically driven pump for microfluidics.[14,15]

A central part of the current work was the stabilization and functionalization of vaterite microspheres with a coating that would allow covalent coupling of biological molecules to some functional group on the surface. While the optical properties of vaterite make it ideal for torsional measurements in optical tweezers,[12] its use in experiments on biological samples such as living cells, proteins, and single DNA molecules has so far been limited. The reason for this is that the typical buffer solutions used for biomolecule attachement tend to dissolve the metastable vaterite crystals rapidly.

We have earlier proposed a method to apply and measure optical torques in optical tweezers with high accuracy.[16] Deufel et al. used this method to apply torque of known magnitude and with high accuracy measure the extension/contraction of B-DNA during positive supercoiling.[17] These findings are important to

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better understand cellular processes such as transcription, replication, DNA recombination, and DNA repair.[17] The particles used were quartz cylinders fabricated by dry-etching of a patterned quartz wafer. However vaterite ($\Delta n = 0.1$) is far more birefringent than quartz ($\Delta n = 0.009$). This allows more torque to be applied or requires lower laser power to be used to apply sufficient torque for a certain experiment. As a rough guide, one can expect to exert approximately 3 pNμm per mW of laser power (i.e., $\hbar$ per photon).

Several papers describe methods for stabilizing vaterite. Kim et al. used fluosilicic acid to enhance acid resistance of calcium carbonate crystals[18] while Walsh et al. coated vaterite microsponges with PVC polymer to stabilize the particles against short-term dissolution.[19] Fuchigami et al. produced hemispherical hollow silica microcapsules by applying a silica coating onto hollow vaterite microspheres,[20] and Nieminen et al. mentioned silica coating of vaterite microspheres for tailoring their optical properties.[21] We used mixed layer coatings of silica and organosilica to stabilize and functionalize vaterite microspheres and make them applicable for biological experiments. The amine functionality of the organosilica allows the immobilization of a variety of biomolecules to the microsphere surface. After functionalizing the microspheres we successfully attached streptavidin onto their surface.

Materials and Methods

Synthesis of Vaterite Microspheres. Vaterite microspheres were synthesized by mixing aqueous solutions of $CaCl_2$ (Sigma), $MgSO_4$ (Sigma) and K_2CO_3 (Sigma). In a typical synthesis 1.5 mL of 0.1 M $CaCl_2$ was pipetted into a 5 mL plastic vial, followed by 120 μL of 0.1 M $MgSO_4$ and 180 μL of 0.1 M K_2CO_3. The solution was then agitated by vigorous pipetting. To stabilize the resulting colloidal solution, agepon (a wetting agent made by Agfa) was added to the solution. This synthesis method is referred to as the agitation method.

After mixing 5 ml of 0.1 M $CaCl_2$, 1.5 ml of 0.1 M $MgSO_4$ and 1 ml of 0.1 M K_2CO_3, approximately 15 μL of a previous sample was added to the solution to promote the seeded growth of the $CaCO_3$ crystals. This sample is referred to as the uncoated sample. A good seed was developed by consecutively producing samples using seed from the previous one where a first seed solution was produced using the agitation method.

Silica Coating. In a typical coating procedure approximately 4 mg of washed microspheres were suspended in a mixture of 940 μL of ethanol, 15 μL of 3-aminopropyltrimethoxysilane (APS, Aldrich), and 50 μL of ammonia (Merck 25% V/V). The microspheres were incubated in a shaker at room temperature. After 2 h, the microspheres were washed three times and resuspended in ethanol.

The procedure for a typical tetraethylorthosilicate (TEOS, Aldrich) coating was basically identical with APS coating, but using TEOS instead of APS for 5 h. TEOS coatings were attempted on uncoated vaterite spheres and on vaterite spheres with a primary APS coating. After TEOS coating, the surface of some microspheres were functionalized with a secondary APS coating.

To verify the quality of the coating several tests were performed. The first of these was a dye-test in which a small amount of each sample was incubated with BODIPY 530/550 succinimidyl ester (Invitrogen) dissolved in ethanol. After incubation, the microspheres were washed in ethanol at least three times to remove all unbound dye.

Second and most importantly a stability test was carried out. In this test 1 mL of LBB^- buffer (10 mM Tris−HCl (pH 7.4), 200 mM KCl, 0.1 mM EDTA, 0.1 mg/mL α-casein) was added to dried vaterite samples and placed in a mixer to suspend the microspheres in the buffer. The samples were then studied in an optical microscope.

These two tests were followed by attempting attachement of streptavidin. The streptavidin coating of the microspheres is achieved in a two step procedure in which initially glutaraldehyde (Ajax Finechem) is linked to the amine groups on the surface and then streptavidin to glutaraldehyde in the second step. To simplify verification of the streptavidin coating, streptavidin labeled with a fluorescent dye (Streptavidin, Alexa Fluor 594 conjugate, Invitrogen Corporation) was used.

