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Preparation and Tracking of Oblate Core-Shell PMMA Ellipsoids

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Anisotropic colloids, 3D-tracking, Core-shell colloids, Diffusion, Fluorescence Microscopy

ABSTRACT: Although single particle level studies on prolate ellipsoidal colloids are relatively abundant, similar studies on oblate ellipsoids are rare because suitable model systems are scarcely available. Here, we present the preparation of monodisperse hard core-shell oblate ellipsoids that can be imaged and tracked in 3D with confocal laser scanning microscopy. Using a thermomechanical squeezing method, we transform spherical core-shell PMMA particles into oblate ellipsoids. We show how the shape polydispersity as well as the aspect ratio of the obtained oblate ellipsoids can be controlled. In addition, we discuss how the core-shell geometry limits the range of aspect ratios due to the different viscoelastic properties of the crosslinked PMMA core and linear PMMA shell. We further demonstrate imaging of the core-shell oblate dispersions on a single particle level in real space and time and the tracking of position and orientation using our recently developed tracking algorithm for anisotropic core-shell colloids. Our results thus provide the tools for the future investigation of the behavior of oblate ellipsoids, especially in dense suspensions.

INTRODUCTION

In contrast to their spherical counterparts, ellipsoidal particles possess orientational degrees of freedom that lead to an intricate behavior of their suspensions. Firstly, simulations and theory predict complex phase diagrams for suspensions of prolate and oblate ellipsoidal particles, with the appearance of isotropic and nematic fluids, plastic solids, stretched fcc-crystals, and SM2 structures.¹⁻⁶ Secondly, ellipsoidal particles have been shown to have very high random packing densities⁷⁻⁸ and to exhibit jamming properties different from spherical particles⁹. Finally, many biological particles with near ellipsoidal shape, such as red blood cells and thrombocytes, but also drug carriers, exhibit shape-dependent physical properties.¹⁰⁻¹² These few examples highlight the importance of improving our understanding of this wide range of physical phenomena. Experiments, however, rely on the availability of monodisperse ellipsoidal model colloids.

The synthesis of colloidal particles with non-spherical shapes is not straightforward and extensive efforts to develop new synthetic methods for ellipsoidal polymeric colloids have been made over the past decades. As a result, today the synthesis of prolate ellipsoidal colloids via stretching of spherical particles in a polymer matrix is well established. This was first shown for both polystyrene particles¹³ and polymethyl-methacrylate (PMMA) particles¹⁴ and has since even been extended to particles with core-shell structure.¹⁵⁻¹⁷ The general procedure

involves embedding spherical precursors in a polymeric film which is heated above the glass transition temperature of the particles. The film is subsequently stretched such that the particles adopt an ellipsoidal shape, which is retained after cooling the matrix film. The availability of such prolate ellipsoidal colloids has allowed 2D and 3D studies that have addressed isotropic fluid structures,¹⁸ dense phases,¹⁹ nematic liquid crystal formation,²⁰ glass formation,²¹ the influence of electric fields,²²⁻²³ and even patchy intercalations.²⁴⁻²⁵

Similar studies on well-defined oblate ellipsoids are, however, still scarce. The first reason is the difficulty of fabricating monodisperse oblates with high yields. While for obtaining prolate ellipsoids, the controlled deformation of a sphere in one direction is necessary, oblate shapes require uniform deformation in two directions. To date, several methods have been developed for the preparation of oblate colloids, such as uniaxial stretching,²⁶⁻²⁸ film bowing processes,²⁹ magnetic stirring of swollen particle dispersions,³⁰ thermal annealing³¹ and thermomechanical squeezing.³²⁻³³ In this way oblate colloids have been prepared from polystyrene (PS) and polymethyl methacrylate (PMMA) spheres as well as from core-shell compositions. Of these methods, the thermomechanical squeezing is the most suitable for scaling up as it simply involves squeezing disks of particles embedded in a polymer matrix between two plates that can easily be expanded. In addition, several experimental aspects for obtaining monodisperse particles have been resolved,

most notably the shearing effects close to the plate by either employing buffer films³² or lubrication of the plates.²⁷ A second reason limiting single particle resolved studies of oblate colloids, is the challenging nature of visualizing, besides the position, also the orientation of the oblate particles when employing confocal laser scanning microscopy (CLSM). To date, only two quantitative CLSM studies have performed with oblate ellipsoids. Cohen *et al.* investigated the structure of a simple oblate fluid but the shapes were polydisperse and particle orientations were not resolved.¹⁸ Hsiao *et al.* showed the assembly of oblate ellipsoids induced by depletion interaction and while positions as well as orientations were resolved, this was only done for particles in strands and small clusters.³³