In the first step of this procedure approximately 2 mg of amine functionalized vaterite microspheres were suspended in 2 mL of ethanol and sonicated for 5 min. A 0.25 mL portion of H_2O was then added together with 1 mL of glutaraldehyde (25% in H_2O), and the mixture was incubated in a shaker for 2 h. The microspheres were washed three times in ethanol to remove the glutaraldehyde and three times in H_2O with 0.5% Tween to remove the ethanol.

In the second step, streptavidin was stored in frozen aliquots each containing 100 μg of streptavidin and 100 μL of PBS buffer. The glutaraldehyde coated microspheres were suspended in $^1/_2$ aliquot and H_2O with 0.5% Tween in a 1.5 mL Eppendorf tube and incubated at 4 °C for 5 h. The microspheres were then washed three times in H_2O with 0.5% Tween.

Methods. *Electron Microscopy*. Scanning Electron Microscopy (SEM) images of platinum coated samples were collected on a JEOL JSM 6400F and a Joel JSM 6460LA, equipped with a quantitative Energy Dispersive Spectrometer (EDS). EDS was used to observe any increase in the abundance of silicon on the surface.

Crushed vaterite were prepared by grinding the ethanol suspension with a mortar and pestle before putting it on the sample holder.

X-ray Diffraction. X-ray diffraction (XRD) was used to determine the abundance of the different calcium carbonate polymorphs in our samples and to estimate the crystallite sizes of the different polymorphs. XRD spectra were obtained using Cu Kα radiation in a Bruker Axs diffractometer.

To prepare the samples for XRD, the microspheres were suspended in ethanol which was dropped on a silicon wafer and left to evaporate. This was repeated until the wafer was covered by a thin layer of dry sample.

X-ray Photoelectron Spectroscopy (*XPS*). Data was acquired using a Kratos Axis ULTRA X-ray Photoelectron Spectrometer incorporating a 165 mm hemispherical electron energy analyzer. Monochromatic Al X-rays (1486.6 eV) at 150 W (15 kV, 10 ma) were used for sample irradiation. Survey (wide) scans were taken at analyzer pass energy of 160 eV and multiplex (narrow) high resolution scans at 20 eV. Survey scans were carried out over 1200−0 eV binding energy range with 1.0 eV steps and a dwell time of 100 ms. Narrow high-resolution scans were run with 0.05 eV steps and 250 ms dwell time. Base pressure in the analysis chamber was 1.0×10^{-9} torr and during sample analysis 1.0×10^{-8} torr.

Fluorescence Microscopy. Fluorescent microspheres were imaged using a SPOT Diagnostic Instruments camera attached to an inverted Olympus IX70 fluorescence microscope. The microscope was fitted with a mercury lamp as the excitation source and equipped with numerous filters in excitation and emission pathways.

Optical Tweezers. The setup for the optical torque measurements is explained elsewhere.[13,22]

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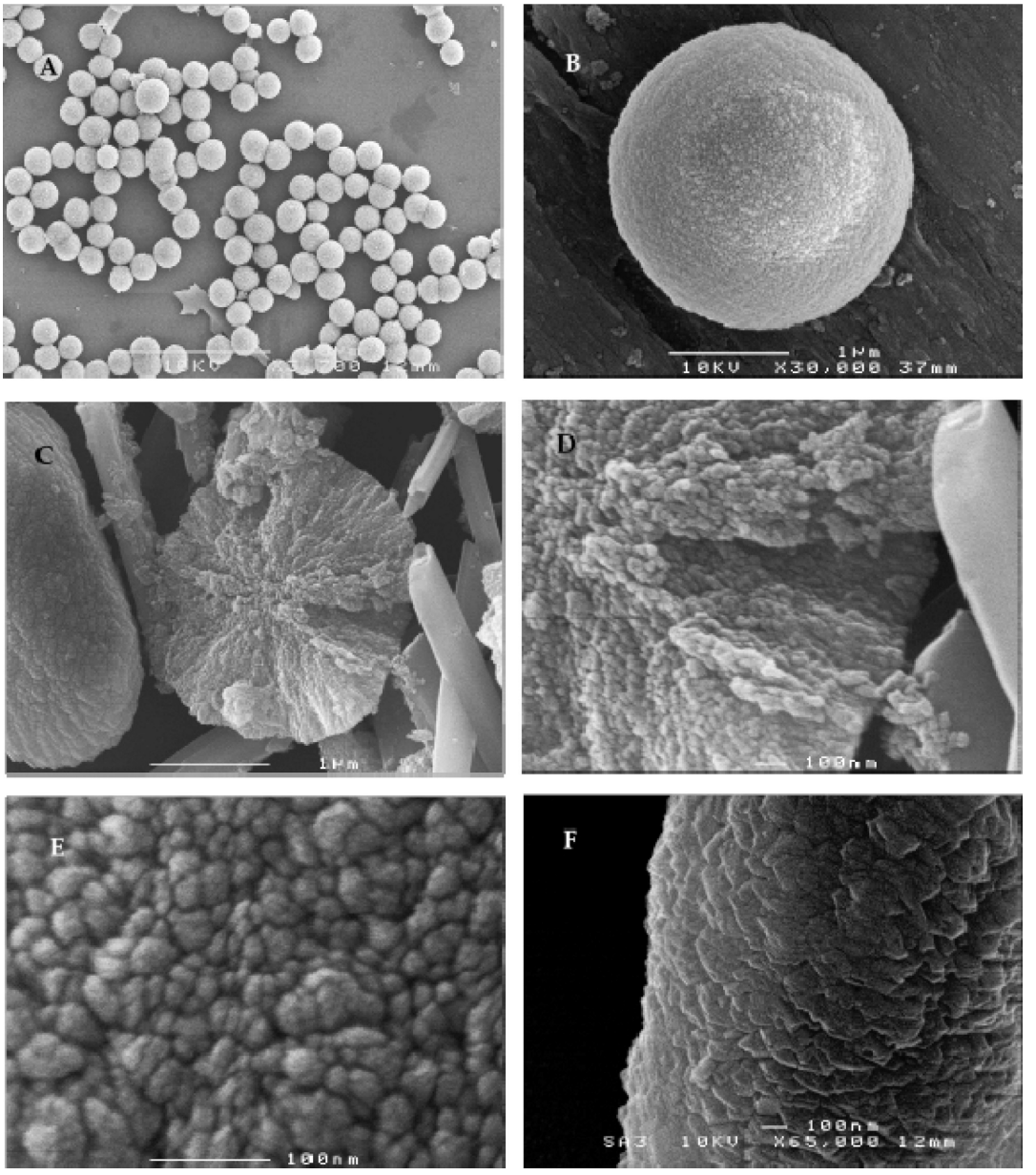

Figure 1. Scanning Electron micrographs showing uncoated vaterite spheres at different magnifications (A), (B), and (E), crushed vaterite spheres together with the two other forms of calcium carbonate, calcite to the left and aragonite to the right (C), crushed vaterite at higher magnification (D), and a calcite particle at high magnification (F).

Results and Discussion

(a). Synthesis of Vaterite Microspheres. Vaterite usually appears in a mixture with various amounts of the two other polymorphs calcite and aragonite. Figure 1 shows micrographs of the different morphologies of calcium carbonate obtained with the seeded growth method. Three distinct structures can be found in most samples. As can be seen in the low magnification image (A), the predominant form is spherical vaterite, but small amounts of polycrystalline egg-shaped particles (C, left) and smooth rod like crystals (C, right) can also be found in most of our samples. The rod shaped crystals are identified as aragonite.[23] High magnification images of the surface of a vaterite sphere (E) and an egg-like particle (F) reveal different shapes of the crystallites that form the particles. The vaterite sphere consists of small smooth crystallites with an estimated diameter ranging from 20 to 40 nm. This is in agreement with the vaterite crystal size calculated from XRD measurements described below as well as with literature.[3] The egg shaped particle in (F) on the other hand, seems to consist of larger plate like crystals (> 100 nm) with sharper edges and is believed to be calcite.[24]

XRD was used to further investigate the composition of the different calcium carbonate polymorphs in our sample. A typical XRD pattern of the precipitate is shown in Figure 2. It displays strong diffraction peaks indicative of the dihexagonal dipyramidal structure of vaterite. The vaterite peaks are indexed as (004), (110), (112), (114), (211), (008), (300), (304), and (118). Smaller amounts of calcite and aragonite also appear to be present in the precipitate, represented by the unlabeled diffraction peaks.

The fwhm of vaterite and calcite peaks were determined to calculate the crystal sizes (L) of the two polymorphs using the

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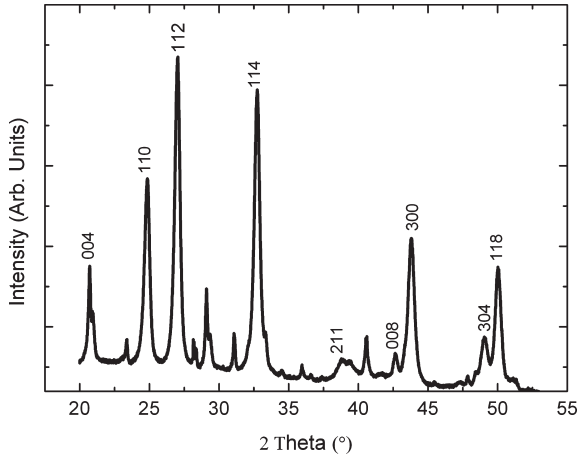

Figure 2. XRD spectrum of uncoated sample, showing predominantly vaterite (diffraction peaks are indexed) and to a lesser degree calcite and aragonite polymorphs represented by the unlabeled diffraction peaks.