The main aim of this study is to develop a system of monodisperse oblate core-shell PMMA colloids that is suitable for quantitative investigations of dense particle suspensions. with CLSM. To achieve this, we employ the thermomechanical squeezing method and a system of fluorescently labelled core-shell PMMA particles. The advantages of these core-shell PMMA colloids are that they can be sterically stabilized with polyhydroxystearic acid (PHSA) coatings and refractive index matched with the dispersion medium. This allows investigations of dense systems with hard-body interactions³⁴⁻³⁵. Spherical particles of this type have been used abundantly for detailed investigations of fundamental processes in dense systems.³⁶⁻³⁹ The particles presented here possess a crosslinked core, to allow accurate tracking of the particles' center of mass, and non-crosslinked PMMA shells, to allow deformation into an ellipsoidal shape. We have previously shown that this is a successful strategy for synthesizing prolate ellipsoids.^{17, 20, 40} We employ the thermomechanical squeezing method to convert these spherical core-shell colloids into oblate shapes and investigate how the particle shape polydispersity can be controlled. Further, we investigate how the core-shell geometry influences the achievable aspect ratio's and the deformation process. Finally, we study how the positions and orientations of these core-shell oblate colloids in suspension can be imaged with CLSM and show quantitative tracking in real space and time with a recently developed tracking algorithm.⁴⁰ We conjecture that the availability of core-shell oblate ellipsoids opens up the possibility to gain unprecedented insights into the role of orientational freedom and aspect-ratio of oblate ellipsoids on phenomena such as crystallization, glass formation and jamming.

EXPERIMENTAL

Materials. Particle synthesis and smoothing process. Methyl methacrylate (MMA), methacrylic acid (MA), ethyl acetate (EA), butyl acetate (BA), ethylene glycol dimethacrylate (EGDMA), 2,2'-azo-bis-isobutyronitrile (AIBN), dodecane, hexane, octyl mercaptane (OctSH) and acetone were purchased from Sigma-Aldrich, Germany. MMA was purified from the inhibitor by filtration over basic aluminium oxide (pH ~ 10, Woelm Pharma, Germany), and MA was distilled under reduced pressure. The PHSA stabilizer, based on the PHSA-*comb*-PMMA copolymer, was

synthesized according to established procedures.^{27, 41-42} We used the PHSA stabilizer as a 40% (w/w) solution in BA/EA (1:2 (w/w)).

Reagents used for dye modification. Quasar 670 Carboxylic Acid (Q670) was purchased from LGC Biosearch Technologies, USA and vinyl-functionalized by esterification with 4-vinylbenzyl alcohol. Bodipy (4,4-difluoro-8-(4-methacrylatephenyl)-3,5-bis-(4-methoxyphenyl)-4-bora-3a,4a-diaza-s-indacene) was synthesized according to literature.⁴³ Bodipy was vinyl-functionalized by esterification with methacrylic acid. The structures of the modified dyes are shown in SI Figure 1.

Reagents for preparing and degrading films. Hydroxy terminated poly-(dimethylsiloxane) (PDMS) ($M_n \approx 110000$, viscosity $\approx 50\,000$ cSt), n-hexane, poly(dimethylsiloxane-co-methylhydrosiloxane) ($M_n \approx 950$, methylhydroxysiloxane 50 mol %, crosslinker), stannous 2-ethylhexanoate (catalyst), n-hexane, isopropanol and sodium methoxide (NaOMe) were purchased from Sigma-Aldrich, Germany, and used as received.

Density-matching of colloids. For the diffusion measurements, the particles were dispersed in a mixture of cis-decalin and cyclohexylbromide (CHB), to match the refractive index and the density of the particles. Tetrabutylammoniumbromide was used to screen charges on the particles. All chemicals were purchased from Sigma-Aldrich, Germany, CHB was degassed by the freeze-pump-thaw technique and stored under nitrogen before use.

Core-Shell Particle Synthesis. Fluorescent core particles. PMMA core particles were synthesized by dispersion polymerization.⁴¹ A non-aqueous precursor solution of 9 g of MMA, 0.2 g of MA, 3 g of dodecane, 6 g of n-hexane, 1 g of PHSA stabilizer, 100 mg of AIBN, 65 μ L of OctSH, and 3 mg of Q670 dissolved in 300 μ L acetone, was placed in a 50 mL round-bottom flask, equipped with a reflux condenser and magnetic stirring. The mixture was gently purged with nitrogen for 1-2 min. Before initiation of the reaction, AIBN has to be dissolved completely. The reaction was started by submerging the flask into a preheated oil bath (90 °C). After 2-3 min, the reaction mixture became turbid, indicating nucleation. Next, a solution of 0.145 g EGDMA, 2 g of dodecane, and 4 g of n-hexane was added dropwise (35.5 mL/h). After the complete addition of the crosslinker, the mixture was stirred for 2h. Finally, the dispersion was cooled to room temperature and washed three times with acetone to remove the unreacted dye. The particles were restabilized by adding 5 % PHSA-stabilizer solution (w/w) in dodecane/n-hexane (1:2 (w/w)) and applying ultrasonication for 5 min. After a final wash step with dodecane/n-hexane, the particles were redispersed in dodecane/n-hexane to a final concentration of ~50% (w/w).

Fluorescent PMMA/PMMA core-shell particles. PMMA core particles were used for seeded dispersion polymerization. 2 g of the particle dispersion (~ 50 % (w/w)) was placed in a 50 mL round-bottom flask and diluted with 1 g of dodecane/n-hexane (1:2 (w/w)). 300 mg of AIBN and 70 μ L of OctSH was added and dissolved. The mixture was heated to 90°C. 20 mL of a monomer mixture consisting of

10 g of MMA, 0.2 g of MA, 3.5 g of dodecane, 7 g of n-hexane, 1.2 g of PHSA stabilizer, and 3 mg of Bodipy were added dropwise (12 ml/h). After an additional 1 h of stirring, the particle dispersion was cooled to room temperature and washed twice with decalin to remove unreacted chemicals and secondary nucleation particles. After a final washing step with dodecane/n-hexane, the particles were redispersed and concentrated in dodecane/n-hexane to a final concentration of ~50% (w/w). This seeded growth step was repeated several times to obtain PMMA/PMMA core-shell particles with a diameter of 4.7 μm .

After synthesis, the particles were often found to have a rough surface that is maintained even after the deformation process.¹⁷ Therefore, a solvent mediated smoothing procedure is employed. In this process the dispersion medium becomes a better solvent for the shell and liquefies the polymer network. The tendency to reduce the surface energy and increased polymer mobility leads to efficient smoothing of the particle surface. For the process, 1 g of PMMA/PMMA core-shell particles were dispersed in 10 g decalin and mixed with a homogeneous mixture of 9 g acetone and 50 g decalin. The dispersion was stirred for 1 h, after which the smoothing process was quenched by diluting the mixture to 50 g of decalin. The smoothed particles were obtained after washing three times with decalin.

Squeezing of oblate ellipsoids. Film preparation. The preparation of PDMS films for thermomechanical deformation procedure has previously been described.^{17, 36} At the beginning, up to 2.0 g of the core-shell particles were dispersed in a mixture of 200 g hexane and 76 g PDMS. After the addition of 0.29 g of the crosslinker methylhydro-dimethyl siloxane copolymer and 0.35 g of the catalyst stannous 2-ethylhexanoate, each dissolved in 5 mL hexane, the solution was mixed carefully. After the complete disappearance of air bubbles, the mixture was poured into a rectangular PTFE coated mold. To avoid thickness gradients in the film, the planarity of the mold was adjusted with a water level. The film was dried overnight in air. Most of the hexane evaporated during this process. In the next step, the PDMS based particle-film was crosslinked at 120 °C for 6 h. This process was performed under nitrogen atmosphere to prevent oxidation of the catalyst. The resulting film was stored between two PTFE coated plates. The same routine was performed to make the PDMS buffer film. The remaining films have a thickness of 1.0 mm.

Squeezing procedure. Circular films with a diameter of 3 and 4 cm were cut from the PDMS films. The PDMS particle film was placed between two metal disks, with or without PDMS buffer films. Metal spacers with defined sizes between 0.5-1 mm in steps of 0.1 ± 0.005 mm were placed near the edges of the metal disks. The spacer allowed the exact tuning of the amount of film compression while they did not hinder the film deformation. By changing the spacer height L for 3 cm films with buffers, we explored squeezing factors between $0.4 < SF < 0.9$. In addition, we studied the role of the thermoelastic expansion of the PDMS films by investigating $SF \geq 1.0$. For $SF = 1.0$ the