Debye−Scherrer formula:

$$L = \frac{K\lambda}{\beta \cos \theta} \tag{0.1}$$

where K is the Scherrer constant, λ is the X-ray wavelength (1.5418 Å), θ is the Bragg angle, and β is the fwhm of the diffraction peaks corrected for the instrumental line width. The Scherrer constant depends on the size distribution of the crystallites and may vary between 0.5 and 1.1. This has to be estimated, which adds a relatively large uncertainty to the calculation. A crystal size of 27 nm was obtained for vaterite and of 91 nm for calcite using $K = 0.8$. These sizes are in agreement with the sizes typically observed using electron microscopy (Figure 1 (E), (F)). This gives further indication that the different shapes observed in Figure 1 are indeed different polymorphs of calcium carbonate.

Since the different shapes can easily be distinguished with optical microscopy, the content of unwanted polymorphs can easily be determined in our lab directly after production. This statement should be made with care as spherical calcite particles have been also reported.[25] However, being negatively birefringent,[26] such particles, if perfectly spherical, will not rotate when trapped in optical tweezers with circularly polarized light, which was not the case for our microspheres.

Electron microscopy was also used to examine the internal structure of the vaterite microspheres. For this purpose, some microspheres were crushed by grinding the sample using a mortar and pestle. Figure 1 (C) and (D) show a vaterite microsphere cracked in halves using this method. These pictures show how the crystallites form needle or fiber like structures that appear to radiate from the center of the microsphere. This observation is in agreement with the general description of spherulites.[27] It also agrees with the description of the vaterite structure by other authors.[8]

A detailed study of the structure based on optical measurements is being prepared for publication elsewhere.

(b). Stabilization and Functionalization of Vaterite Microspheres. The main objective of the current work was the stabilization and functionalization of vaterite microspheres through a coating that allows covalent coupling of biological molecules to some functional group on the microspheres' surface. We have shown in our earlier work that a spherical vaterite is ideal for torsional measurements in optical tweezers. However, the investigation of biological samples such as living cells, rotormolecules, and single DNA molecules is hardly possible. Normally buffer solutions are used with these biological samples, usually PBS or LBB$^-$ buffers. When vaterite microspheres are put into these buffers they tend to dissolve rapidly. Therefore to be able to use these microspheres for rotational measurements in biologically relevant environments, they have to be chemically modified. First, they have to be coated with a material dense and thick enough to prevent or at least radically reduce the ionic exchange between the calcium carbonate and the medium. Second, these modified particles have to be further functionalized to allow covalent attachment of biomolecules onto the microsphere surface.

Table 1. Lifetimes of Uncoated and Coated Vaterite Microspheres

sample	lifetime in LBB$^-$ buffer
uncoated	$<$ 1 min
APS-coated	$<$ 3 min
1×TEOS with no primary APS	$\approx$ 30 min
1×TEOS with primary APS	$\approx$ 4 h
2×TEOS with primary APS	$\approx$ 6 h
4×TEOS with primary APS	$\approx$ 10 h

With an isoelectric point of pH 8.5[28] vaterite is negatively charged at elevated basic conditions reducing particle aggregation during the coating process. APS was used to modify the vaterite surface with organosilica and acting as a primer for further silica coatings. As a common agent for the surface modification of silica, metal, and other inorganic and organic materials, it is known to form very thin coatings.[29,30] Short chain aminosilanes such as APS are among the most extensively studied self-assembled monolayer systems (SAMs).[31] These SAMs frequently contain microstructural defects which lower the surface homogeneity.[31] Hence the APS coating (also referred to as the primary APS coating) alone was not expected to provide sufficient protection for the vaterite microspheres against dissolving in buffer solutions. This hypothesis was supported by lifetime studies (Table 1) discussed later. For this purpose, TEOS was used to form a stable silica coating which later could be functionalized with a second APS coating (also referred to as the secondary coating). Similar mixed layer coatings of silica and organosilica have been applied to silica microspheres to create core−shell structures where the shell may have different properties such as variable refractive index[32] or incorporated dye molecules.[33]

Several methods were used to verify and characterize the different coatings. SEM micrographs of the APS coated microspheres show surface structures more or less identical to those of uncoated spheres (Figure 3). It was also verified that they are still trappable and rotate in circularly polarized light. This indicates that the inner structure of the microspheres was intact and birefringence maintained after coating.

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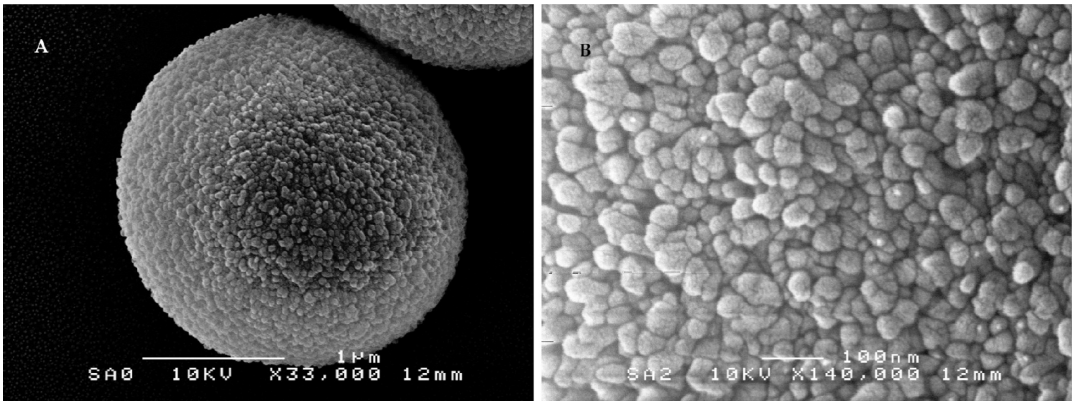