spacer height matches L_0 , while $SFs > 1.0$ were obtained with $L > L_0$ and $SF_{\min} \sim 1.2$. This was achieved by not using a covering steel plate. We further investigated the limits of squeezing to see the effect of the crosslinked core on the final oblate shape. For this we achieved $SF_{\max} \sim 0.3$ by not employing a spacer and by exerting the maximum force possible. The setup was pressed until both metal plates were touching the spacers and the setup was heated up to 165 °C for 1 h, unless stated otherwise. Next, the films were cooled down to room temperature, and were subsequently degraded by mixing 3.5 % PDMS-film, 78.8 % hexane, 17.7 % isopropanol and 0.04 % NaOMe by weight. This mixture was stirred at room temperature for 20 h and finally washed 3 times by centrifugation and redispersion with isopropanol, twice with hexane, and once with decalin. The obtained oblate ellipsoids were restabilized by adding 5 % PHSA-stabilizer solution (w/w) in decalin and applying ultrasonification for 5 min. To remove unused stabilizer, the colloids were subsequently washed with decalin and finally dispersed in 5 mL of decalin. More detailed information about the design of the mechanical press can be found in the SI.

Locking process. During the degrading process of the PDMS matrix, the steric PHSA stabilizer is removed completely from the particle surface. To ensure the steric stabilization of the resulting colloids in further applications, the stabilizer was locked covalently on the particle surface. To this end, 2 μL of N,N-dimethyldodecylamin were added to 20 mL of an approximately 1% (w/w) stabilized particle dispersion. The reaction mixture was stirred by refluxing at 80 °C for 24 h and after cooling, the colloids were washed twice with hexane. The reaction was performed under inert atmosphere to prevent side reactions of the fluorescent dyes.

Particle characterization. Scanning electron microscopy measurements. Scanning electron microscopy (SEM) was performed on a Crossbeam 1540XB (Zeiss, Germany) at an acceleration voltage of 1 kV. Particles were deposited on plasma cleaned silicon wafers from dilute dispersion in decalin and allowed to slowly dry at ambient conditions. We found that with this process drying effects could be avoided and dilute monolayers of particles were obtained. SEM images of particles deposited and dried on silicon wafers were recorded by using the secondary electron detector of the microscope. Typically, the particles deposited on the silicon wafer were found to be slightly tilted with respect to the surface of the wafer. Yet, due to the circular symmetry of the particles' circumferences, it is possible to determine the length of the semi-major axis a . We carefully examined different parts of the silicon wafer to make sure images of the particles were representative for the full system and drying effects did not influence the particle shape.

Particle sizes and shapes of at least 100 particles were analyzed using the open source software ImageJ (Version 1.51). To determine a of the oblate particles, an elliptical selection mask was placed over images of individual particles. The semi-minor axis was calculated assuming volume preservation during the squeezing process. This was done by equating the ellipsoid volume ($V_E = 4/3\pi a^2b$)

to the volume of the original sphere ($V_s = 4/3R^3$) and calculating the length of the semi-minor axis according to $b = V_s/(4/3\pi a^2)$.

Figure 1. Typical (A) SEM and (B) fluorescence images of the spherical core-shell particles. (C) Schematic illustration of the squeezing process. Core-shell particles are embedded in a PDMS matrix, placed between two PDMS buffer films. Next the films are squeezed by a mechanical press and heated above the glass temperature of the PMMA shell which transforms the shell into an oblate shape that is retained upon cooling the films. Typical (D) SEM and (E) fluorescence images of the core-shell particles with a spherical core and oblate shell that can be obtained. Scale bars in (A, B, D, E) are 10 μm .

Confocal microscopy measurements. Confocal laser scanning microscopy imaging was performed on an inverted laser-scanning microscope (TCS SP5, Leica Microsystems, Germany) equipped with a resonant scanner (8 kHz, bidirectional mode), a glycerol objective (63 $\times$, 1.3 NA, Leica Microsystems, Germany), and a temperature stabilization unit (Ludin cube, Life Imaging Services, Switzerland) at 22 ± 0.05 $^{\circ}\text{C}$ for optimizing the density match between the particles and the solvent. For illumination we used a He-Ne laser with $\lambda = 633$ nm to excite the Q670 dye and an Ar laser with $\lambda = 514$ nm to excite the Bodipy dye. Samples of diluted colloid dispersions were prepared in a refractive index and density matching CHB/decalin mixture. To achieve this, samples were centrifuged multiple times over different increasing time steps. Between additions of CHB or decalin, either the particles swim or sink after the centrifugation process. After 4 h and a centrifugation speed of 4000 g, the colloid suspension is nearly density matched. The dispersions were placed into a home-made sample cell, based on a 160 μm thick cover glass. The sample was placed on the microscope and left to rest for 16 hours to reduce drift. Subsequently, xyz -stacks of $144 \times 36 \times 16$ μm with a resolution of 1024×256 pixels and a z -step of 0.21 μm were recorded with a lag time of $\tau = 3$ s between stacks. This spatial and temporal resolution was found to be fast enough to resolve individual particle orientations and resolve their dynamics. Samples were

imaged between 30 μm and 80 μm above the sample bottom wall to avoid wall effects.

RESULTS AND DISCUSSION

Squeezing core-shell colloids. The oblate core-shell ellipsoidal PMMA particles are obtained via the thermomechanical squeezing process as schematically shown in Figure 1. To make sure that the ellipsoids possess a spherical core after this procedure, we used crosslinked, spherical PMMA core particles with a diameter of $d = 1.004 \pm 0.038$ μm and fluorescently labeled by a vinyl-modified Quasar670. The crosslinker provides the necessary mechanical stability of the cores in the thermomechanical squeezing process. These cores were coated with a non-crosslinked Bodipy labeled PMMA shell that can be deformed in the squeezing step. To obtain the desired shell thickness of $R \sim 2$ μm , the PMMA shell was grown in several non-aqueous seeded dispersion polymerization steps. Figure 1A shows an SEM image of the final core-shell particles with $d = 4.724 \pm 0.028$ μm . Figure 1B shows a CLSM image of the same particles, where the core-shell structure is clearly visible.

The thermomechanical squeezing process to transform the core-shell spheres into oblate ellipsoids is schematically depicted in Figure 1C. For this process, the core-shell colloids are embedded in a PDMS matrix, from which circular pieces are cut out. These pieces are placed in the

middle between two steel plates of a mechanical press. Buffer layers are placed on top and below the film, which reduces the shape and size polydispersity. This method was first reported for the thermomechanical squeezing of polystyrene beads in a PVA matrix.³² The buffer layers place the particle film in the plane of an approximately homogeneous elongation that occurs between the two pressing plates, as can be seen from calculations of the squeeze flow for the case of Bingham⁴⁴ and Herschel-Bulkley⁴⁵ fluids. In addition, spacers are placed on the bottom plate that allows us to tune the squeezing factor (SF) of the film. The SF ultimately controls the oblate ellipsoids aspect-ratio, $AR = a/b$, where a is the semi-major axis and b the semi-minor axis of the ellipsoids. With this setup a relatively high yield of particles can be obtained because of the design of the mechanical press allows it to be easily expanded in the z-direction by adding more pressing layers (for more details see SI Figure 2). In this way, the same amount of particle film as typically used for investigations of dense suspensions of prolate ellipsoids can be processed.

To start the squeezing process of the particle film, a force is applied perpendicular to the films with a mechanical press. This step is done at room temperature, which is well below the glass transition temperature of the PMMA particles of 105°C. Since Young's modulus for PDMS is about a thousand times smaller than for PMMA,²⁵ in this step we assume that only the PDMS matrix but not the particles are deformed. The squeezing process will thus result in complex stress fields around the particles. Next, the setup is heated to 165 °C, which brings the particles above the glass transition temperature of the non-crosslinked PMMA shell. The relaxation of the stress fields in the PDMS matrix will then induce the transformation of the PMMA shell into an oblate shape. After 1 h, the system is cooled down, the applied force removed, and the squeezed particle film extracted. The core-shell oblate ellipsoid particles are obtained by degrading the PDMS matrix and performing subsequent washing steps. Figure 1D shows a typical SEM image of the oblate ellipsoids obtained with this squeezing procedure. Here, the oblate ellipsoidal shape can be clearly distinguished, especially when compared to Figure 1A. Figure 1E shows a CLSM image of the oblate particles where the core-shell structure, with a spherical core and ellipsoidal-shell, is clearly visible, confirming the transformation of the shell into an oblate shape.

Shape and polydispersity control. We found that several aspects the squeezing process need to be controlled to obtain monodisperse oblate particles in terms of shape from these core-shell PMMA particles. First, employing PDMS buffer layers – which are used to have the same chemical nature as the particle films – were found to provide similar homogeneous elongation of the particle films as previously found for PVA films.³² Figure 2 shows the particles and their aspect-ratio polydispersity obtained without and with the use of buffer films. The absence of buffers not only leads to particles with different oblate shapes but also ellipsoidal particles with three different axis lengths ('surfboards') as indicated with the white

arrows (Fig. 2A and 2B). We further explored the full shape polydispersity index (PDI) of the particles in terms of aspect ratio (AR) and found that the use of buffer films significantly reduces the spread in the AR (Fig 2C and 2D).