Figure 3. Scanning electron micrograph of an APS coated vaterite microsphere.

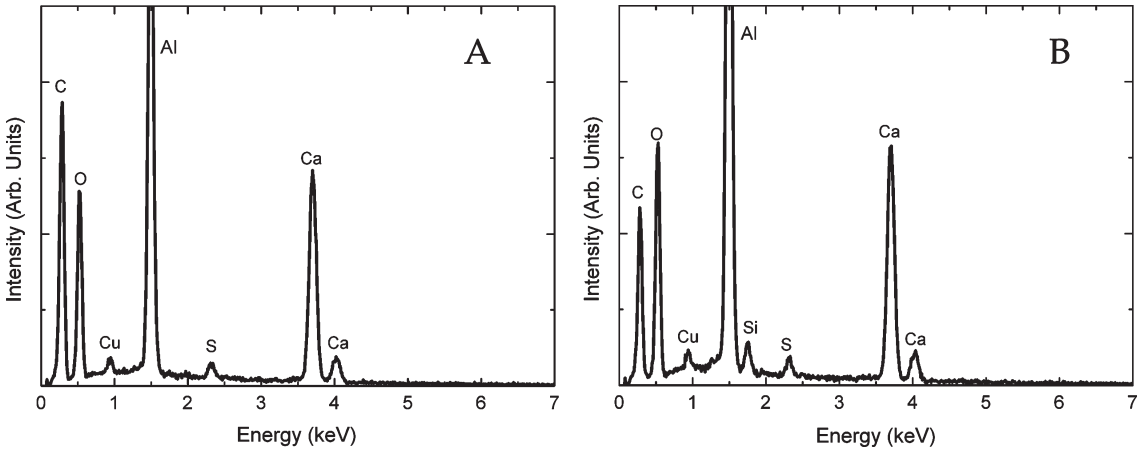

Figure 4. EDS spectra of an uncoated (A) and an APS coated (B) vaterite microsphere.

EDS was used to analyze the content of different atomic elements. The change in abundance of silicon for coated and uncoated microspheres was observed to confirm that some organosilica was applied onto the microspheres. Figure 4 shows EDS spectra of uncoated and APS-coated vaterite. As expected, the spectrum of the uncoated microsphere (A) shows peaks for carbon, oxygen, and calcium and also a high peak for aluminum caused by the sample holder. After APS coating (B), a microsphere from the same sample, with similar acquisition parameters, shows a small but distinct peak for silicon indicating the presence of silica on the surface. It also shows an increase in the oxygen/carbon ratio, which is expected because of the higher relative content of oxygen in silica compared to vaterite. Several coated and uncoated microspheres were examined giving similar results. The coated microspheres show similar silicon/calcium ratios for different samples and for different microspheres in the same sample (Figure 4 B). Even after sonication for approximately 1 h before measurement the coating was still intact showing similar Si/Ca ratios.

These results suggest that APS penetrates into the vaterite microspheres and coats each individual nanocrystal with a very thin layer. If the APS were to coat only the outer surface of the vaterite microspheres with a very thin coating, the EDS results would not show any significant presence of Si.

These results were further supported by XPS analysis. Figure 5A and 5B show XPS spectra of uncoated and APS-coated samples, respectively. From the Ca2p and Si2p peaks the atomic ratio of Ca to Si of a typical APS coated sample was calculated to be 1.83. XPS is a surface specific technique that probes only the top 5−10 nm of the material, which is called its information depth.[34] It should be noted that the calculated Ca to Si ratio is underestimated within the information depth. This is because APS forms the very top surface layer and covers the underlying $CaCO_3$. For these reasons the APS coating must be very thin, of the order of at the most several molecular layers. If the coating were to be thicker, no Ca would be appearing in the XPS scan. As expected according to the stoichiometry of APS the Si to N ratio of a typical APS-coated sample was calculated to be approximately 1 (0.95).

Further a characteristic double peak is apparent (399.6/401.4 eV) in the N1s spectrum (inset of Figure 5B) as a result of APS coating. This peak has been attributed to a mixture of primary amines and protonated amines.[35]

The EDS and XPS results provide good evidence that the APS coating was a successful method to amine-functionalize the vaterite with high reproducibility. However, to make the microspheres useful for experiments on biological samples, where salt buffer solutions are commonly used, the coating also has to stop or at least radically slow down ionic exchange between the vaterite and the buffer. It was found that the APS coating alone did not provide such protection.