Figure 2. (A, B) SEM images and (C, D) aspect ratio distributions of the squeezed oblate particles obtained with 0 buffer layers (A, C)) and with 5 buffer layers (B, D)). The arrows highlight particle shapes deviating from oblate symmetry. Scale bars in A, B are 10 μ m.

In addition, we found that the use of one pair of buffer layers already leads to a reduction of the AR-PDI from > 30% to < 10% and that the use of additional buffer layers did not lead to a further improvement of the AR-PDI (See SI Figure 3).

Figure 3. (A, B) SEM images and (C, D) AR-distribution of oblate particles obtained from the inner ring (3 mm) and the outer ring (14 mm) of a 3 cm film. Scale bars in (A,B) are 10 μ m.

Secondly, due to pressing a round film, radial stresses play an important role and exclusion of the outer rim of the particle films as well as spacers are needed to obtain monodisperse oblates shapes. Figure 3 shows the particles obtained from the inner and outer ring of a 3 cm diameter film pressed without spacers. Here, a clear difference in oblate shapes can be observed, with similar oblate and spherical

shapes in the outer ring (Fig. 3A and 3B). The extreme shapes are the result of biaxial deformation as indicated with white arrows. The distribution of aspect ratios for the inner and outer ring shows a clear spread of the AR in the outer ring (Fig 3C and 3D). This confirms that the stress field is much more homogeneous in the center of the film than at the edge, motivating the use of spacers. We also found that the AR-PDI is largest in the outer ring of the film, with a jump AR-PDI from $\sim 10\%$ to $\sim 20\%$ in the outer 3 mm. By employing spacers, the film is pressed more homogeneously and reduces the AR-PDI, although the particles in the outer rim ($\sim 15\%$) irrespective of total film radius will still experience an inhomogeneous stretching (See SI Figure 4). Clearly, throughout most of the film the particles are deformed homogeneously, but in the outer edges shear-effects deteriorate the obtainable AR-PDI and thus need to be excluded.

Aspect ratio control. Besides controlling the polydispersity with buffer layers and spacers, we found that the range of oblate ARs that can be achieved for these core-shell PMMA particles with the squeezing setup has limitations due to their core-shell geometry. The AR is controlled by the compression of the film given by the SF, which is defined as the ratio L/L_0 , where L is the spacer height and L_0 the film thickness before squeezing, where we assume that the film thickness after squeezing is equal to L . Figure 4 shows SEM images of oblate particles obtained with SFs between $SF_{\max} \sim 0.3$ and $SF_{\min} = 1.2$, where $SF_{\max}$ was achieved with maximum pressure and no spacers, $SF\ 0.4 \leq SF \leq 1.1$ with different spacer heights, and $SF_{\min} \sim 1.2$ without the use of a covering steel plate. With decreasing SF, i.e. increased squeezing, a clear increase in the length of the semi-major axis a , can be observed as well as a more pronounced oblate shape. We find that between $SF = 0.5$ and $SF = 0.9$ the particles possess a clear oblate shape (Fig 4D – 4H), while for the other SFs the particles are slightly deformed. For very limited to no squeezing (SFs ≥ 1.0) oblate but not uniform shapes are visible (Fig. 4A – 4C). An explanation for these deformations is the thermoelastic expansion of the PDMS film during heating as well as the formation of complex stress field around the particles during polymerization of the PDMS matrix. Although both forces supposedly are weak, they are clearly strong enough to deform particles embedded in the matrix. For the highest squeezing factors (SFs ≤ 0.4) the crosslinked core becomes visible (Fig. 4I) and particles become almost UFO-like shaped for $SF_{\max}$ (Fig. 4J). This protrusion of the core confirms that the crosslinked core retains its spherical shape after the squeezing method but also shows there is a limit to how much the core-shell colloids can be deformed. Figure 5A shows the obtained ARs for each SF. The highest AR value that is achieved is $AR = 0.91$ for $SF = 1.1$ and the lowest $AR = 0.13$ for $SF_{\max}$. When volume preservation of the particles during the film squeezing is assumed the AR is expected to have an $SF^{3/2}$ dependence (dashed-line). It is clear that the experimental AR values follow the prediction quite accurately over a large range of SF. Only for the larger $SF \geq 1.0$, which are not truly squeezed particles, the AR deviate from the curve as expected. Surprisingly, the oblates with visible core

protrusions still accurately match the prediction. However, considering only the particles without visible core protrusion, we conclude that we can obtain oblate particles with an AR-range from $AR = 0.81$ for $SF = 0.9$ to $AR = 0.37$ for $SF = 0.5$, as indicated by the green shaded area in Figure 5A.