Successive coating with TEOS was attempted to overcome the stability issue. The choice of alcohol and catalyst as well as the alcohol/water/TEOS ratio may affect the porosity and thickness of the coating. Several methods were therefore attempted in

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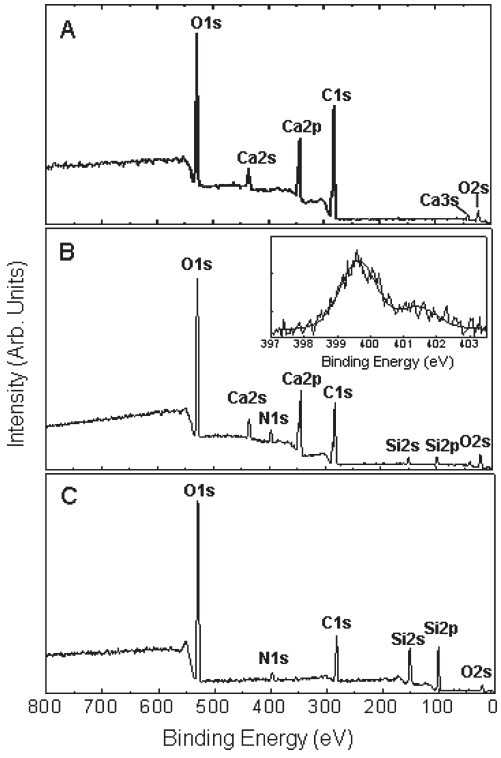

Figure 5. XPS spectra of uncoated (A), APS-coated (B), and APS/2×TEOS/APS coated vaterite microspheres. The inset in B shows the N1s high-resolution scan of APS-coated microspheres.

finding the most protective coating. Other requirements were that the surface of the coated microspheres should remain reasonably smooth and the amount of nucleated silica particles should be kept to a minimum if the microspheres were to be used for optical tweezer experiments.

To avoid any dissolution of the vaterite microspheres during the coating procedure, we used ammonia as catalyst instead of acid. In the current study TEOS coating of uncoated as well as previously APS coated samples was attempted. APS has been used as a primer for TEOS coating of gold particles which normally do not allow silica coating.[36]

Figure 6 shows an electron micrograph of a crushed microsphere coated with TEOS. A general change in the texture of the surface can be seen, indicating that some coating has occurred. The surface no longer consists of the 25−35 nm crystallites that normally can be seen on vaterite microspheres and still appear on the break surface.

These results were supported by XPS analysis of TEOS-coated microspheres with both primary and secondary APS coating (Figure 5C). Compared to uncoated (Figure 5A) and APS-coated microspheres (Figure 5B), no Ca is present in the APS/2×TEOS/APS coated microspheres (Figure 5C). For reasons, discussed before, this is an indication that the TEOS-coating is thicker than 10 nm. It should be noted that the Si to N ratio was calculated to be larger than 10. This again is proof that the APS-coating is of the order of at most a few molecular layers.

LBB^- buffer was used to monitor the stability of the coated vaterite microspheres. The reason for using LBB^- instead of PBS buffer was that it dissolved the uncoated vaterite much faster and hence gave a quicker indication of how well the coating protected the microspheres and prevented leakage of ions between the

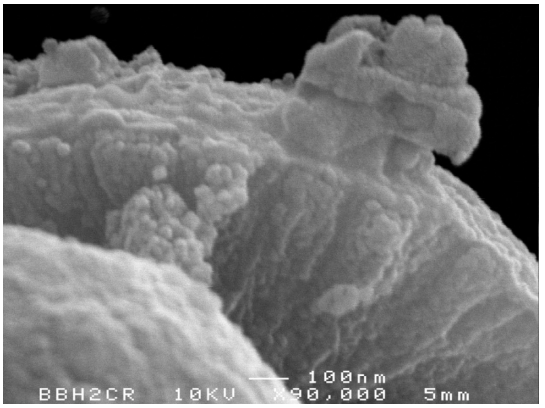

Figure 6. Scanning electron micrograph of crushed TEOS coated vaterite microsphere.

vaterite core and the solution. LBB^- buffer is typically used in experiments involving attachment of biotinylated DNA.[37]

Stability tests showed that uncoated vaterite microspheres dissolved completely in less than 1 min. The APS + TEOS coated microspheres survived for at least 4 h (Table 1), while the TEOS coated microspheres dissolved in less than an hour (Table 1, both significant improvements in stability compared to the uncoated microspheres. Hence, the initial APS coating seems to make a difference in the efficiency of successive TEOS coatings. It was further shown that applying multiple TEOS coatings onto the APS coated microspheres increased their stability of the microspheres to at least 10 h (Table 1). The lifetime of the microspheres scaled with the number of consecutive silica coatings and hence with the thickness of the final coating.

Various coated and uncoated microsphere samples were incubated with BODIPY 530/550 succinimidyl ester to observe differences in adsorption of the dye molecules to the surface. BODIPY 530/550 succinimidyl ester is a fluorescent dye molecule linked to an amino-reactive group and thus binds specifically to the primary amine groups on the APS surface. It can therefore be used to verify an APS coating directly. By removing all unbound dye by washing the microspheres in ethanol, only the dye molecules bound to amine groups should remain and the APS-coated microspheres should show much higher fluorescence than the uncoated ones.

If, on the other hand, a TEOS coating is applied on the APS surface and the resulting silica layer has low enough porosity, no dye molecules should reach the functional groups and could easily be washed off. After a secondary APS coating, they would again stay fluorescent after washing. This way, the same dye could be used to verify each step of the functionalization process.

Samples of APS coated vaterite microspheres were examined using this procedure, and each experiment showed a very high yield of fluorescent microspheres while the uncoated microspheres showed almost no fluorescence. Figure 7 shows a typical set of images from a dye-test. The right column (B, D, F, and H) shows epi-fluorescence micrographs of microspheres in different states of the coating process in blue excitation light. The left column (A, C, E, and G) shows the corresponding bright field micrographs. The samples used in this dye-test were uncoated vaterite for Figure 7 A and B, APS coated vaterite for Figure 7 C,

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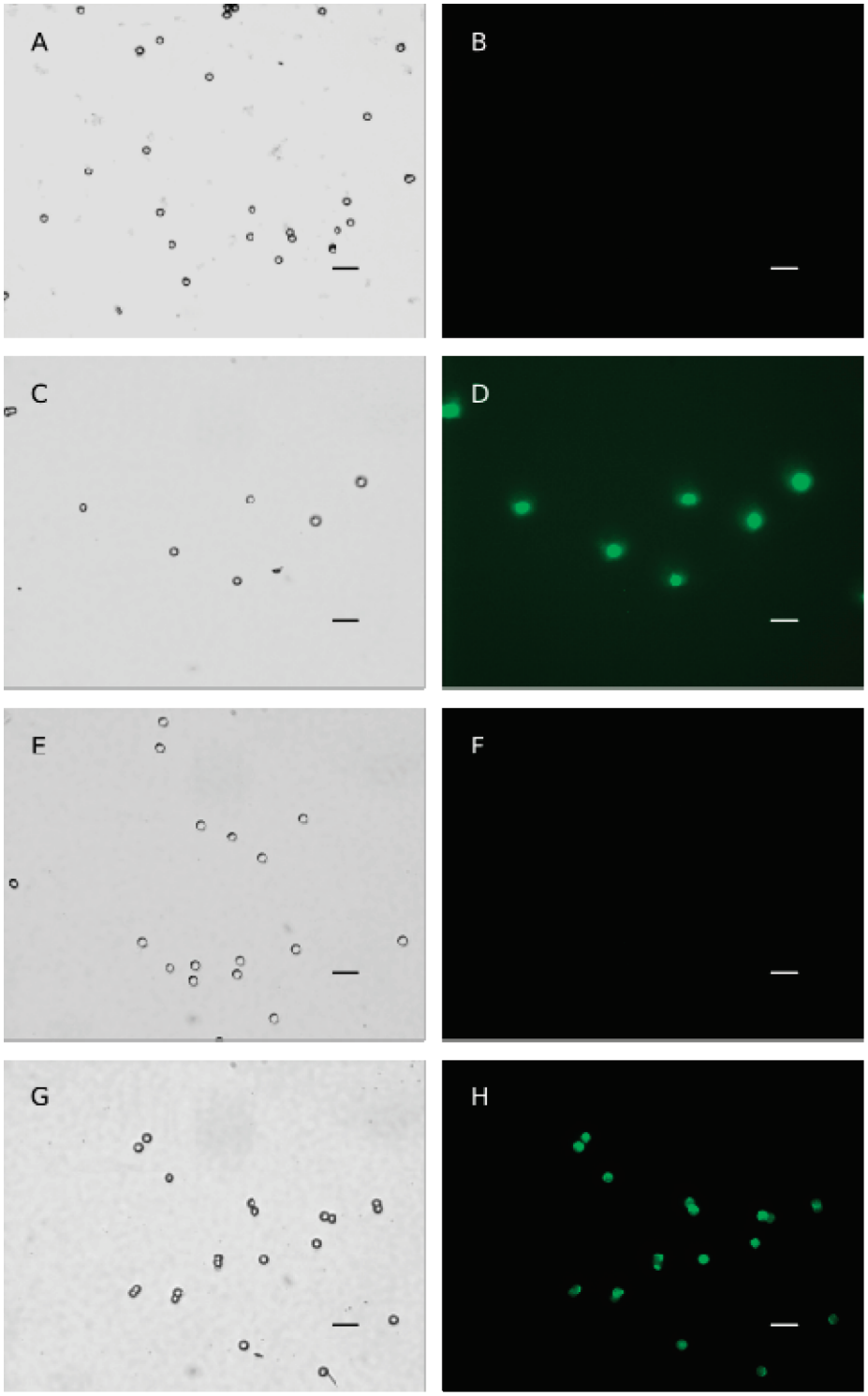

Figure 7. Bright field (left) and fluorescence (right) micrographs showing the change in binding of an amine reactive dye to the surface of vaterite microspheres after each step of the functionalization process. The samples used are uncoated microspheres (A, B), APS coated microspheres (C, D), APS + TEOS coated microspheres (E, F), and APS + TEOS + APS coated microspheres (G, H). The scale bars are 10 μm.