Figure 4. (A-J) SEM images of the oblate colloids obtained with different squeezing factors indicated in the top right corner. Scale bars are 10 μm .

The lower limit of the accessible ARs without core protrusion, i.e. the point where the core diameter equals the oblate minor semi-axis, is given by the ratio of the core diameter with respect to the shell thickness. Figure 5B shows how the minimum AR depends on the employed core-shell ratio. For our core-shell PMMA particles the core-shell size ratio is 3.72 (dashed vertical grey line) and hence the achievable minimum AR should be 0.15. However, we find that the core already starts to protrude for $AR = 0.25$ ($SF = 0.4$). Closer inspection of the UFO-like particles ($AR = 0.13$, $SF_{\max}$) reveals that the core protrusion is caused by a ~ 600 nm thick layer of PMMA shell remaining around the spherical core (inset in figure 5B).

When taking into account the remaining shell thickness, we define a protrusion limit for the minimum AR obtainable by squeezing core-shell colloids with a 1 μm core (blue line Figure 5B). This clearly reduces the minimum ARs possible with these core-shell particles. In addition, also the minimum AR limit achievable for particles obtained with $\text{SF} = 0.4$ are shown (green line Figure 5B), for which the core-protrusion is minimal.

Figure 5. (A) The obtained aspect-ratios in relation to all squeezing factors investigated for the employed particles with a core-shell size ratio of 3.72. The black dashed line shows the $\text{AR} = \text{SF}^{3/2}$ prediction. Green shaded area indicates the region in which oblates shapes were successfully achieved. (B) The accessible minimum aspect-ratio as a function of core-shell size ratio. The theoretical limit in the case of homogenous shell transformation (black line), the limit due to remaining shell surrounding the core for a 1 μm core (blue line), and the limit based on $\text{SF} = 0.4$ where slightly protruding cores are visible (green line). The dashed line indicates the influence of polydispersity onto the limit. Inset: SEM image of side view of oblates obtained with SF_{max} .

The presence of the shell layer on top of the cores is unexpected, especially as no such observation has been made for core-shell prolate ellipsoid before. We found that the PMMA layer is caused by incomplete shell deformation as well as compression of the particles in the PDMS matrix when the external pressure is applied. Figure 6A to 6E show the oblate ellipsoids obtained with the same film

thickness, buffer layers, spacer height and external force, but differing heating times. It can be observed that the particles become increasingly oblate in shape with heating time and their AR decreases accordingly (Fig. 6F). In addition, the core can be seen to start to protrude after 180 min (Fig. 6C) which disappears after 720 min of heating (Fig 6E). From this time dependence, we conclude that the liquefaction of the shell occurs upon heating above T_g but that the response of the shell to the local stresses is a relatively slow process. This slow deformation can be explained by the high elastic modulus of the linear PMMA leading to rubbery flow.⁴⁶

The continued deformation also reveals that for longer periods of time the $\text{SF}^{3/2}$ dependence does not hold anymore. Why the squeezing continues remains unclear although it might be related to the thermal expansion of the PDMS. However, we can conclude that to explore different ARs with PMMA core-shell particles it is crucial to control both spacer height and heating times. Moreover, the appearance and disappearance of the core-protrusion in combination with the slow deformation of the shell indicates that not only the PDMS matrix but also the particles must be deformed when the external pressure is applied. The shell PMMA layer on the core is present because the core was compressed and did not return to its spherical shape during the heating time but only after removal of the external pressure. Clearly, the viscoelastic properties of the PMMA shell plays an important role. Thus, the squeezing of these core-shell PMMA particles into oblate shapes, although reproducible and controllable with the same heating time and spacer height, is relatively complex and has several limitations due to the core-shell geometry of the particles.

Figure 6. (A-E) SEM images of the oblate colloids obtained with different heating times indicated in the top right corner. (F) AR-distribution of oblate particles. Scale bars in (A-E) are 10 μm .

Detection and tracking in 3D. The main reason to choose core-shell oblate PMMA colloids is that core and shell can be labeled with two different fluorescent dyes. This gives the advantage that the particle positions and orientations in suspensions can independently and simultaneously be tracked with high accuracy. With CLSM we imaged a dilute dispersion of core-shell oblates with $a = 6.58 \pm 0.19 \mu\text{m}$ and $\text{AR} = 0.37 \pm 0.087$ in a refractive and density matching CHB/decalin mixture (see Movie SI. 1). The CLSM imaging volume of $144 \times 36 \times 16 \mu\text{m}$ was captured with a xy -resolution of 1024×256 pixels and a z -step of $0.21 \mu\text{m}$ for a full duration of 180 s and a lag time of $\tau = 3$ s between stacks. The resolution of the $xyzt$ -stacks allows us to extract the positions and orientations of the oblates over time with high accuracy and subpixel resolution. To this end, we employ our recently developed tracking algorithm for prolate core-shell ellipsoids³⁴ that recognizes the spherical particle core as the particle center of mass and can be easily adapted to recognize an oblate shell shape. Figure 7A shows a 3D reconstruction of the oblates of part of the imaged sample volume where the RGB color code indicates the particle orientation. From the particle positions and orientations, we derived spatio-temporal trajectories. Figure 7B and 7C show typical translational and rotational motion trajectories obtained that show that

the oblates are free to explore different positions and orientations in space. We further determined the mean squared displacement (MSD) and the mean squared angular displacement (MSAD, using the definition of Hunter *et al.*⁴⁷ as shown in Figure 7D and 7E. As expected for free translational and rotational diffusion in dilute suspensions, we find a linear dependence of $\langle \Delta r^2 \rangle \sim t$ and $\langle \Delta \theta^2 \rangle \sim t$. To determine the respective diffusion constants, we fitted the MSD curves according to Roller *et al.*⁴⁰ with

$$\langle \Delta r^2 \rangle = 6D_t t + 6\epsilon_t^2$$

and the MSAD curve with

$$\langle \Delta \theta^2 \rangle = 4D_r t + 2\epsilon_r^2$$

From the best fits to the data we find for the translational diffusion coefficient $D_t = 0.032 \mu\text{m}^2 \text{s}^{-1}$ and for the rotational diffusion coefficient $D_r = 0.0034 \text{rad}^2 \text{s}^{-1}$ with uncertainties of $\epsilon_t = 47 \text{nm}$ and $\epsilon_r = 5.9^\circ$, which is comparable to our previous results for prolate ellipsoids.⁴⁰ The comparison further confirms subpixel accuracy for the positional tracking and high resolution for the rotational tracking. Our results thus show that the developed core-shell oblate colloids combined with our tracking algorithm are very well suited for quantitative 3D CLSM studies and will allow us to study dense phases in the future.

Figure 7. (A) 3D reconstruction of positions and orientations of a dilute dispersion of oblate core-shell colloids with $\text{AR} = 0.37$, in part of the volume imaged with the CLSM. The RGB color code indicates orientation of the minor semi axis of the oblate ellipsoids. Typical (B) translation and (C) rotation trajectories for a single particle over $t = 180$ s with a lag time of $\Delta t = 3$ s. (D) mean squared displacement (MSD) curve and (E) mean squared angular displacement (MSAD) curve calculated from all particle tracks, showing free diffusion.

CONCLUSION

In this study, we demonstrate that the thermomechanical squeezing method can be employed for the facile transformation of core-shell PMMA spheres into oblate ellipsoids. We found that to obtain monodisperse oblate ellipsoids buffer films, spacers, and exclusion of the outer rim of the squeezed particle film is necessary. Our experiments further revealed that due to the core-shell geometry there are specific preparation factors that must be controlled to achieve a full range of aspect ratios. These factors including the core-to-shell ratio, spacer height, and heating time, where the latter controls how much the PMMA shell reshapes and if the crosslinked core, which gets compressed by the external pressure, relaxes back to its spherical shape. In addition, we showed that the core-shell oblates can be imaged with the CLSM and that combined with our recently developed analysis algorithms for anisotropic core-shell colloids can be accurately tracked and traced in real space and time.

The oblate colloids consist of the same materials PMMA sterically stabilized with PHSA, as the system of nearly hard spheres used extensively to explore theoretical predictions.⁴³⁻⁴⁶ Therefore, the successful synthesis of these traceable core-shell oblate ellipsoids opens up the possibility to quantitatively explore the diffusion and phase behavior of hard oblates on a single particle level in 3D. Future work will address the role of particle aspect ratio on the diffusion of ellipsoidal colloids, a problem that can now be explored from oblate ellipsoids all the way to prolate ellipsoids.^{17, 20} In addition, it will be possible to test theoretical and simulations results for the dense phase behavior of oblate ellipsoids, such as the formation of nematic and columnar phases as well as the role of orientational order on glass phases and jamming.

ASSOCIATED CONTENT

Supporting Information

Setup of the colloidal press. Retrieval of particle size distributions from SEM images. Calculations of the aspect-ratios from the particle sizes. Effect of buffer layers. Role of radial distance.

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