D, TEOS with primary and secondary APS coating before the secondary APS coating for Figure 7 E, F, and after the secondary APS coating for Figure 7 G, H. Although the TEOS coating seems to provide a high yield of coated microspheres (Figure 7 E), the absence of fluorescent microspheres in Figure 7 F shows that there were no or very few amine binding sites available for the dye molecules implying that the APS surface has been completely coated with silica.

Please note that by a simple modification of the coating procedure (using other organosilanes than APS as the outer shell), other functional groups may be incorporated on the surface to allow attachment of virtually any molecule of interest.

(c). Coating Functionalized Vaterite Microspheres with Streptavidin. Streptavidin is a well-characterized protein which binds biotin with one of the highest affinities known in biochemistry.

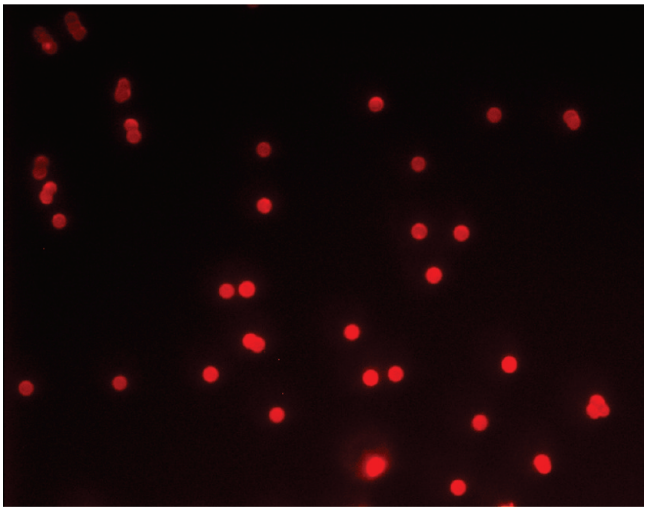

Figure 8. Fluorescent micrograph of the streptavidin coated microspheres. This image was taken in red excitation light (590 nm) in which the glutaraldehyde treated microspheres show no fluorescence.

The streptavidin−biotin affinity is used in many areas of biotechnology, and the interactions are very well characterized.[38−40]

The attachment of streptavidin to amine fuctionalized vaterite microspheres was done using the homobifunctional linker molecule glutaraldehyde. Homobifunctional means that the molecule can bind to the same type of functional group, in this case amine, on each of the two sides of the molecule.

When reacted with amine, glutaraldhyde becomes weakly fluorescent in a broad spectrum of excitation wavelengths from blue to green. Since it was not fluorescent in red excitation light, streptavidin labeled with a dye fluorescing in red excitation was chosen to verify the streptavidin attachment with fluorescence microscopy (Figure 8).

Please note that all coated microspheres look intact and maintain their initial birefringence, as verified in optical tweezers. The measured rotation rates for uncoated and fully coated vaterite spheres (APS, 2×TEOS, APS) were comparable and typically of the order of approximately 100 Hz per Watt of laser power at the beam focus. This result was expected since a thin coating will not significantly affect the optical properties of the microspheres, and will not alter their trapping properties.[41] Even if the coating were thicker, acting as an anti-reflection layer,[42] the optical torque would be little affected, since the microspheres are not highly reflective to start with. What matters for the practical use of the microspheres as rotating birefringent probe particles is that they can be trapped, and remain strongly birefringent after coating, which has been clearly shown in this paper. Other minor changes in the optical properties of the microspheres are unimportant.

Hence using streptavidin coated vaterite microspheres, torque measurements on single molecules, such as botinylated DNA molecules can be carried out.

Conclusion

A new method for the synthesis of spherical vaterite particles has been introduced. The microspheres were shown to have a narrow size distribution, maintain their birefringence and be stable in aqueous agepon solution. The strong birefringence and spherical shape make these particles a first choice for spin angular momentum transfer in optical tweezers.

Immersed in buffers typically used for biomolecule attachment the particles dissolved almost instantly. A method for coating vaterite with several organosilica and silica shells has been developed that was shown to significantly increase the stability in buffer solutions. Streptavidin was attached to the amine functionality of the modified microspheres to demonstrate their usability for applications such as the micromanipulation of single biological molecules in an angular optical trap or biosensors.

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